The Second International Symposium of Molecular Pathology

2001.8.18-23, Chengdu, the People's Republic of China

Supported by
Japanese-Chinese Medical Association
China Medical University

Organizing Committee
Deparment of Pathology, China Medical University

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CONTENTS

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Organizing and Scientific Committee

President	Jia Xin Shan	(China Med. Univ.)
	Nagura Hiroshi	(Tohoku Univ.)

Japanese scientific committee

Hata Junichi	(Keio Univ.)
Takashi Saku	(Tsaku Univ.)
Abe Masafumi	(Fukushima Univ.)
Yoshiyuki Osamura	(Tokai Univ.)
Aooi Akishi	(Yamanashi Med. Univ.)
Shamoto Mikihiro	(Fujita Health Univ.)
Tsutsumi Yutaka	(Fujita Health Univ.)
Inai Kouki	(Hilocima Univ.)
Kikuchi Masahiro	(Fukuoka Univ.)
Motohiro Takeya	(Kumamoto Univ.)
Watanabe Masatoshi	(Mie Univ.)
Sato Eiichi	(Kagoshima Univ.)
Hasui Kazuhisa	(Kagoshima Univ.)

Chinese science committee

Li Gan Di	(Sichuan Univ.)
Zhengguohao	(anhui Med. Univ.)
Liu Yan Fang	(The 4th Army Med.Univ.)
Si Lue Sheng	(Xian Med. Univ.)
Wangjilun	(Senyang Med. Col.)
Sunhanxiao	(Jinan Univ.)
Zhang Yue E	(Fudan Univ.)
Yangxianghong	(China Med. Univ.)
Fangweigang	(Beijing Univ.)
Dengzhongduan	(Tongji Med. Univ.)
Liyulin	(Jilin Univ.)
Jia Xin Shan	(China Med. Univ.)

Secretary

Japanese	Hasui Kazuhisa	(Kagoshima Univ.)
Chinese	Yangxianghong	(China Med. Univ.)
	Mixiaoyi	(China Med. Univ.)

Supported by
Japanese Chinese Medical Association
China Medical University

General Information of The Second International Symposium of Molecular Pathology

Date: August 18-24,2001

Venue: The symposium will be held in the meeting room of Tianshi Hotel in Chengdu.

All the participants must arrive Tianshi Hotel in Chengdu by August 17,2001 from Japan or the other areas. Promoting the symposium in success and in ecomonic aspect for attending the symposium, the organizing committee plan a group travel for the Japanese participants.

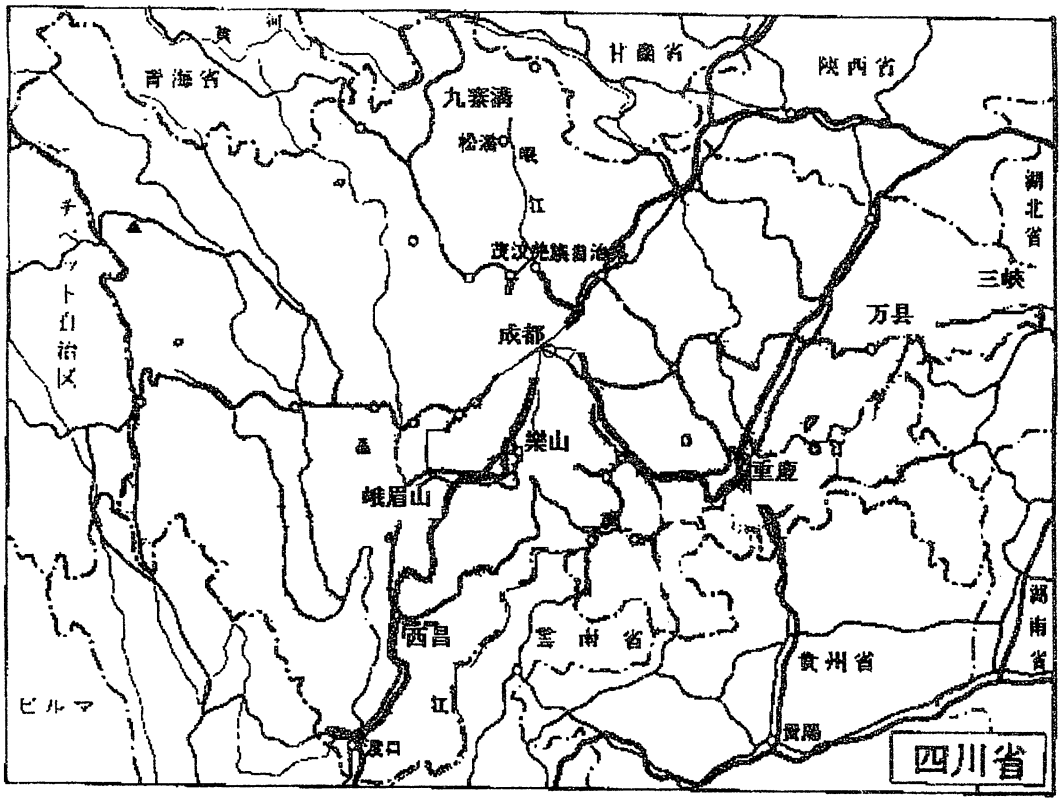
Language:
The official language of the symposium is English.

Wear: Informal. Considering the climate in Chengdu is very hot and moist, please all the participants prepare suit wears.

Registration:
Most of the participants are pre-registered. People who want to attend the symposium must contact the Chinese side President of the symposium, Prof. Jiaxinshan at the meeting room in Tianshi Hotel in Chengdu.

Presentation in the scientific program:
Special lecture: The presenter must finish the oral presentation within 25 min and left at least 5 min for discussion. **Lecture:** The presenter must finish the oral presentation within 25 min and left 5 min for discussion. **Oral presentation:** The presenter must finish the oral presentation within 12 min and left at least 3 min for discussion. **Poster presentation:** The presenter must prepare the poster in 90(W) X 150(H)cm on the plate where will be indicated before the opening of the symposium (in the morning August 18, 2001). The presenter must stand by the presentation and answer the questions in the discussion time according to the program. The presentation must be removed after the closing of the symposium.

Group travel (To Chengdu and Jiuzhaigou natural legacy of world s) for all participants and social and associating persons to attending The Second International Symposium of Molecular Pathology, planed by the Chinese Organizing Committee.
(The travel will be organized by Chinese International travel society in Liaoning
Tel: 86(024)-86809796
Fax: 86(024)-86806890
E-mail: lncitsn@pub.sy.ln.cn



August 17,2001 Registration of participants

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August 18,2001 International Symposium of Molecular Pathology in Tianshi Hotel Chengdu

Welcome party

August 19,2001 Scientific meeting in Tianshi Hotel

August 20,2001 Going to Jiuzaigou by bus

Scientific meeting in Jiuzaigou

August 21,2001 Scientific meeting in Jiuzaigou

August 22,2001 Scientific meeting in Jiuzaigou

The closing ritual of this meeting

August 23, 2001 Returning of all participantss

Opening Remarks

It is my great honor and privilege to declare the opening the Second International Symposium of Molecular Pathology to all participants from China and Japan. Previous symposium was held in 1998 in Dunhuang, the People's Republic of China by Dr. Jia Xin Shan, Professor of China Medical University and Dr. Eiichi Sato, Professor of Kagoshima University.

There are growing impacts of the molecular biology on pathology as well as clinical medicine during these three years. These are many interesting contributions from molecular biological investigations on both neoplastic and immuno-inflammatory diseases in our program. Each participant brings the latest results of his work to the symposium, and I hope all participants will enjoy the discussion during the symposium. It is also important for the symposium to promote collaborative studies and to strengthen personal friendships among the participants from China and Japan.

Finally, this symposium was made possible by the kind and valuable assistance of the most outstanding pathologists in China and Japan as well as the many efforts of Dr. Kazuhisa Hasui of Kagoshima University.

Japanese Side Present of the Symposium
Hiroshi Nagura, M.D. & Ph.D.
(Professor, Tohoku University)

Ladies and gentlemen:

At first, thank you for attending the meeting toilfully. Three years ago, we hold the first International Symposium of Molecular Pathology on Aug 26, 1998 in Dunhuang. Three years is only a wink of history, but it leaves the great promotion in molecular biology. Among which, the most remarkable is Human Gene Map, new technique of DNA chips and the research about embryonic stem cells. These will push the development of the whole pathology, which has been partly communicated in the meeting and did great good to us. Since WHO claimed new histologic classification of lymphoma and lung cancer, both Japanese and Chinese researchers face amending the diagnostic criterion. It is a rather vital task and also has been debated in this meeting. This symposium deepened our comprehension to each other, enhanced communication. While committee will exchange views about reinforcing intercourse of pathology between Japan and China. All of those lay a foundation for cooperation and communication in the future. With Japanese amicable gentry attending, I believe this symposium will produce more great influence for Japanese-Chinese amicability. After meeting, we will tour World Natural Legacy-Jiuzhaigou and some showplaces in Chengdu.

At last, I want to express a lot thanks to Prof. Ligandi and his postgraduates, Huaxi Medical College and Tianshi Hotel for their hard work and warm acceptation.

Wish your good health and happy traveling! I am looking forward to seeing everyone next time.

President of the symposium:

Jia Xin Shan. M. D.

(Professor, China Medical University)

2001. 8.

List of Chinese participants

Academic part

Acce. Prof.	Wuqiang	Anhui Medical University
Prof,	Zhengguohao	Anhui Medical University
Mr,	Zuoguangwu	The central Hospital of Chaoyang
Mr。	Zhangxiguang	The first Hospital of Beipiao
Prof.	Ligandi	Huaxi Med.Col. of Sichuan University
Miss.	Zhangwenyan	Huaxi Med.Col. of Sichuan University
Miss.	Penghui	Huaxi Med.Col. of Sichuan University
Prof.	Sunhanxiao	Jinan University
Mr.	Zengzihua	Jinan University
Miss.	Caiqi	Neoplasma Hospital of Fudan University
Prof.	Jiaxinshan	China Medical University
Miss.	Hanyuchen	China Medical University
Prof.	Heanguang	China Medical University
Mr.	Guoyi	China Medical University
Prof.	Yangxianghong	China Medical University
Prof.	Zhanghong	China Medical University
Acce. Prof.	Lijianhua	China Medical University
Acce. Prof.	Songmin	China Medical University
Acce. Prof.	Mixiaoyi	China Medical University
Mr.	Xiechengyao	China Medical University
Mr.	Liqingchang	China Medical University
Mr.	Jiajunyong	China Medical University
Miss.	Wuhui	China Medical University
Miss.	Wu'anhua	China Medical University
Miss.	Cuishuang	China Medical University
Miss.	Guodongli	China Medical University
Mr.	Wangxuguang	China Medical University
Mr.	Liguosheng	China Medical University
Mr.	Liugefei	China Medical University
Acce. Prof.	Huangdongyang	China Medical University
Prof.	Xinyan	China Medical University
Miss.	Gaohua	China Medical University
Prof.	Fangxiubin	China Medical University
Mr,	xuhuinian	China Medical University
Prof.	Fanshuduo	China Medical University
Acce. Prof.	Zhaoyujie	China Medical University
Prof.	Wangguizhen	China Medical University
Prof.	Wangsufen	China Medical University
Prof.	Liujunting	China Medical University
Prof.	Zhanlifen	China Medical University
Prof.	Shiyuxiu	China Medical University
Prof.	Lijinming	China Medical University
Mr.	Jiangtao	China Medical University
Miss.	Zhangying	China Medical University
Miss.	Daiwenying	China Medical University
Miss.	Lilina	China Medical University
Prof.	Liubaoyi	China Medical University
Mr.	Hanjingsong	The Public Security Bureau Clinic ,Liaoning
Prof.	Wangjialun	Shenyang Medical college
Mr。	Cuifeilun	205 Hospital in Jinzhou
Mr.	Liujun	The 1 st Hospital of Sujiatun Shenyang
Miss.	Liudong	The Liaoning Tumor Hospital
Mr.	Kangjunling	The hospital of Woman and Infant, Shenyang

Prof.	Zhanghong	Siping Center Hospital
Mr.	Yangbo	General Hospital of Shenyang Military Distr.
Miss.	Hanlihua	Shengyang Medical University
Mr.	Libaoqiang	202 Hospital in Shenyang

List of Japanese participants

Academic part

Prof.	Nagura hiroshi	(Tohoku Univ.)
Prof.	Hata Junichi	(Keio Univ.)
Prof.	Takashi Saku	(Tsaku Univ.)
Prof.	Abe Masafumi	(Fukushima Univ.)
Prof.	Yoshiyuki Osamura	(Tokal Univ.)
Prof.	Aooi Akishi	(Yamanashi Med. Univ.)
Prof.	Shamoto Mikihiro	(Fujita Health Univ.)
Prof.	Tsutsumi Yutaka	(Fujita Health Univ.)
Prof.	Inai Kouki	(Hilocima Univ.)
Prof.	Kikuchi Masahiro	(Fukuoka Univ.)
Prof.	Motohiro Takeya	(Kumamoto Univ.)
Prof.	Watanabe Masatoshi	(Mie Univ.)
Prof.	Hasui Kazuhisa	(Kagoshima Univ.)
Miss.	Suliying	(Kagoshima Univ.)
Mr.	Nakayama Junkon	(Jikei Univ.)
Mr.	Chengjun	(Niigata Univ.)
Prof.	Sato Eiichi	(Kagoshima Univ.)

Social part

Prof.	Takahashi Kiyoshi	(Kumamoto Univ.)
Prof.	Hayata Takashi	(Kagoshima Woman Univ.)
Pres.	Hidaka Umashi	(Kagoshima Television Station)
Pres.	Nozoe Yoshitaka	(Nozoe Dental Clinic)
Pres.	Hirayama Kosei	(Hirayama Dental Clinic)

Associating persons

Mrs.	Inai Junko	Mrs.	Watanabe Noriko
Mrs.	Aooi Shizue	Mrs.	Sato Yuriko
Mrs.	Takahashi Kazuko	Mrs.	Hayata Kumiko
Mrs.	Hidaka Hisako	Mrs.	Nozoe Keiko
Mrs.	Hirayama Hideko	Mrs.	Shamato Mihoko

Scientific Program

2001.8.18

Am 9:00 **Opening**
Greeting — Japanese side President : **Nagura Hiroshi**
Introduce the participants — Chinese side President : **Jia Xin Shan**
Photography of the symposium (All the participants attending the symposium)

Am 9:30 SPECIAL LECTURE 1 Chairman: **Nagura Hiroshi, Li Gandi**
Junichi Hata, Hajime Okita, Mariko Fukuma:
Department of Pathology, Keio University School of Medicine, Shinjuku, Tokyo, Japan
National Children's Medical Research Center, Setagaya, Tokyo, Japan.
Pathological and molecular characteristics of Ewing family of tumors.

Am 10:00 SPECIAL LECTURE 2 Chairman: **Hata Junichi, Jia Xinshan**
Zhaoyujie , Hequn, Houweijian, Maruhai:
Department of Cell Biology, China Medical University, Shenyang, China.
Gene-chip technologies

Am 10:30 **Coffee break**

Am 10:40 LECTURE 1 Chairman: **Masahiro Kikuchi, Huang Dongyang**
Akishi Ooi, Takuo Takehana, Kazuyoshi Kunitomo:
Department of Pathology, Yamanashi University, Japan.
Status of c-erbB-2 in gastric adenocarcinoma: A compararive study of immunohistochemistry, fluorescence in situ hybridization and enzyme-linked immuno-sorbent assay .

Am 10:55 LECTURE 2
Zhangwenyan[1], Ligandi[1], Liuweiping[1], Ouyangqin[2], Renxingchang[1], Lifengyuan[1], Zhangshangfu[1]:
1)Department of Pathology, Sichuan University, Huaxi Hospital, Chengdu, P.R.C.
2)Department of Gastroenterology, Sichuan University, Huaxi Hospital, Chengdu, P.R.C.
Intestinal T-cell lymphoma: a study of its neoplastic cell lineage and relationship to Epstein-Barr virus

Am 11:10 LECTURE 3 Chairman:**Masatoshi Watanabe, Yang Xianghong**
Kouki inai, Hajime Ishida, Mayumi Kaneko, Yukio Takeshima:
Second Department of Pathology, Hiroshima University School of Medicine.
The effects of hyaluronic acid and CD44 to invasive character of malignant mesothelioma

Am 11:25 LECTURE 4
Wuqiang , Zhangguihong, Raohuirong, Zhengguohao:
Department of Pathology, Anhui Medical University, Hefei, China.
The study of cell cycle regulatory relevant genes and the mechanisms of carcinogenesis in breast cancer

Am 11:40 LECTURE 5 Chairman: **Sato Eiichi, Xu Huimian**
Masahiro Kikuchi, Ohshima K, Suzuki K:
First Department of Pathology, School of Medicine Fukuoka University, Fukuoka, Japan.
Chronic active EBV infection: clinicopathological and molecular studies.

Am 11:55 LETURE 6
Mixiaoyi [1], Wangyan[1], Cuishuang[1], Jiangyi[1], Songjiye[1], Shirai Toshikazu[2]:
1)Department of Pathology, China Medical University, Shenyang.
2)Department of Pathology, Junteodo University School of Medical, Tokyo, Japan.
Gene mapping of IgG hypergammaglobulinemia and sequence analysis in SLE model-New Zealand mice

Am 12:10 **Rest**

pm 1:30 SPECIAL LECTURE 3 Chairman: **Inai Kouki, Xin Yan**

Hiroshi Nagura[1], Haruo Ohtani[1], Seiji Arihiro[2], Takayuki Matsumoto[3]:
1)Department of Pathology, Tohoku University Graduate School of Medicine, Sendai, Japan.
2)Department of Internal Medicine Jikei University School of Medicine, Tokyo, Japan.
3)Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan.

Immuno-inflammatory responses and tissue injury in inflammatory bowel disease: Roles of the mucosal immune system.

Pm 2:00 LECTURE 8

Wangguizhen[1], Chengying[2], Zhouzhengren[1], Antti vaheri[3], Hilkka Lankinen[3]:
1)Department of Microbiology, China Medical University, Shenyang
2)Department of Pathology, the university of Sydney, NSW2006. Australia
3)Department of Virulogy, Helsinki University FIN-00014, Finland

Screening of genes which interaction with nucleocapside protein of Hantavirus in Vero E6 cells with Yeast two-hybrid system

Pm 2:15 **Coffee break**

Pm 2:30 LECTURE 9 Chairman: **Tsutsumi Yutaka, Zhao Yujie**

Motohiro Takeya, Junko Hayashida, Katsunori Jinnouchi, Ryu-ichiro Tomokiyo,Kiyoshi Takahashi:
Second Department of Pathology, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto, 860-0811 Japan

Macrophage scavenger receptor（CD204），a new differentiation marker for macrophages.

Pm 2:45 LECTURE 10

Caiqi[1], Sunmenghong[1],Luhongfen[1],Moshanjing[1],Caisanjun[2],Zhuxiongzeng[1],Shidaren[1]:
1)Department of Pathology, Shanghai Cancer Hospital, Medical Center of Fudan University.
2)Department of Abdominal Surgery, Shanghai Cancer Hospital, Medical Center of Fudan University

Clinicopathological and molecular genetic analysis of 4 Chinese typical hereditary nonpolyposis colorectal cancer families.

Pm 3:00 LECTURE 11 Chairman: **Akishi Aooi, Mi Xiaoyi**

Yutaka Tsutsumi:
Department of Pathology, Fujita Health University, School of Medicine, Toyoake, Japan.

Diagnosiss of emerging and re-emerging infectious diseaseses

Pm 3:15 LECTURE 12

Masatoshi Watanabe, Ryuichi Yatani, Taizo Shiraishi:
Second Department of Pathology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie514-8507, Japan

Analysis of genetic heterogenity in human prostate cancer

Pm 3:30 **Poster and Disscusion**

Poster 1

Mikihiro Shamoto[1], Keiji Sugiura[1], Mariko Sugiura[2], Ritsuko Hayakawa[2], Rika Hashimoto[2], Yoshimi Kato[2] :
1) Division of Pathological Cytology, Institute for Comprehension Medical Science, Fujita Health University, Kutsukake-cho, Toyoake, Aichi470-1192, Japan
2) Department of Environmental Dermatology, Nagoya University School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.

An immunohistological study of a lesion similar to human atopic dermatitis in NC mice.

Poster 2

Kazuhisa Hasui[1,2], Kiyohiro Sakae[3], Shin-ichi Akiyama[2], Takashi Hayata[5], Shuji Izumo[6], Suguru Yonezawa[1], Fusayoshi Murata[2], Eiichi Sato[1]:
1)Second Department of Pathology, Kagoshima University.
2)Second Department of Anatomy, Kagoshima University.
3)Department of Basic Physical Therapy, School of Health Sciences.
4)Department of Chemotherapy, Cancer Institute.
5)Kagoshima Women's Junior College, Kagoshima, Japan

Synovial cells express thymidine phosphorylase in inflammatory and neoplastic synovial tissue.

Poster 3

Junkon Nakayama[1], Masatoshi Watanabe[2], Hiroyuki Takahashi[1], Masahiro Ikegami[1], Takashi Nikaido[1], Hiroshi Hano[1]:

1)Department of Pathology, Jikei University School of Medicine, Tokyo 105-8461, Japan.

2)Second Department of Pathology, School of Sciences, Mie University, Mie 514-8507, Japan

Enhanced expression of E2F1 in hepatocellular carcinoma.

Poster 4

Suliying[1 2], Kazuhisa Hasui[1], Shinichiro Tsyama[1], Fusayoshi Murata[1]:

1)Second Department of Anatomy, Faculty of Medicine, Kagoshima University

2)Department of Histology and Embryology, Faculty of Medicine, Kagoshima University

The selection of oligonucleotide probe for in situ hybrization from already known genes by means of DNA database on the web

Poster 5

Chengjun[1], Satoshi Maruyama[1], Takefumi Hayashi[2], Wulanyan[3], Zhouzhiyu[3], Takashi Saku[1]:

Divisions of [1]Oral Pathology and [2]Radiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan.

[3]Department of Oral Pathology, Faculty of Stomatology, Huaxi University of Medical Sciences, Chengdu, China.

Biological background for differential diagnosis of salivary pleomorphic adenoma from myoepithelioma by CT images and characteristic histopathology of paucivascular stroma

Poster 6

Jiaxinshan[1], Heanguang[2], Zhangdaorong[1], Wangenhua[1], Songjiye[1]:

1) Department of Pathology, China Medical University, Shenyang, China.

2)The first department of the first affiliated hospital of China Medical University,Shenyang.

Study of pathogenetic condition and histopathology of 1224 cases of lung cancer

Poster 7

Heanguang[1], Jiaxinshan[2], Lifang[2]:

1)The first department of the first affiliated hospital of China Medical University,Shenyang.

2)Department of Pathology, China Medical University, Shenyang, China.

Pathological analysis to 21 cases of early lung cancer.

Poster 8

Yangxianghong, Sundonghui, Liuyumei:

Department of Experimental Pathology, China Medical University, Shenyang, China.

Endothelial cell injuried by lipid peroxidation induce the proliferation and apoptosis of vascular smooth muscle cell

Poster 9

Kazuhisa Hasui[1], Jiaxishan[2], Takashi Hayata[3], Eiichi Sato[4]:

1)Second Department of Anatomy, Kagoshima University.

2)Department of Pathology, China Medical University.

3)Kagoshima Women's Junior College.

4)Second Department of Pathology, Kagoshima University.

Oncogenic factors in gastric malignant lymphoma.

Poster 10

Libaoqiang[1], Wangguizhen[2], Dongzhanshuang[2]:

1)Chinese People's Liberation Army, 202 Hospital, Shenyang, China.

2)Department of Pathologenic Biology, China Medical University, Shenyang, China.

Study on cloning of P1 attachment protein gene of mycoplasma pneumoniae.

Poster 11

Zhaoyujie [1], Hequn[1], Houweijian[1], Maruhai[1], Jiaxinshan[2]:

1)Department of Cell Biology, China Medical University, Shenyang, China.

2)Department of Pathology, China Medical University, Shenyang, China.

Investigation of the melting curve of immobilized oligonucleotide.

Poster 12
Gaohua, Daiwenying, Yuanyuan:
Cancer Institute, China Medical University, China
Research of the proliferation characteristics in H.pylori gastric diseases.

Poster 13
Xinyan [1], Y.Chen[1], YP.Wang[1], SM.Zhang[1], DY.Wu[1], M.Leader[2],E.Kay[2]:
1)The Fourth Laboratory, Cancer Institute, China Medical University,Shenyang,China.
2)Department of Pathology, Royal College of Surgeons in Ireland, Dublin.
Relationship between CD44V6 expression and metastasis and prognosis in gastric cancers.

Poster 14
Sunhanxiao[1], Fenglixia[2], Liyicheng[1], Hewenfang[1]:
1)Medical College of Jinan University, Guangzhou, China.
2)Department of Pathology, University of Montreal, Canada.
vMIP- α encoded by human herpesvirus 8 blocking HIV co-receptor CCR5 of peripheral blood monocytes.

Poster 15
Zengzihua[1], Weijiwu[2], Zhengpeie[1]
1)Department of Pathology, Medical College, Jinan University, Guangzhou, China.
2)Department of Surgery, Zhuhai People's Hospital, Zhuhai, Guangdong, China.
The expression of apoptosis-related gene caspase-4 in HD and ALCL.

Poster 16
Penghui, Yangguanghua:
Department of Pathology, West China University of Medical Sciences.
Molecular cytogenetic changes in soft tissue leiomyosarcoma

Pm 5:30 **End**
Pm 7:00 **Welcome party**

2000. 8.19

Am 9:00 Oral 1 Chairman: **zhanghong, Nakayama Junkon**
Wuhui, Yangxianghong, Dongyulan:
Department of Experimental Pathology, China Medical University, Shenyang, China.
Influence of lipid peroxidation on NO in human vascular endothelial cell and the effect of vitamin E

Am 9:15 Oral 2
Cuishuang, Mixiaoyi, Songjiye:
Department of Pathology, China Medical University, Shenyang, China.
The relationship of cAMP content and CAMs expressions of EC with monocytes' adhesion to lipoperoxidated endothelials

Am 9:30 Oral 3
Hanyuchen[1], Jiaxinshan[1], Minyongfen[1], Zhanglihong[1], Peihuimin[1], Sukuru Yonezawa[2], Sato Eiichi[2]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Second Department of Pathology, Faculty of Medicine, Kagoshima University.
Expression and localization of thrombomodulin in fetal lung tissue and in lung carcinoma

Am 9:45 Oral 4
Jiajunyong, Jiaxinshan:
Department of Pathology, China Medical University, Shenyang, China.
The study of LEA(large external antigen) in non-small cell lung cancer

Am 10:00 **coffee break**

Am 10:10 Oral 5 Chairman: **liubaoyi, Shamoto Mikihiro**

Zhanghong, Zhangjun:
Siping Center Hospital, Jilin, China.

Regulatory effects of cAMP analogs on growth and differentiation of metastatic human lung cancer cells.

Am 10:25 Oral 6

Jiangtao[1], Wangwei[2], Mixiaoyi[1], Wangenhua[1], Songjiye[1]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Computer Center, China Medical University, Shenyang, China.

Expression of P^{27Kip1},CDK4, Rb in biopsy lung and the significances on the prognosis of carcinoma of lung.

Am 10:40 Oral 7

Liudong[1], Zhangdaorong[2], Liyi[1], Zhanglihong[2]:
1)Department of Pathology, Liaoning Province Tumor Hospital, Shenyang, China.
2)Department of Pathology, China Medical University, Shenyang, China.

The expression and significance of cell cycle regulators in non-small cell lung cancer.

Am 10:55 Oral 8

Xiechengyao, Liqingchang, Yangxiu, Qiuxueshan, Wangenhua:
Department of Pathology, China Medical University, Shenyang, China.

Expression of MMP-2,MMP-9,TIMP-1 protein and its relation with metastasis in NSCLC.

Am 11:10 **Rest**

Pm 1:30 Oral 9 Chairman: **lijianhua, Takeya Motohiro**

Liqingchang, Wangyan, Xiechengyao, Qiuxueshan, Wangenhua:
Department of Pathology, China Medical University, Shenyang, China.

A study on protein and mRNA of MMP-2, MMP-9,TIMP-1 in non-small cell lung cancer.

Pm 1:45 Oral 10

Zuoguangwu, Hanzhipeng, Zhangguirong:
Department of Pathology, The Center Hospital, Chaoyang, Liaoning, China.

Expression of estrogen and progestesterone receptors in Non-small-cell lung cancer and its clinical significance.

Pm 2:00 Oral 11

Liguosheng[1], Zhangdaorong[1], Liudong[2], Wangenhua[1], Qiuxueshan[1]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Liaoning Province Cancer Hospital, Shenyang, China.

The study on the expression and significance of cyclinB_1 and p^{34CDC2} in human non-small lung cancer.

Pm 2:15 Oral 12

Liugefei, Huangdongyang, Wangbo, Dujing, Zhuli:
The Key Laboratory of Cell Biology, China Medical University, Shenyang, China.

Cloning of human angiostatin and construction of its recombinant baculovirus.

Pm 2:30 **Rest**

Pm 2:40 Oral 13 Chairman: **songmin, Kikuchi Masahiro**

Guoyi[1], Jiaxinshan[1], Jiajunyong[1], Kazuhisa Hasui[2]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Second Department of Anatomy, Kagoshima University.

The study on the histologic type and CD44 expression in gastric lumphoma.

Pm 2:55 Oral 14

Jiaxinshan[1], Kazuhisa Hasui[2], Lifang[1], Eiichi Sato[3]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Second Department of Anatomy, Kagoshima University.
3)Second Department of Pathology, Kagoshima University.

Study on 80 cases of EBER-1 ISH of malignant lymphoma.

Pm 3:10 Oral 15

Sunhanxiao[1], Fenglixia[2], Liyicheng[1], Hewenfang[1]:
1)Medical College of Jinan University, Guangzhou, China.
2)Department of Pathology, University of Montreal, Canada.

vMIP-α encoded by human herpesvirus 8 blocking HIV co-receptor CCR5 of peripheral blood monocytes.

Pm 3:25 Oral 16

Zhanghong[1], Yangbo[2]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)General Hospital of Shenyang Military District, China.

Diagnosis significance of serum β -glucuronidase detection in patients with early gastric carcinoma.

Pm 3:40 **Coffee break**

Pm 3:50 **Poster and Disscusion**

Poster 1

Hanjingsong[1], Shiyuxiu[2]:
1)Clinical of Liaoning Provincial Public Security, Shenyang
2)Department of Histology and Embryology, China Medical University,Shenyang

Ultrustructural study of diffuse axonal injury

Poster 2

Shiyuxiu, Tianguang, Liudongjuan:
Department of Histology and Embryology, China Medical University, Shenyang ,P.R.China

Effect of PMA and membrane fluidity on the nematolysosomes in rat neuron of spinal cord

Poster 3

Jiangtao[1], Wangwei[2], Mixiaoyi[1], Wangenhua[1], Songjiye[1]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Computer Center, China Medical University, Shenyang, China.

Expression of P^{21Waf1},CyclinE in biopsy lung and the significances on the prognosis of carcinoma of lung.

Poster 4

Kangjunling[1], Liguosheng[2], Zhangdaorong[2]:
1)Shenyang Women and Infants Hospital, Shenyang, China.
2)Department of Pathology, China Medical University, Shenyang, China.

Expression and significance of vascular endothelial growth factor in human non-small-cell lung cancer.

Pm 4:30 **End**

2000. 8. 20

Daytime **Going to Jiuzhaigou**

Pm 7:00 SPECIAL LECTURE 4 Chairman: **Nagura Hiroshi, Jia Xinshan**

Yoshiyuki Osamura:
Department of Pathology, Tokai University School of Medicine, Boseidai Isehara-city Kanagawa 259-1193

Differentiation of pituitary cells and adenomas: approaches by molecular morphology.

Pm 7:30 **Committee of The Second International Symposium of Molecular Pathology Dosscusion**

Pm 8:30 **End**

2000. 8.21

Pm 1:30 Oral 1 Chairman: **Wangshufen, Tsutsumi Yutaka**

Yangbo[1], Zhanghong[2]:
1)General Hospital of Shenyang Military District, China.
2)Department of Pathology, China Medical University, Shenyang, China.

Expression and significance of β -glucuronidase in hepatocellular carcinoma.

Pm 1:45 Oral 2

Guodongli, Yuanyuan:
Cancer Institute, China Medical University, China

Gastric tumor-associated antigen MG7 expression in the gastric cancer, precancerous lesions and H.pylori-related gastric benign lesions.

Pm 2:00 Oral 3

Wangxuguang, Yuanyuan:
Cancer Institute, China Medical University, China

Expression of PI glutathion S-transferase in intestinal metaplasia of gastric mucosa and its relationship with H.pylori infection

Pm 2:15 Oral 4

Daiwenying, Gaohua, Yuanyuan:
Cancer Institute, China Medical University, China

Telomerase activity in normal gastric mucosa, H.pylori associated gastric diseases and gastric cance

Pm 2:30 **Coffee break**

Pm 2:40 Oral 5 Chairman: **zhanlifen, Hasui Kazuhisa**

Lilina[1], Yinliyuan[1], Panyaping[1], Jiaxinshan[2]:
1) College of Stomatology, China Medical University, Shenyang, China.
2)Department of Pathology, China Medical University, Shenyang, China.

Analysis of 72-kDa and 92-kDa gelatinases activity in chronic adult periodentitis.

Pm 2:55 Oral 6

Songmin, Mixiaoyi, Libailin, Gaoyingxian, Songjiye:
Department of Pathology, College of Basic Medical Sciences, China Medical University.

Study on expression of telomerase genes and apoptosis related genes in mammary atypical ductal hyperplasia

Pm 3:10 Oral 7

Zhangying, Songmin, Mixiaoyi, Wanghui, Songjiye:
Department of Pathology, College of Basic Medical Sciences, China Medical University.

Activation of extracellular signal-regulated kinase and phosophatidylinositol 3-kinase in human breast tumors.

Pm 3:25 oral 8

Hnlihua, Wangjialun:
Department of Pathology, Shenyang Medical College, Shenyang, China.

The research of transforming growth factors effect on the disease of prostate hypertrophy

Pm 3:40 **Coffee break**

Pm 3:50 **Poster and Disscusion**

Poster 1

Fangxiubin , Lidongpei, Liuxiaoxiang:
Department of Neurobiology, China Medical University, Shenyang

The effect of nerve growth factor in the regulation of neuropeptides in the pathogenesis of the asthmatic guinea pig

Poster 2
Wangshufen , Lanni, Xieqiwen:
Neuroendocrine Research Laboratory, Brain Research Institute, China Medical University
The relationship between c-fos and prolactin gene expression in the rat pituitary after restraint stress

Poster 3
Wuanhua , Lijinming, Yangguoru:
Department of Pharmacology, College of Basic Medical Sciences, China Medical University
The protect effect of K^+ channel openers on ischemic brain injury in rat

Poster 4
Kongxiangzhu[1], Zhangyunbiao[2],Zhuqiumei[3]:
1) The Hospital for Gynaecology and Paediatrics, Sujiiatun, Shenyang, China.
2) The Center Hospital, Sujiatun, Shenyang, China.
3) The First Hospital, Sujiatun, Shenyang. China.
Estrogen and progesterone receptors p53 in endometrial carcinoma.
Pm 4:30 **End**

2000. 8. 22
Pm 1:30 Oral 1 Chairman: **shiyuxiu, Watanabe Masatoshi**
Cuifeilun [1], Wangwenyi[1], Fangxiubin[2], Luyao[1]:
1)Department of Urology, 205th Hospital of PLA, Jinzhou, Liaoning.
2)Department of Anatomy, China Medical University, Shenyang, Liaoning.
Telomerase activity in bladder cancer with reference to their features

Pm 1:45 Oral 2
Kongxiangzhu [1], Liujun[1], Zhuqiumei[2]:
1)Department of Pathology, The Hospital for Gynaecology and Paediatrics, Sujiatun,Shenyang.
2)The First Hospital, Sujiatun, Shenyang, China.
A case of rich-cell neurilemoma easily made mistaken for malignant tumor

Pm 2:00 Oral 3
Zhangxiguang [1], Hanzhipeng[2], Liuliquan[1], Luzhenjun[3]:
1)Department of Pathology, The first Hospital ,Beipiao, Liaoning.
2)Department of Pathology, The Center Hospital, Chaoyang, Liaoning.
3)Department of Pathology, The Chinese Traditional Medical Hospital, Beipiao, Liaoning.
Clinicopathological and immunohistochemical study of 30 cases of Rhabdomyosarcoma

Pm 2:15 Oral 4
Fanshuduo[1], L.wang[2], P.R.Vulliet[2], S.Schaefer[2]:
1)Department of Physiology, China Medical University,Shenyang
2)Div. Cardiovasc. Med. and Sch. of Vet. Med., Univ. of Calif., Davis, CA
Fasting limits norepinephrine release on reperfusion following ischemia: A mechanism of reduced injury?

Pm 2:30 **Coffee break**

Pm 2:40 Oral 5 Chairman: **Sun Hanxiao, Inai Kouki**
Liubaoyi, Liujiahui, Liuyinghui:
Department of Pathology, China Medical University, Shenyang, China.
A study on morphological changes of the aortic endothelial cells in artherogenesis. (Observed by en face, Scanning and Transmission Electron Microscopy)

Pm 2:55 Oral 6
Lijianhua[1], Fuzhimin[1], Liushijun[2]:
1)Pathological Diagnosis Center, First Hospital of China Medical University, Shenyang.
2)Research Institute of Chinese Medicine, Liaoning, Shenyang
Differentiation between malignant mesothelioma and metastatic carcinoma cells in human serous cavity effusion by monoclonal antibodies

Pm 3:10 Oral 7

Zhanlifen, Fanshuduo, Sunli, Zhangyuzhi:
Department of Pharmacology, College of Basic Medical Sciences, China Medical University

Experiment study for the effect of Qing Shen Tiao Zhi tablet on MDA and SOD in hyperlipoidemia animal

Pm 3:25 Oral 8

Shiyuxiu, Huangying:
Department of Histology and Embryology, China Medical University, Shenyang ,P.R.China

The observation of double-labeling lysosome and neurofilament in motor neuron of anterior spinal cord in rat

Pm 3:40 **Coffee break**

Pm 3:50 **Poster and Disscusion**

Poster 1

Liujun[1], Kongxiangzhu[2], Zhangyunbiao[3]:
1) The First Hospital, Sujiatun, Shenyang, China.
2) The Hospital for Gynaecology and Paediatrics, Sujiiatun, Shenyang, China.
3) The Center Hospital, Sujiatun, Shenyang, China.

Pathological research on breast cancer with vimentin expression

Poster 2

Kongxiangzhu[1], Liujun[2], Zhangyunbiao[3]:
1) The Hospital for Gynaecology and Paediatrics, Sujiiatun, Shenyang, China.
2) The Hospital for Gynaecology and Paediatrics, Sujiiatun, Shenyang, China.
3) The Center Hospital, Sujiatun, Shenyang, China.

The expression and clinical significance of vascular endothelial growth factor in breast cancer.

Poster 3

Jiangtao[1], Wangwei[2], Mixiaoyi[1], Wangenhua[1], Songjiye[1]:
1)Department of Pathology, China Medical University, Shenyang, China.
2)Computer Center, China Medical University, Shenyang, China.

Expression of P^{21Wafl},CyclinE in biopsy lung and the significances on the prognosis of carcinoma of lung.

Pm 4:20 **President greeting**
Pm 5:00 **Closing**

Abstracts

4 Special lectures
12 Lectures
32 Oral presentations
27 Posters

CATALOG

PATHOLOGICAL AND MOLECULAR CHARACTERISTICS OF EWING FAMILY OF TUMORS

Jun-ichi Hata, Hajime Okita and Mariko Fukuma
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Shinjuku, Tokyo
National Childrens Medical Research Center, Setagaya, Tokyo, Japan

Ewing's sarcoma is one of most malignant tumors of children and young adult. Ewing's sarcoma expresses specific chimeric genes, e.g. EWS-FLI-1, EWS-ERG and EWS-ETV1 generated through characteristic chromosomal translocations, t(11; 22), t(21; 22) and t(7; 22) Recently, we isolated a novel fusion gene, EWS-E1AF, and established a cell line from a tumor of Ewing's sarcoma with t(17; 22)EWS-E1AF gene is conclusively found to be another fusion gene available for the diagnosis of Ewing's sarcoma and participate in the oncogenesis of Ewing's sarcoma. Furthermore, we have done the genetic analysis of Ewing family of tumors in correlation with expression of neuronal phenotypes at mRNA level, immunohistochemical analysis, and ultrastructural analysis. From these analysis, we are going to discuss following points:

1.diagnosis of Ewing family tumors from both genetic analysis and histopathological analysis,

2. roles of these chimeric genes for oncogenesis. Present findings provide us significant information concerning the diagnosis and the oncogenesis of Ewing family of tumors.

gene-chip technologies

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The draft human genome sequence published on 9 Feb 2001 is the culmination of 15 years of work, involving 20 sequencing centres in six countries. The genome project constructs a higher-resolution map that is used to sequence and assemble the human genome.The first complete human chromosome sequence — number 22 — is published in December 1999. Chromosome 21 follows in May 2000. Not only include the yeast *Saccharomyces cerevisiae* (May 1997), the nematode *Caenorhabditis elegans* (December 1998), the fruitfly *Drosophila melanogaster* (March 2000, right), but also the plant *Arabidopsis thaliana* (December 2000, left)were sequenced.The complete genome sequences of model organisms are proving immensely valuable to biologists working on these species, and will also help interpret the human genome sequence. Till now, we have more and more information of different species genome sequencing . How did using the mass sequencing information for biology and for medicine? this problem is still puzzle ourselves.that is to say that methods are needed to detect specimens which are interested by us. Human genome project will move on to the post genome sequencing era, including single nucleotide polymorphisms (SNPs) analysis, functional genomics and proteome analysis. In the post Human Genome sequencing era, further development of analytical technology for nucleic acid is highly required for high-throughput screening of disease-causing gene from 3.2 Gbp human genome and high speed analysis of analysis of genetic polymorphism. Genechip is a modern technique, can quickly, correctly and largely sequencing some specimens' DNA/RNA sequences.[1]

Research fundamentals

Is the adoption of genechip technologies the next medical revolution? IVD Technology explores the potential of this emerging technology for diagnostics. As is often the case with breakthrough technologies, a great deal of hyperbole has surrounded the development of micro- and nanoarray diagnostic technologies, popularly known as DNA or genetic chips. Sometime early in the next decade DNA chips will usher in a new era in medical care.

Researchers in the field expect that DNA chips will enable clinicians--and in some cases even patients themselves--so quickly and inexpensively detect the presence of a whole array of genetically based diseases and conditions, including AIDS, Alzheimer's disease, cystic fibrosis, and some forms of cancer. Moreover, the technology could make it possible to conduct widespread disease screening cost-effectively, and to monitor the effectiveness of patient therapies more effectively.

Theoretical Principles

A variety of recent technological breakthroughs have made possible the development of genechips. Fundamentally, however, genetic chips are the result of achievements in

two fields: molecular biology and microfabrication technology.

Molecular Biology. Especially as it has been catalyzed by the work of the Human Genome Project (HGP), research in molecular biology has laid the groundwork for the development of clinical laboratory tests and therapies involving genetic probes. Fundamental advances include the use of polymerase chain reaction (PCR) or other amplification techniques to make copies of a nucleic acid sample, which can then be tested using a genetic probe. Hybridization can be performed either in solution or on a solid support. genechip manufacturers are also exploring variations of both solid-phase and solution-based hybridization for use in their. genechips are designed to identify hybridization products in the same fashion as with traditional sequencers. Once hybridization has been completed, phosphorescent chemicals that bind to the hybridized sequences are scanned with a light source, making it easy to detect their presence with automated colorimetric or fluorimetric equipment.

Microfabrication Technologies. The second technological trend that is making genechip products possible encompasses the steady improvements in nano- and microscale fabrication techniques. DNA microassays are fabricated onto glass or plastic wafers or are placed in tiny glass tubes and reservoirs. To produce its genechip, Affymetrix (Santa Clara, CA) bonds hundreds of genetic sequences onto the surface of a microchip using photolithographic processes such as photosensitive masks, chemical doping layers, and other techniques used in computer chip fabrication.

How Genetic Sequencing Works

Sequencing, the process of finding the molecular structure of a DNA fragment, employs the Watson-Crick rules of hybridization, whereby each strand of DNA can bond only to a chemical mirror image via two sets of four bases: adenine (A), cytosine (C), guanine (G), and thymine (gT).

Step 1: Determine chemical structure of fragment.

Representing all or part of a DNA strand of interest, short fragments of DNA are identified.

Step 2: Separate strands.

DNA is denatured (separated) and placed in solution or on a solid substrate, forming a reference segment for the DNA fragment of interest.

Step 3: Introduce sample.

Unknown DNA sample is introduced to the reference segment. If present, the complement of the reference segment will hybridize (bond) to it.

Step 4: Identify result.

Chemicals that bond to successful hybridization help researchers identify results. Such chemicals are typically photosensitive (fluorescent or chemiluminescent), which helps researchers confirm results.

Probe arrays are manufactured by Affymetrix's proprietary, light-directed chemical synthesis process, which combines solid-phase chemical synthesis with photolithographic fabrication techniques employed in the semiconductor industry. Using a series of
photolithographic masks to define chip exposure sites, followed by specific chemical synthesis steps, the process constructs high-density arrays of oligonucleotides, with

each probe occupying a predefined position in the array. Multiple probe arrays are synthesized simultaneously on a large glass wafer. The wafers are then diced, and individual probe arrays are packaged in injection-molded plastic cartridges, which protect them from the environment and serve as chambers for hybridization.
Another manufacturing approach involves the deposition of gene probes onto the chip substrate using a tiny droplet sprayer that resembles an ink-jet printer. This approach is being used by Combion (Redwood City, CA), Rosetta (Seattle), ProtoGene Laboratories (Palo Alto, CA), and Affymetrix.
Some companies, such as Nanogen (San Diego), use robots to deposit the gene probes onto the substrate. Nanogen uses electrophoresis to speed up hybridization.Yet another approach is the use of gels in a solution-based process. Genechips will give researchers the ability to analyze thousands of genes at once, and may also make it possible to conduct very elaborate diagnostic procedures in such small settings as a physician's office or even with mobile equipment used at the point of care.

Genechip application

1.genetic analysis

The genechips will enable researchers to accomplish the genetic analysis, and single base-pair discrimination capabilities(SNPs and genetic mutation). The doctors would then prescribe therapies for a disease and later, repeating the above steps, monitor the effectiveness of treatment. Ultimately, fewer trips to doctors' offices, pharmacies, hospitals, and labs would be required, and prescriptions would be more accurate. Measured across a large population, the total cost of healthcare would be substantially reduced, as well as the time people spend away from work due to illness. Healthcare itself would improve, as a consequence of the convenience and availability of diagnosis and testing

2.gene expression monitoring

*Gene expression microarrays enable researchers to rapidly generate and explore huge quantities of gene expression data. It can help researchers:

*Identify and validate promising gene targets for drug development.

*Identify previously unknown genes.

*Understand the behavior and interactions of genes in both diseased and healthy cells.

*Assess the efficacy and toxicity of compounds before beginning clinical trials.[2]

However, dene expression technologies are a challenge to use. Access to and application of the technologies provides tools to interrogate physiological processes that result in overwhelming amounts of data, not necessarily information.Evaluation of the disease state requires a comprehansion of these massive amounts of data to provide the understanding, insights, and perspectives. The advantages of the genechips are the speed of investigation, economy of reagents,and the ability to observe gene action in unison.. [3-5]

References

[1].Ideker T, Thorsson V, Siegel AF, Hood LE.Testing for differentially-expressed genes by maximum-likelihood analysis of microarray data.J Comput Biol. 2000;7(6):805-17.

[2]. Stuart RO, Bush KT, Nigam SK. Changes in global gene expression patterns during

development and maturation of the rat kidney.Proc Natl Acad Sci U S A. 2001 May 8;98(10):5649-54.

[3]. Yasui W, Tahara E. Gene diagnosis-introduction of new technology: the tenth international symposium of the Hiroshoma cancer seminar, October 2000.Jpn J Cancer Res. 2001 Feb;92(2):239-42.

[4]. Gonzalez IL, Sylvester JE. Human rDNA: Evolutionary Patterns within the Genes and Tandem Arrays Derived from Multiple Chromosomes.Genomics. 2001 May 1;73(3):255-63.

[5]. Cadet JL, Jayanthi S, McCoy MT, Vawter M, Ladenheim B. Temporal profiling of methamphetamine-induced changes in gene expression in the mouse brain: Evidence from cDNA array. Synapse. 2001 Jul;41(1):40-8.

Immuno-inflammatory Responses and Tissue Injury in Inflammatory Bowel Disease: Roles of the Mucosal Immune System

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Introduction

The inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the intestinal tract with unknown aetiology. The mucous membrane covering the intestinal tract represents the most frequent portal of entry for infectious agents and food allergens, and a large and highly specialized immune system called the mucosal immune system surveys these antigens in the lumen and protects the mucosal surface from their entry. Recently an abnormal immuno-inflammatory response detected against enteric microflora and food antigens in a genetically susceptible host was proposed, and familial clustering of disease strongly suggests that IBD is a genetic disorder. Genome-wide researches for IBD-susceptibility genes have identified several foci related to alter mucosal immunologic homeostasis in the intestinal tract in pericentromeric region of chromosome 16.

Abrogation of this mucosal immunogic homeostasis may be involved in the pathogenesis and exaggerated chronic active inflammation of IBD.

This study therefore, aimed to determine abnormal immuno-inflammatory responses specific to UC and CD in the intestinal mucosa with IBD.

Chronic Mucosal Inflammation Induced by Defects of Mucosal Immune Regulation

Long-standing chronic active inflammation appears to be a hallmark of pathophysiology for the IBD colonic mucosa, and induce characteristic mucosal injury and tissue remodeling in UC and CD respectively. A consensus exists that characteristic gastrointestinal inflammation is driven by disorders of immuno-inflammatory regulation specific to UC and CD in the gastrointestinal mucosa. immune system and that impaired and/or excessive immuno-inflammatory responses appear and continue in these colonic mucosa.

Abrogation of mucosal B-cell responses and augmented B-cell proliferation: the apparent intestinal predominance of IgA plasma cells in the normal intestinal mucosa suggests that the relationship of IgA antibodies to local inflammation is an important factor in homeostasis of the intestinal mucous membrane where abundant microbes and diatary antigens are exposed. In IBD colonic mucosa, particularly UC, however, IgG plasma cells are much increased and reach to the equal number of IgA

plasma cells. IgG immune responses in the inflamed mucosa may facilitate epithelial injury by activation of the complement cascade and therefore is deterious to host. In addition, abnormal proliferation aggregations of B-cells without germinal center are identified in UC, particularly at the ulcer base, but not in CD. Their Ki67-labeling indeces are almost 42% at the ulcer base, and most of them are CD19+CD20- immpature B-cells. Therefore, these exaggerated abnormal B-cell responses in UC may result from the disruption of mucosal barriers by disorders of the mucosal immune system in a certam genetic background of the patient.

MAdCAM (mucosal addressin cell adhesion molecule)-1 on the endothelial cells in the intestinal lamina propria plays a role for the recruitment of lymphocytes derived from the inductive site for the mucosal immune system into the intestinal mucosa. Interestingly, MAdCAM-1 expression by endothelial cells in the ulcer base of UC is almost absent in spite of their proliferation. This suggests that lymphocytes participating in the mucosal inflammation in UC come from non-mucosal immune tissues by the disruption of the mucosal defense mechanism.

Augmented macrophage activation and T-cell responses: During active IBD, particularly CD, $CD68^+$ macrophages and $S100^+$ dendritic cells together with T-cells were much increased in the lamina propria correlated with its histogical and clinical gradings. These T-cells show high Ki-67 labeling index up to 4%. Our immunohistochemical studies revealed that actively inflamed intestinal tract in CD contained abundant B7-1(CD80)/B7-2(CD86) macrophages, which tended to form aggregates and granulomas. They were also $HLA\text{-}DR^+$, $ICAM\text{-}1(CD54)^+$ and $transferring\ receptor^+$, but less active acid phosphatase. T-cells positive for CD28, ligand for B71-/7-2, are closely attached to these macrophages. This suggests that exaggerated chronic immuno-inflammatory responses in CD is coupled with antigen presentation via a B7-1/7-2-CD28 pathway at the site of inflammation. In addition, $B7\text{-}1/B7\text{-}2^+$ macrophage aggregates are occasionally present in lymphatic vessels in the lamina propria, and may form granuloma in the intestinal tract distant from the primary lesion. Recently a frameshift mutation in NOD2, of which gene product confers susceptibility to CD by altering the recognition of microlial pathogen and/or by over activating NF- κ B in monocyte/macrophages, was reported is CD patients.

Conclusion

IBD is a chronic inflammatory disorder of the intestinal tract resulting from the effect of antigenic stimuli from the intestinal lumen in a genetically predisposed host. This disorder leads altering immunologic homeostasis maintained by the mucosal immune system and characteristic abnormal immuno-inflammatory responses and tissue injuries in UC and CD, respectively.

The Effects of Hyaluronic Acid and CD44 to Invasive Character of Malignant Mesothelioma

Kouki INAI, Hajime ISHIDA, Mayumi KANEKO and Yukio TAKESHIMA
Second Department of Pathology, Hiroshima University School of Medicine

1. Background

Malignant mesothelioma is one of the tumors showing the worst prognosis. Extensive invasiveness and wide metastasis are characteristics as its biological behaviors. However, those characters has not been explained at the molecular level, so far.

Histologically, malignant mesothelioma, especially epithelioid type, produces abundant hyaluronic acid. Some other carcinomas including breast cancer also produce hyaluronic acid, and the content is abundant at the invading site.

On the other hand, CD44 is a transmembrane protein and has an adhesive function at the sites of cell to cell and cell to extracellular matrix and it might play a role on cellular motility and transmission of growth signals.

One the basis of these facts, our aim of this study is to investigate the effects of hyaluronic acid and CD44 on motility of malignant mesothelioma cells, as an intial step of invasion.

2. Materials and Methods

Human malignant mesothelioma cell line, HMMME was used. The original tumor is epithelioid type of malignant mesothelioma.

Immunohistochemical staining using anti-CD44 antibody (F10-44-2) was performed. Intracytoplasmic actin filaments was stained by phalloidin. Co-localization of CD44 and actin filaments was observed by confocal microscope.

Wound assay (cell motility analysis) was done as follow: repopulation of denuded culture monolayer was measured at 12 hours interval, and the effect of the blocking antibody to CD44 (MEM85) was also examined. In addition, the same assay was conducted under the presence of 4-metlylumbelliferone (4-MU) in order to block synthesis of hyaluronic acid by the cells.

CD44 expression on malignant mesothelioma cells was analysed by flow cytometry, and the effect of hyaluronic acid was also examined.

3. Results

Immunohistochemical staining showed that CD44 expressed on the free surface membrane of the cells. By means of the confoal view, its was revealed that CD44 on the surface membrance connected to the intracytoplasmic actin filaments.

In the wound assay, without the blocking antibody to CD44, the cells treated by the hyaluronic acid showed significantly higher motility than the control. However, on the case using the blocking antibody to CD44, no significantly different motility was found.

By the flow cytometric analysis, the mean fluorescence intensity of CD44 expression on the cells with hyaluronic acid was about 5 times compared to the control.

4. Summary

In HMMME cells, CD44 was expressed preferentially at migrating front where co-localization of CD44 and actin filament was suggested. Hyaluronic acid activated cellular motility and also increased expression level of CD44.

DIFFERENTIATION OF PITUITARY CELLS AND ADENOMAS: APPROACHES BY MOLECULAR MORPHOLOGY

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The pituitary gland which is derived from the Rathke's pouch give rise to the anterior lobe and intermediate lobe in the rodents. In human, the intermediate lobe is not evident. In the anterior lobe, six cell types have been identified by immuno-histochemistry and in situ hybridization, i.e. growth hormone(GH) secreting cells, prolactin(PRL) secreting cells, thyroid stimulating hormone(TSH) secreting cells, adrenocorticotropic hormone(ACTH) secreing cells and follicle stimulating(FSH)/luteinizing hormone(LH) secreting cells. In contrast, the intermediate lobe in rodents is differentiated only to αMSH secretion. In human, the "invading anterior cells" are equivalent to the rodent intermediate lobe and shows predominant ACTH immunoreactivity. ACTH is known to be produced as a precursor molecule proopiomelanocortin(POMC) which is processed to not only ACTH but αMSH, endorphin and LPH by prohormone convertase(PC) 1 and 2. TSH, FSH and LH are glycoproteins in which α subunit is common and functional specificity lies in β subunit. Recently, molecular mechanisms for the differentiation of the pituitary cells hav been clarified by cloning various transcription factors. It has been also emphasized that the cellular differentiation follows cell lineages, i.e. GH-PRL-TSH lineage, POMC(ACTH) lineage, and FSH/LH lineage. The transcription factors for these different lineages have been clarifed,

The human pituitary adenomas have been classified according to the functions of their tumor cells, i.e. GH secreting adenomas,PRL secreting adenomas, TSH secreting adenbomas, ACTH secreting adenomas, FSH secreting adenomas and Non-functioning adenomas. The functional differentiation of the human pituitary adenomas apparently follow the cell lineages disclosed in the rodent pituitary glands. Interestingly, GH secreting adenomas and TSH secreting adenomas are multihormonal tumors,i.e. GH secreting adenomas also produce RPL and TSH β subunit as well α subunit., TSH secreting adenomas also produce GH and PRL. Ptx1 has been localized frequently in all types of adenomas and is speculated to function as a universal transcription factor as it

has been shown in the rodent pituitary glands(Tahara et al.) In these adenomas, Pit-1 has been shown to be frequently localized in the nuclei. As it has been reported in the rodent pituitary glands, GHRH-R has been detected as a synergistic factor in GH adenomas. In PRL secreting adenomas and TSH secreting adenbomas, ER and RXR have been reported from our results as synergistic factors respectively. NeuroD1 has been frequently and specifically detected in ACTH secreting adenomas. It has not clearly been identified whether "intermediate zone" derived adenomas exist. In non-functioning adenomas, they have been shown to be positive for glycoprotein subunits by IHC, i.e. α subunit and FSH/LH β subunits. In human, LH secreting adenomas are relatively rare and in contrast, FSH secreting adenomas are more commonly occur in male. Some suprresive mechanism for LH β subunhit may exist in the tumors. And it is considered that FSH secreting adenomas and many glycoprotein positive adenomas in non-functioning adenomas are probably in the same cell lineage in differfentiation. SF-1 and GnRH-R in conjunction with Ptx1 are the key factors in the differentiation toward FSH differentiation. Negative GATA-2 has been also proposed for this differentiation.

In human pituitary gland and adenomas, GH secreting cells also show immuno-positivity for α subunit and further occasionally FSH β subunit. This is considered to be unique phenomena in the human pituitary gland. And some non-functioning adenomas with glycoprotein subunhit positivity are positive for Pit-1. It has been proposed that in human a special cell lineage with both GH and gonadotropin production may exist and may undergo neoplastic transformation.
Very rarely, the pituitary adenomas show functional differentiation which overlaps two different cell lineages, i.e. GH and ACTH. Pathologic combination of transcription factors, Pit-1 and NeuroD1 have been proposed.

As a summary, recent molecular and histochemical techniques have disclosed that the anterior pituitary cells and human pituitary adenomas follow three distinct cell lineages, i.e.GH-PRL-TSH, POMC, and FSH/LH. Various cell lines with distinct cell lineages have been established. The differentiation for these cell lineages has been clarified to be dependent on the combination of transcription factors and synergistic co-factors. Further detailed molecular mechanisms for the synergistic actions for these factors remains to be further investigated.

Endothelial Cell Injuried by Lipid Peroxidation induce the Proliferation and Apoptosis of Vascular Smooth Muscle Cell

Xianghong Yang, Donghui Sun, Yumei Liu
Department of Experimental Pathology, China Medical University

Objective: That VSMC in the media replicate and migrate into the intima is the very important step in atherosclerosis (AS) occurrence. The apoptosis of VSMC is predominant to the replication of it in advanced lesion of AS. The increase of apoptosis of VSMC has some effect on the stability of the plaque. It is well accepted that the vessel endothelial cell dysfuction and injury contributes to the atherogenesis. We investigate weather or not dysfuction or injury of EC induced by lipid peroxidation can induce proliferation or apoptosis of VSMC.

Methods: HUVECs were isolated by 0.25% typsin, and HVSMCs were cultured from tissue segments. EC were exposed in different concentration of cumene hydriperoxide (0, 0.01, 0.05, 0.1, 0.2, 0.5, 1.0mmol/L) and endothelial cell conditional medium (EC-CM) were collected. EC-CM was added into the cultured VSMC to detect the DNA replication of VSMC by [H^3]-TdR incorporation and to observe apoptosis of VSMC through transmission electron microscope and gel electrophoresis.

Results: [H^3]-TdR incorporation rate increase gradutely with lower concentration of cumene hydriperoxide (0.01~0.1mmol/L). the promotion to [H^3]-TdR incorporation rate is enlarged with time dependent when VSMC were exposed in EC-CM (cumene hydriperoxide 0.2mmol/L). Cumene hydriperoxide (0~0.2mmol/L) can't induce the increase of LDH level in EC culture medium,so the dysfunction of EC have no significant change in morphology. Apoptosis of VSMC were seen when exposed in EC-CM of higher concentration of cumene hydriperoxide (0.1~1.0mmol/L). TEM: chomosomes condense and accumulate and some fragments of chomosomes in cytoplasma with membrane intact. Apoptotic bodies can be seen also. Gel eletrophoresis show DNA ladder. The level of LDH in EC culture medium sharply increase, and show that EC have morphological change.

Disscution: Ross'Response-to injury hypothesis is the proposal that the different risk factors somehow lead to endothlial cell' dysfunction, which can elicit series of AS. Recently investigation found that proliferation of VSMC is important in early phase, but in advanced lesion, apoptosis of VSMC are prodominant and has some effect on the stability of the advanced lesion. Our study show that EC can promote proliferation of VSMC when EC induced by lipid peroxidation have no significant morphological change; however EC also can induce apoptosis of VSMC when EC were injuried strongly and have morphological change. In conclusion, EC dysfunction or injury can control the VSMC proliferation and apoptosis in different stages of AS to promote the occurrence and development of it.

Influence of lipid peroxidation on NO in human vascular endothelial cell and the effect of vitamin E

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Objective: The current study was designed to explore the influence and mechanism of lipid peroxidation on NO in endothelial cell (EC) and the effect of vitamin E. **Methods:** The human umbilical vein endothelial cells (HUVECs) were cultured in our experiments. Cumene hydroperoxide was added to the culture to excite the lipid peroxidation. Then the cultured HUVECs were divided into 8 groups according to different concentration of cumene hydroperoxide. We determined the NO content in the EC media by Enzyme Method, and we examined the expression of eNOS, iNOS , activated NF-κB in EC by immunohistochemical method. **Results:** After the HUVECs were incubated with comene hydroperoxide for 3h, the content of NO decreased as the concentration of cumene hydroperoxide increased ($p<0.01$). After that, the HUVECs were further incubated in cumene hydroperoxide-free media for 10h, the content of NO had increased proportionate to the concentration of cumene hydroperoxide raised ($p<0.01$). The immunohistochemical results suggest that as the concentration of cumene hydroperoxide increased, the ratio of NOS_3 positive cells decreased ($p<0.01$), but the number of NOS_2 positive cells increased.($p<0.01$), stains of NF-κBp65 shift from the cytosol to the nucleus , and the number of positive cells increased ($p<0.01$). When vitamin E was introduced into the media, positive staining rate of NOS_3increased($p<0.01$), but the positive staining rate of NOS_2 decreased($p<0.01$), and also the positive rate of NF-κBp65 decreased($p<0.01$). **Discussion:** Under the physiological conditions, the NOS in EC is mostly eNOS which acts rapidly and shortly after stimulation. And iNOS expressing in induced condition in EC acts slowly and need long hours. So we speculated that the decrease of NO content in EC stimulated with cumene hydroperoxide for a short time may correlate with its inhibiting effect on eNOS, and that the increase of NO content after a long period of cumene hydroperoxide stimulation may correlate with iNOS production. From our experiment, we speculate that lipid perioxidation objects can activated NF-κB, and it may act as an initial factor and further promote atherosclerosis(AS) by decreasing the eNOS expression and increasing the iNOS expression. In summery, lipid peroxidation may promote the development of AS by regulating the produce of NO in different phase in EC. vitamin E can prevent the change of NO content by lipid peroxidation to some extent.

The Relationship of cAMP Content and CAMs expressions of EC with Monocytes' Adhesion to Lipoperoxidated Endothelials

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The injury of endothelial cells (EC) and adhesion of monocytes (MC) to EC are considered as initial factors in the development of atherosclerotic lesions. Varies agents can elevate the amount of MC adhering to EC, but the mechanism of signal transduction and the change of adhesion molecules are unclear. cAMP as a second messenger perhaps plays an important role. In order to investigate the association between cAMP content of EC and the adhesion of MC to EC, cAMP and MC adhesion were measured after the addition of diamide. In another series of experiments, cAMP inhibiting agent Galanin was added and cAMP, MC adhesion were measured. In these two series of experiments adhesion molecule ICAM-1, VCAM-1 of EC was detected by immunohistochemstry and the adhesion rate of MC to EC was measured under LM.

The results indicated that diamide could lead to the injury to EC as a result of lipid peroxidation. cAMP content of EC increased after diamide was added, but dropped after Galanin was added. Both MC adhesion rate to EC and expression of ICAM-1 VCAM-1 of EC were paralleled to the changes of cAMP content. It could be concluded that cAMP as a second messenger might play an important role in the mechanism of increasing adhesion of MC to EC by elevating the expressions of ICAM-1 and VCAM-1.

Expression and localization of thrombomodulin in fetal lung tissue and in lung carcinoma

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Abstract

Aims: To investigate the expression and the significance of thrombomodulin(TM) in fetal lung tissue and in lung cancer.

Methods and results: Using immunohistochemical staining to detect thrombomodulin protein in 18 weeks fetal lung tissue and 56 cases lung cancer and cultured human lung adenocarcinoma cell line AGZY-83 a . This study was carried in accordance with the Regional Ethics Committee on development and reproduction

In 18 weeks human fetal lung, TM expressed in the endothelial cells which surrounding the primary alveoli, but absent in broncheal epithelium cell. Except expressed in endothelial cells, TM also existed in all 35 cases of lung squamous cell carcinoma along the cell membranes and the intercellular bridges; but it was not detected in 14 cases of adenocarcinoma and 3 cases of small cell carcinoma and normal bronchial epithelium. TM also expressed in human lung adenocarcinoma cell line AGZY-83 a .

Discussion: Result showed that in 18 weeks fetal lung, TM existed in the vascular endothelial cell surface and cytoplasm which surrounding the primitive alveoli mass, but in the monolayer ciliate-columnar cells of terminal bronchiole and cuboid cells of respiratory bronchioles and the primitive alveoli and the cartilage cells, TM was absent .

TM did not express in human fetal lung tissue except in vascular tissues. Perhaps, TM is transient, or expressed in the glandular stage (7-16 weeks) when the lung tissue was tubular in form and the bronchial division were differentiated and their air conducting system began to establish.

When we examined 53 cases human lung cancer for TM expression using immunohistochemistry, we found that 100% (35/35) SQCCs , 11.1% (2/18) ACs and 0% (3/3) small cell lung cancer showed immunoactivity. This differential expression of TM between AC and SQCC is in agreement with previous report, this suggest that anti-TM immunostaining is a useful marker for squamous cell carcinoma in the differential diagnosis of pulmonary carcinoma.

Conclusions: Anti-TM immunostaining is a useful marker for squamous cell carcinoma in the differential diagnosis of pulmonary carcinoma. TM do not express in fetal lung tissue after the primitive alveoli formation.

Study of the pathogenetic condition and histopathology of 1224 cases of lung cancer

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[Abstract] Objective: To compare the changes of pathogenetic condition and histopathology of lung cancer between 1980's and 1990's of Shenyang district. Discussing the changes of new WHO classification and diagnostic key points of rare types of lung cancer. **Methods:** HE staining of 572 cases in lung cancer in 1981-1990 and 652 cases of 1991-2000. Parts of these samples were stained with immunohistochemistry and *in situ* hybridization.

Results and Conclusions: Comparing 1980's and 1990's, the results show:1The incidence in women has been increaseing .2The highest incidence age is between 61 and 70, it is one decade later than before.3 Adenocarcinoma, especially papillary carcinoma and Bronchioloalveolar carcinoma increased remarkably, which may be a result of contacting more fibrous carcinogens. In addition, we also discuss the diagnostic points of well-differentiated fetal adenocarcinoma, large cell neuroendocrine carcinoma, lymphoepithilioma-lide carcinoma, pleomorphic carcinoma, carcinosarcoma and pulmonary blastoma.

PATHOLOGICAL ANALYSIS TO 21 CASES OF EARLY LUNG CANCER

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[Abstract] We researched 21 cases of early lung cancer by collecting sputum during 1985~1990, which was confirmed by pathological diagnosis. The complete gross and material for microscope is still conserved.

In the 21 cases, the ratio of male to female is 17:4. The age ranges from 46~79 with average of 58.8. In clinical manifestations: 6 persons have no symptom and 13 persons coughed and expectorated with blood contained in 5 cases. By X-ray, we found mottled in 3 cases, atelectasis in 1 case, string shadow in 1 case and nothing in 2 cases. Tumor cells of 16 cases (76.1%) were detected by PAT assay, and another 3 cases by PT washing. Using fine-needle aspiration biopsy (FNAB), we found 4 cases of cancer. At last, by pathological examination, we confirmed 8 cases of adenocarcinoma, 1 case of scar carcinoma, 1 case of undifferentiated small cell carcinoma, 1 case of in situ cancer and 10 cases of squamous carcinoma including 1 case of that companied by alveolar epithelial carcinoma. The size of tumor range from 0.1x0.3~2.2x2.0.

Through all of above, we can see: 1. FNAB and PAT take important effects in scanning early lung cancer. 16 (76.1%) of 21 cases were found by using them. The detective incident is rather high. 2. Early lung cancer occurs most frequently above 45 and male to female preponderance of about 4:1. And it always companied by cough and expectoration. 3. 12 (92.3%) of 13 cases taking X ray was found abnormal in radiography, so we suggest those patient whose radiography is abnormal should take sputum cytology or PT washing and PAT, and may take FNAB if good condition.

In generally, PAT and PT cytological washing have great importance in early diagnosis for early lung cancer. And FNAB can also help much to it.

The study of LEA(Large External Antigen) in Non-Small Cell Lung Cancer

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Jia Junyong Jia Xinshan

Objective: To study the expression of LEA(Large External Antigen) by monoclonal antibody ND-1(Provided by Jindan Song Professor) in NSCLC(Non-Small Cell Lung Cancer) and discuss the relation between the expression of LEA and clinical characters of NSCLC. **Methods:** Study the qualitative and quantitative expression of LEA by S-P immunohistochemistry and Western Blotting. The software used is SPSS(Statistical Package for Social Science) for window 8.0. **Results:** The result of immunohistochemistry: There were 56 cases of positive expression and occupied 73.7% of the 76 cases; In the 43 cases of adenocarcinomas, 34 cases were positive (79.1%); 22 cases were positive in 34 cases of lung squamous cell carcinoma(68.8%). Results of Western Blotting: Consistent with the results of immunohistochemistry, the results of Western Blotting showed the relation between expression of LEA and differentiation: the higher differentiation, the higher expression. In normal lung, there is very little expression of LEA. **Conclusions:** 1 LEA is a kind of tumor associated antigen that is also expressed in NSCLC. 2 The expression of LEA is related with adenocarcinoma differentiation of lung cancer.

【Key Words】 NSCLC(Non-Small Cell Lung Cancer)

LEA(Large External Antigen)

Regulatory effects of cAMP analogs on growth and differentiation of metastatic human lung cancer cells

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Abstract Objective To study the effects of two cAMP analogs with different site-selectivity on growth and differentiation of metastatic human lung cancer cells.

Methods The methods used include cell culture, in vitro invasion assay, soft agar colony formation assay, immunocytochemisty and electron microscopy. A metastatic human lung cancer cell line gwas treated with dibutyrbl cAMP (db-cAMP, non-site-selective) or 8-chloro-cAMP(8-cl-cAMP, site-selective for type II PKA).

Results Treatment of PG cells with 1 mmol/L of db-cAMP for 7days resulted in 48% growth inhibition, while treatment with 20 μ mol/L of 8-cl-cAMP gave 70% growth inhibition. The growth inhibitory effect of db-cAMP was shown to be reversible, while that of 8-cl-cAMP was not. The ability of PG cells to penetrate matrigel-coated membrane and to form colonies in soft agar was also significantly inhibited by treatment with these two drugs, Microscopic observation showed that cells formed elongated cytoplasmic processes and increased expression of neuron-specific enolase as well as chromogranin after treatment.

Conclusion The objective to inhibit malignance could be reached by activation of specific PKA with site-selective cAMP analogs.

Key words lung neoplasms Genes, suppressor, tumor protein kinases

Expression of P^{27Kip1}, CDK4, Rb in biopsy lung and the significances on the prognosis of carcinoma of lung

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Abstract **Objective**: To study the expressions of P^{27Kip1}, CDK4, Rb in fiber optic bronchoscopic squamous cell carcinoma specimens, their correlations to the clinical pathological features and the prognosis of carcinoma of lung.

Methods: Histochemical staining of ABC.

Results: The rate of positive staining of P^{27Kip1} protein expression was 28/54(51.9%), the expression level of P^{27Kip1} was positively related to the prognosis and multi—variance proportional hazard shown that its correlation significance was one of the highest ones. The rate of positive staining of CDK4 was 26/54(48.1%). The expression rate of Rb was 33/54(61.1%), its expression level was positively related to the prognosis.

Conclusions: The lower expression of P^{27Kip1} could independently be a valid marker for the poor prognosis of squamous cell carcinoma of lung. The expression of CDK4 was significant for the differential diagnosis of benign and malignant tumor in some extent. The expression of Rb could be a valuable marker for the prognosis of squamous cell carcinoma of lung.

Key Words: Fiber optic bronchoscope; P^{27Kip1}; CDK4; Rb; Squamous cell carcinoma of lung; Prognosis

Expression of P^{21Waf1}, CyclinE in iopsy lung and the significances on the prognosis of carcinoma of lung

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Abstract **Objective**: To study the expressions of P^{21Waf1}, CyclinE in fiber optic bronchoscopic squamous cell carcinoma specimens, their correlations to the clinical pathological features and the prognosis of carcinoma of lung.

Methods: Histochemical staining of ABC.

Results: The expression level of P^{21Waf1} was lower in squamous cell carcinoma of lung specimens, the rate of positive staining of P^{21Waf1} was only 11/54(20.4%). The rate of positive staining of CyclinE was 17/54 (31.5%) in squamous cell carcinoma of lung specimens. The expression of CyclinE in the case of poor differentiation or lymph node metastasis was significantly higher than that in those cases of well and moderate differentiation or no lymph node metastasis.

Conclusions: The expression level of P^{21Waf1} was lower in squamous cell carcinoma of lung. The expression of CyclinE could be a marker to predict the malignant degree of squamous cell carcinoma of lung.

Key Words: Fiber optic bronchoscope; P^{21Waf1}; CyclinE; Squamous cell carcinoma of lung; Prognosis

The Expression and Significance of Cell Cycle Regulators in Non - Small Cell Lung Cancer

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[**Abstract**] **Objective** In cell cycle of normal cell, there exists p53 - $p21^{WAF1}$ - cyclinECDK2 regulatory pathway . The article studied the expression and significance of the three regulators in non - small cell lung cancer **Methods** 69 NSCLC cases with neighboring noncancerous tissue and normal lung tissues were selected at random . Expression of p53, $p21^{WAF1}$ and cyclinE in 69 cases of NSCLC were detected using immunohistochemical S - P method **Results** There were low - level protein expression of p53, $p21^{WAF1}$ and cyclin E in normal bronchi epithelial. The expression of neighboring proliferation tissue was stronger than that of normal tissue but weaker than that of cancer tissue ($P<0.05$); The positive rates of p53, $p21^{WAF1}$ and cyclinE protein in NSCLC were 62.32%, 42.03% and 46.38% respectively; the expression of $p21^{WAF1}$ was negative correlated with clinical stage ($P<0.05$), The overexpression of cyclinE in NSCLC is correlated with sex and histological type ($P<0.05$). The expression of p53 and cyclinE is positive correlation ($P<0.05$) . **Conclusion** The abnormality of p53 may exist a mechanism of inducing cyclinE expression, No correlation between $p21^{WAF1}$ and p53 protein expression may indicate $p21^{WAF1}$ induction expression existing a p53 non - dependent pathway, The $p21^{WAF1}$ protein expression may become a reference factor in clinical stage and may benefit the judgement of treatment or calculation of the prognosis . The overexpression cyclinE may have some significance in judging the histological type.

Expression and significance of vascular endothelial growth factor in human non-small-cell lung cancers

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Objective: To determine the expression of vascular endothelial growth factor (VEGF) in human non-small-cell lung cancer (NSCLC) and its clinical significance. **Methods**: Paraffin sections from 65 human NSCLCs including 32 adenocarcinomas, 28 squamous cell carcinomas, 2 large cell carcinomas and 3 adenosquamous carcinomas. All lesions were resected at surgery. The immunohistochemistry S-P method was used to examine the expression of VEGF. **Results**: The frequencies for positive VEGF expression were 27 of 32 (84.4%) adenocarcinomas, 15 of 28 (53.6%) squamous cell carcinomas, 2 of 2 (100%) large cell carcinomas and 2 of 3 (66.7%) adenosquamous carcinomas. The positive ratio was significantly higher in patients with adenocarcinoma than in those with squamous cell carcinoma. The degree of positivity was generally greater in well differentiated tumors. VEGF expression did not correlate with clinicopathological factors such as tumor size or pathological stage. **Conclusions**: The results indicated that VEGF expression was frequently detected in human non-small-cell lung cancers and VEGF might be related with the pathogenesis and progress of human non-small-cell lung cancers.

EXPRESSION OF MMP-2, MMP-9, TIMP-1 PROTEIN AND ITS RELATION WITH METASTASIS IN NSCLC

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OBJECTIVE: To observe the expression of MMP-2, MMP-9, TIMP-1 protein in Non-Small Cell Lung Cancer and to study the relation between the protein expression and metastasis.

METHOD: 65 NSCLC samples were obtained from the patients admitted to the First Affiliated Hospital of China Medical University between 1989-1992, lymphatic metastasis occurred in 31 samples among them. We used immunohistochemical technique to detect the protein expression of MMP-2, MMP-9, TIMP-1 and used the X^2 test to analysis its relation with lymphatic metastasis.

RESULTS: MMP-2, MMP-9, TIMP-1 were mainly expressed in the plasma of tumor cells, also weakly expressed in the interstitial tissue and monocyte or macrophage surrounding the cancer lesions. The positive rate of MMP-2, MMP-9, TIMP-1 in nonmetastatic group was 55.9%(19/34), 35.3%(12/34), 82.4%(28/34) respectively, while in metastatic group the positive rate was 90.3%(28/31), 87.1%(27/31), 45.2% (14/31) respectively. There were statistical relationship between immunoreactivity of MMP-2, MMP-9, TIMP-1 and lymphatic metastasis ($p<0.01$, $p<0.01$, $p<0.01$) .

CONCLUSION: The degree of MMP-2, MMP-9, TIMP-1 expression intensively related to lymphatic metastasis, MMP-2 and MMP-9 were positively related to metastasis, while TIMP-1, negatively.

A STUDY ON PROTEIN AND mRNA OF MMP-2, MMP-9, TIMP-1 IN NON-SMALL CELL LUNG CANCER

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OBJECTIVE: To detect the protein and mRNA expression of Matrix Metalloproteinase (MMP) and Tissue Inhibitor of Metalloproteinase (TIMP) in Non-Small Cell Lung Cancer , to study the relation between protein and mRNA expression.

METHOD: 32 NSCLC samples were obtained from the patients admitted to the First Affiliated Hospital of China Medical University between 2000.3-2000.9. We used immunohistochemical technique to study the expression of MMP-2, MMP-9, TIMP-1 protein and used In Situ Hybridation technique to detect the mRNA level. X^2 test was used to analysis the concordance of mRNA and protein.

RESULT: MMP-2, MMP-9, TIMP-1 were mainly expressed in the plasma of tumor cells, also weakly expressed in the interstitial tissue and monocyte or macrophage surrounding the cancer lesions. In normal bronchial epitheliums and glands, TIMP-1 appeared marked expression. The results of IHC and ISH suggested that the concordant rates of MMP-2, MMP-9, TIMP-1 protein and mRNA were 81.3%(26/32), 84.4%(27/32), 71.9% (23/32) respectively, being of statistical significance. ($p<0.01$, $p<0.005$, $p<0.025$)

CONCLUSION: The protein and mRNA of MMP-2, MMP-9, TIMP-1 were concordant in NSCLC. We can use the antisense technique to down-regulate the expression of MMPs. This can offer a new thought to prevent lung cancer from metastasis.

Expression of Estrogen and Progestesterone Receptors in Non-small-Cell lung Cancer and its clinical Significance

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Objective: We investigated the ralation of Estrogen recepors (ER) and progesterone receptors (PR) in the Lung Cancer with prognsis and tested the feasibility of clinical endocrinotherapy. **Methods:** ER and PR in 116 Cases non-Small-Cell Lumg Cancer tissue specimens Were assayed by immunohistochemical method. **Resuts:** ER and PR Were negative in all normal Lung tissue the positive rate of ER and PR in Lung Cancer Was 48.7% and 50.5% respectively. We found no correlation between ER (PR) Content and age Sex, histologic Subtype, clinical classification or metastaisis of regional lymphnodes, respectively, The 5-year Survival rate for ER positive patients (42.8%) was higher than that for ER negative patients (17.2%) ($P<0.05$), and the 5-year survival rate for positive patients (45%) Was higher than that PR negative patients (14.6%) ($P<0.01$), The positive rate of ER and PR in Well-differentiated Cancer tissue Were higher than that in poorly-differentiated cancer tissue ($P<0.05$). **Conclusions:** We Concluded that ER and PR Could be used as biological markers to judge prognosis and the malignant degree of non-small-Cell Lung Cancer, Selected Case of Lung Cancer might respon favorably to endocrinal therapy.

References:

①Basil A Stoll Endocrine Managernent of Cancer;1;Biological London 1988:53.

②Charles C. Vincent A Penhy L, et al. Sex hormone receptors in non-small-Cell-Lung cancer in humain beings. J Thoral Cardiovase sury. 1994;108.

③Mclendon JE, Appleby D, Claudon DB, et al. Colonic neoplasm tissue estrogen receptor and Carcinoembryonic antigen Arch Sunry. 1977; 112:240.

THE STUDY ON THE EXPRESSION AND SIGNIFICANCE OF CYCLINB$_1$ AND P^{34CDC2} IN HUMAN NON-SMALL LUNG CANCER

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Objective The objective of this report is to observe the expression of cyclin B_1 and $P34^{cdc2}$ in human non-small cell lung cancer(NSCLC), and study the relationship and significance between the expression of cyclin B_1 and $P34^{cdc2}$ and clinicopathological features of NSCLC. **Methods** Totally 100 NSCLC cases with neighboring noncancerous tissues and normal lung tissues were selected at random. Those samples were stained with cyclin B_1, $P34^{cdc2}$ by immunohistochemstry S-P method, and analyzed in conjunction with the clinicopathological features of NSCLC. Statistics study included χ^2-test and the exact probabilities. The significance level chosen was P value less than 0.05($p<0.05$), and all test were two-sided. **Results** the expression of cyclin B_1 and $P34^{cdc2}$ was localizated in the cytoplasm of NSCLC cells and neighboring noncancerous tissues with proliferation in epithelial cell of bronchiole and small bronchus, brownish yellow and yellow can be observed, and no color was found in nucleus. Results indicated the expression of cyclin B_1 and $P34^{cdc2}$ between cancer tissues 、 neighboring noncancerous tissues with proliferation in epithelial cell of bronchiole and small bronchus and normal lung tissues was significant statistical difference($P<0.01$).There was overexpression cyclin B_1 and $P34^{cdc2}$ in NSCLC and the expression cyclin B_1 and $P34^{cdc2}$ in neighboring noncancerous tissues with proliferation in epithelial cell of bronchiole and small bronchus was stronger than that in normal lung tissues. Significant positive correlation was found between the expression of cyclin B_1 and that of $P34^{cdc2}$ in 100 NSCLC cases($P<0.01$),correlation coefficient was 0.866. Positive correlation was also found between the expression of cyclin B_1 and that of $P34^{cdc2}$ in neighboring noncancerous tissues with proliferation in epithelial cell of bronchiole and small bronchus ($P<0.01$),correlation coefficient was 0.638. No statistical significant was found between the different histological types 、 the differentiated degree 、 lymphatic metastasis and the expression of cyclin B_1 and $P34^{cdc2}$ ($P>0.05$) .Statistical significance between the different clinical staging of NSCLC and the positive expression of cyclin B_1 and $P34^{cdc2}$ ($P<0.05$) was obvious. **Conclusions** Overexpression of cyclin B_1 and $P34^{cdc2}$ was found in NSCLC , Before M phase , Overexpression of cyclin B_1 and $P34^{cdc2}$ formed more MPF(maturation promotion factor) to make NSCLC cells across G_2/M checkpoint and proceed into M phase .the Overexpression of cyclin B_1 and $P34^{cdc2}$ might be one of efficient marker in manifesting the dividing and proliferating ability of NSCLC and estimating clinical staging of NSCLC .

Chronic active EBV infection: clinicopathological and molecular studies

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Chronic active Epstein-Barr virus infection (CMEBV) disease shows repeated infectious mononculeosis-like symptom, abnormal elevation of levels of EBV antibodies in serum and poor prognosis. The disease occasionally transforms to lymphoproliferative disorders including malignant lymphoma. From these findings this condition seems to be prelymphomatous state. We examined 29 patients with CAEBV. The patients consists of non-neoplastic (group A), neoplastic (group B) and fulminant clinical course terminating lymphoid malignancy (group C).

Immunological examination for CD20, CD3, CD56 and Southern blotting for TCR gene (C beta, J gamma) was performed to determine the origin of proliferating lymphoid cells. For EBV, EBER-1 in situ hybridization and examination of terminal repeat using Southern blotting were performed.

Group A consisted of 10 patients ranged from one to 34 years with a median of 10 years and three male and seven female. Group B consisted of 16 patients ranged from three to 52 years with a median of 17 years and five males and 11 females, and Group C consisted of three patients ranged from one to four years with a median of one year, and 3 males.

In groups A and B, fever, lymphadenopathy and hepatosplenomegaly were noted in 70 – 90%, and oral and cutaneous lesions were found in 20 – 50 %. Group C showed these findings in all cases. Leukopenia and elevation of AST, ALT and LDH in serum were found about 50% in groups A and B and all cases in group C. Concerning antibody titers of EBV and elevation of VCA IgG in serum were found in almost all cases in groups A and B, but no elevation in group C. Elevation of VCA IgM was not found. A medial overall survival was of 58 months in group A, 45 months in Group B and 1.6 months in group C. Four patients were died of hemophagocytic syndrome in group A, 11 were died usually of lymphoid malignancy in group B and all three were died of peripheral T cell lymphoma with hemophagocytic syndrome in group C. Among them 3 patients with stem cell transplantation were alive in group B. Group B transformed hematological malignancies (four of peripheral T cell lymphoma, 10 of NK or NK/T cell lymphoma/ leukemia, one of malignant histiocytosis and one of AML) averaging

about 22 months after the onset of CAEBV symptom . Group C showed peripheral T cell lymphoma in all cases.

EBV positive cells were distributed through the node, mainly in the paracoretex and clonal proliferation was detected in five of eight examined cases in group A. All cases except AML were positive for EBV in the neoplastic cells in groups B and C.

Conclusions

1,CAEBV induced lymphoid malignancies averaging about 22 months after the onset of clinical symptom

2.Lymphoid malignancy in CAEBV consisted usually of peripheral T cell malignancies of unspecified or NK cell type.

3.Some cases showed a fulminant clinical course with transformation to peripheral T cell lymphoma and hemophagocytic syndrome.

CLONING OF HUMAN ANGIOSTATIN AND CONSTRUCTION OF ITS RECOMBINANT BACULOVIRUS

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ABSTRACT

OBJECTIVES: Angiostatin, a 38KDa protein containing the first 4 of the 5 plasminogen kringles, is one of the most novel antiangiogensis compounds. It has been shown to dramatically suppress the growth and metastases of experimental tumors in animal model. Sufficent amount of angiostatin is essential to examine its mechanism of action and develop a potent candidate of anti-cancer drug. So, we cloned the gene of angiostatin and constructed recombinant baculovirus containing its encoding sequence. This work established the foundation of large scale expression of angiostatin using baculovirus expression system (BVES).

METHODS: The reverse transcription product of mRNA purified from human liver cells was amplified by polymerase chain reaction (PCR) using sequence-specific primers designed according to the full length cDNA of plasminogen in GenBank/EMBL (Forward: 5'-AAAGTGTATCTCTCAGAGT G-3' and Reverse: 5'-ACACTCGCTTCTGTTCCTGAG-3'). After 3' ends being blunted, the PCR product was linked with pCR-Blunt vector (Invitrogen), and then transformed into TOP10 comptent cells (Invitrogen). Length and sequence confirmation of purified plasmid abtained from positive clones were carried out.

The confirmed plasmid was amplified using primers designed to generate angiostatin DNA fragment with *BamH* I sensitive 3' end and *Hind* III sensitive 5' end (Forward: 5'-GGATCCGAAAGTGTATCTCTCAGAGTGC-3' and Reverse:5'-AAGCTTTCATTCTGTTCCTGAGCATTTTTTCAG-3'). The DNA

fragment was inserted into pGEM-T easy vector (Invitrogen). *E. coli* JM109 was transformed by the vector. Plasmid was purified from positive clones and confirmed by *EcoR* I and DNA sequencing.

The pGEM-T easy vector was digested with *BamH* I and *Hind* III after proliferated and purified. The enzymes digested product was subcloned into the pBlueBacHis2B baculovirus expression vector (Invitrogen) then the vector was transformed into *E. coli* JM109. The cloned product was digested by *BamH* I and *Hind* III and sequenced to confirm proper insertion of the angiostatin DNA into the multiple cloning site of the plasmid.

Recombinat baculovirus was generated by cotransfection of the pBlueBacHis2B vector with linerized baculovirus DNA (Bac-N-Blue DNA, Invitrogen) into *Spodoptera frugiperda* 9 (Sf9) cells. The transfection media was used in plaque assay to purified the recombinat baculovirus away from any uncut viral DNA backgroud and/or illegilimate recombinants that did not contain the gene of angiostatin. PCR analysis of blue plaque was carried out to determine the presence of an insert of angiostatin DNA in a putative recombinat virus and confirm the isolation of a pure recombinant plaque. The right plaques were subsequently used to generate high-titer stocks of recombinant virus for future infections of cells grown in protein-free media (SFX-Insect MP, Hyclone)

RESULTS: Baculovirus transfer vector, pBlueBachis2B-Angio contained sequence encoding amino acids 97-458 of human plasminogen, was acquired. The newly cloned and constructed human angiostatin is differed from reported recombinant human angiostatin in the term of number of amino acids and transfer vector. 5 days after infection, the constructed recombinant baculovirus leaded to Sf9 cells lysis.

CONCLUSION: We succeeded in cloning the gene of angiostatin and abtained the recombinat baculovirus with biology capacibility containing the encoding sequence of angiostatin.

Title

Status of c-erbB-2 in gastric adenocarcinoma: A comparative study of immunohistochemistry, fluorescence in situ hybridizationand and enzyme-linked immuno-sorbent assay.

Authors

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Abstract

c-erbB-2 is a proto-oncogene that is located on chromosome 17 (17q12-21.32) and that encodes a 185-kDa transmembrane tyrosine kinase receptor which is a member of the growth factor receptor family. c-*erb*B-2 abnormalities attract great deal of attention because the new adjuvant therapy using an antibody against c-*erb*B-2 gene product, trastuzumab (Herceptin; Genentech, Inc, South San Francisco, CA) is effective to breast cancer with amplification and/or overexpression of c-*erb*B-2. The aberration of c-*erb*B-2 also occurred not only in breast cancers but also in ovarian, lung, and gastric carcinoma in variety of frequencies.

We examined amplification of c-*erb*B-2 locus (17q12-q21), protein overexpression of c-*erb*B-2 protein (p185), and serum level of soluble c-*erb*B-2 fragment (p105) in gastric cancer using fluorescence in situ hybridization (FISH), immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA)

respectively.

Overexpression of c-*erb*B-2 protein (2+ or 3+ immunostaining) was found in 29 (8.2%) of the 352 gastric carcinomas analyzed. In FISH analysis, 23 of 24 tumors with 3+ immunostaining and one of 5 tumors with 2+ staining had high level amplification (more than 20 copies) of c-erbB-2. Pre-operative serum p105 was quantified in the serum specimens obtained from 129 patients with gastric cancer and 28 patients with benign diseases. There was no significant differences between the serum p105 levels of 11 patients with c-*erb*B-2 overexpressing carcinomas, those of 118 patients without overexpression of c-*erb*B-2, and those of 28 control patients, although single case of gastric carcinoma overexpressing c-*erb*B-2 with extensive liver metastasis had higher than the cut-off level.

The mechanisms of high overexpression of p185 in cellular membrane due to high level amplification of c-*erb*B-2 seem similar to those well established in breast cancers. Patients with gastric adenocarcinoma with c-*erb*B-2 amplification are potential subjects of new adjuvant therapy using humanized monoclonal antibody.

Enhanced Expression of E2F1 in Hepatocellular Carcinoma

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Purpose: E2F1, which is one of transcription factor, is an important regulator of cell proliferation, apoptosis, and differentiation. E2F1 is regulated during the cell cycle at the mRNA level by alterations in transcription of *E2F1 gene* and at the protein level by complex formation with proteins such as the *retinoblastoma* gene product (pRB), cyclin A and DP1. To clarify involvement of E2F1 in the carcinogenesis of hepatocellular carcinoma (HCC), we investigated E2F1 expression in HCC using the immunohistochemical method.

Materials and Methods: The 40 HCC specimens obtained from surgical resections were fixed in 10% buffered formalin and embedded in paraffin. All the sections, which were three μm thick, were immunostained by indirect method with anti-E2F1 mouse monoclonal antibody (KH-95, Santa Cruz Biotechnology, CA).

Results and Conclusion: Positive nuclear staining for E2F1 was detected in 33 of 40 HCC cases (82.5%). Of the 33 cases, 22 cases (66.7%) showed focal and scattered positive staining pattern and other 11 cases (33.3%) showed diffuse positive pattern. There was no correlation between the positive nuclear staining of E2F1 and tumor differentiation. However, positive staining was prominently observed in the high cellularity areas of tumor nodules. No positive nuclear staining was detected in non-cancerous liver cells. These data suggest that E2F1 may play an important role in tumor cell proliferation in the carcinogenesis of HCC.

Synovial cells express thymidine phosphorylase in inflammatory and neoplastic synovial tissue

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Synovial cells (SCs) in pathological conditions of synovial tissue were analyzed by means of paraffin-immunohistochemistry in points of immunological phenotypes, which are usually expressed in monocytes/macrophages and dendritic cells. Paraffin sections of 4 cases of non-inflammatory and non-neoplastic synovial tissue, each 1 case of non-specific chronic synovitis and of granulomatous synovitis, 5 cases of rheumatoid arthritis (RA), and 1 case of benign giant cell tumor (GCT) were used. SCs in non-inflammatory and non-neoplastic synovial tissue revealed weak or strong immunoreactivity with CD68 and LN-3 (Ia-like antigen) but did not with thymidine phosphorylase (TP). SCs in RA expressed strong immunoreactivity with TP. GCT tumor cells showed strong immunoreactivity with CD68, LN-3 and TP. T- and B-cell lymphoproliferative lesions were noted in RA, whereas T-cell-dominated one was seen in the other conditions. Germinal centers (GCs) in RA associated some CD3-positive T-cells. Consequently, it was recognized that SCs are of monocytes/macrophage lineage. In pathological conditions SCs expressed TP especially in RA. The expression of TP in SCs in RA suggested non-neoplastic genetic alteration in the expression of TP in the pathological conditions. The unusual expression of TP may induce unusual lymphoproliferative lesion in RA. But the expression of TP in GCT might be as a tumor growth inhibitory factor.

Oncogenic Factors in Gastric Malignant Lymphoma

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(This study is the co-operative study with Masanori Nakagawa, Shinji Yashiki, Suguru Yonezawa, Shuji Izumo and Fusayoshi Murata in Kagoshima University.)

It has been clarified in the epidemiological retrieval that the infection of Helicobacter pylori (HP) is a cause of the gastric mucosa-associated lymphoid tissue (MALT) type lymphoma. But, mechanism of the gastric MALT type lymphoma-genesis under infestation of HP has not yet been explained. Recently, it was reported that nitric oxide (NO) which HP produces may modulate the active oxygen metabolism in the mucosa. The enzyme that produces NO and is induced in various cells in disease states is inducible nitric oxide synthase (iNOS). In order to understand the relationship between actual condition of the NO production and the gastric MALT type lymphoma-genesis under the HP infestation, this study included analysis of iNOS expression in gastric MALT and regional lymph nodes, stromal cells in the gastric MALT type lymphoma, somatic mutation in immunoglobulin heavy chain gene (IgH) CDR3 region, and Epstein-Barr virus (EBV) infection detected by EBER-1 in-situ hybridization.

Four cases of MALT developed in the stomach with HP-related ulcer, 40 Chinese cases of gastric malignant lymphoma and some cases of lymph nodes and tonsil tissue were used. The expression of iNOS was evaluated by means of immunohistochemistry. In order to know immunological characteristics of lymphocyte and lymphoma cells and HP infestation, anti-HP antibody, anti-Lewis X/Y antibody, and several antibodies characterizing lymphocytes and stromal cells were applied. Lewis X/Y is lipopolysaccharides (LPS) of the cell membrane of HP. Direct DNA sequencing analysis of IgH CDR3 region was performed, extracting DNA from paraffin sections of the malignant lymphomas and amplified the DNA by means of Fr3A-LJH, -VLJH semi-nestic polymerase chain reaction (PCR).

In the HP-related gastric ulcer, HP could be detected in the mucous coat and in the glands of the gastric mucosa. The anti-Lewis X/Y antibodies labeled HP bodies in the mucous material and in the glandular epithelial cytoplasm and follicular dendritic cells (FDCs) in the regional lymph nodes. Anti-human IgM/G/A antibodies also labeled the HP bodies. In MALT, IgM-positive cells were seen in among germinal centers (GCs) but IgG or A-positive centroblasts were detected. In a case, IgM-positive cells revealed pseudonodular growth with proliferating centers in the MALT. INOS is expressed in FDCs weakly in the MALT and strongly in the regional lymph nodes. Forty cases of gastric lymphomas comprised 11 cases of MALT type, one case of plasmacytoma, 24 cases of diffuse large B-cell lymphoma (DLBC), 2 cases of T-cell lymphoma and 2 cases of nodal B-cell lymphoma. The GCs in the MALT type lymphomas revealed colonization of lymphoma cells, appearance of CD68-positive and/or thymidine phosphorylase (TP)-positive dendritic cells, and expression of iNOS. In DLBL, CD68-positive and /or TP-positive stromal cells and few stromal cells expressing iNOS were noted among lymphoma cells. The GCs in DLBC manifested collapsed network of FDCs and weak expression of TP and iNOS. A few lymphoma cells expressed signals of EBER-1 in the MALT type and DLBL. The expression of iNOS in FDCs was recognized in small number of tonsil and lymph nodes and in many gastric regional lymph nodes.

Consequently, it was suggested that Lewis X/Y deposited in GCs of the regional lymph nodes induced iNOS in FDCs. iNOS produces NO that is a mutagen and disordered membrane receptor functions, although NO is easily catalyzed in a water. Then, the NO induced by HP is one of the factors in the gastric malignant lymphoma-genesis. In addition, there was a possibility that gastric MALT type lymphoma came from the GCs expressing iNOS in the regional lymph nodes. IgH CDR3 DNA sequence suggested on-going somatic mutation that may reveal homology with various human DNAs or would not indicate any homologies with known DNA sequences. Therefore, the gastric MALT type lymphoma-genesis includes accelerated on-going somatic mutation and the NO mutagen rather than an immunoreaction against the specific antigen. The microenvironment with iNOS-positive glandular epithelial cells and/or -stromal cells, TP-positive dendritic cells and CD68-positive cells is also the factors in the gastric MALT type lymphoma-genesis. EBV infection may be one of the factors in Chinese cases. The gastric MALT type lymphoma-genesis must be studied further, because there are undiscovered genetic mutation or pathological conditions that correspond to the API2-MALT-1 chimerical gene formation in the MALT type lymphoma without relation of HP infestation.

STUDY on 80 CASES of EBER-1 ISH of MALIGNANT LYMPHOMA

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Objective To detect Epstein-Barr virus (EBV) in 80 cases of malignant lymphoma by immunohistochemistry and EBV-encoded small RNA-1(EBER-1) *in situ* hybridization(ISH), and study the relationship between part of Chinese malignant lymphoma and EBV infection.
Method 70% formalin-fixed paraffin wax-embedded and HE-stained material from 80 cases of malignant lymphoma.1.Immunohistochemistry: T cells and B cells were detected by antibody MT1,UCHL1, L26, BerH2 from DAKO and stained by Number method and ABC. 2. EBER-1 ISH: DIG-EBER-1 probe was hybridizated by method as reference, positively controlled by infectious moncytopoiesis and negatively controlled by EBV-negative lymphadenitis and detected by RNA probe. 3. Histological classification of malignant lymphoma is based on 1997 WHO Classification.
Results 25 of 80 cases(31.3%) show EBER-1 positive, in which 18%(9) in 50 cases of B cell lymphoma、 50%(9) in 18 cases of T cell lymphoma and 58.1%(7) in 12 cases of Hodgkin's disease were EBER-1-positive (HD)(see tablet 1).The positive signals were distributed in tumor nuclei with negative plasma and cell membrane. It also can be seen in most of RS cells and Hodgkin's cells. This result suggests that part of Chinese malignant lymphoma is related to the infection of EBV.
Discussion : EBER-1 may be one million in EBV infectious cells. So it is a highly sensitive EBV detective method. The fact that 25 of 80 cases is EBER-1 positive showed closely relationship between partly malignant lymphoma and EBV. This suggests that EBV is very important to genesis of part lymphoma. EBER-1 in 18% of B cell lymphoma 、 50% of T cell lymphoma and 58.1% of HD were positive. Among which, HD is the highest, then is T cell lymphoma and B cell lymphoma. Compared with nasopharyngeal carcinoma and gastric cancer, the distribution of EBER-1 is different. In the former two, it can be seen in almost all tumor cell; while in malignant lymphoma, it only can be found in part tumor cell. In generally, this paper provides important foundation for studying the relationship between Chinese each type of malignant lymphoma and EBV. The positive and negative cases of EBV influence to prognosis will be researched in further.

The study on the histologic type and CD_{44} expression in Gastric Lymphoma

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【Abstract】 Objective: To study the expression of CD_{44s} and CD_{44v6} in gastric lymphoma and correlated this with several clinicopathologic parameters. **Method:** Histologic sections cut from paraffin blocks were stained routinely with hematoxylin and erosin. The haenmatopoietic origin of lymphomas was confirmed by immunohistochemistrical staining with monoclonal antibodies against CD_3, CD_5, CD_{79a}. Accessory cells in stroma were determined by monoclonal antibodies against CD_{21}, S_{100}, and CD_{68}. **Result:** (1)According to the new WHO classification criterion, among the 38 cases of gastric lymphoma, 23 were classified as low grade, 10 MALToma, 1 plasmacytoma, 2 T-cell lymphoma, 2 lymph node metastasis of lymphoma. (2)One of the 2 T-cell lymphomas were both stained by CD_3, CD_5, CD_{79a}; There were 2 B-cell lymphomas with co-expression of CD_3 and CD_{79a}. The double immunostaining studies show unequivocally that co-expression is occurring on the same lymphoma cell population. There were a lot of macrophages and dendritic cells stained by CD_{20}, S_{100} and CD_{68} in stroma. (3) The positive expressions of CD_{44s} and CD_{44v6} were 47.3% and 23.7% respectively. Differences in the intensity of expression were related to the histologic subtype, low grade

gastric lymphoma were often weakly positive for CD_{44s}(33.3%). Whereas high grade gastric lymphoma were characterized by a strong reactivity for CD_{44s}(63%)(P=0.02). We observed a substantial positive expression of CD44v6 in high grade gastric lymphoma(37%), Whereas it is negative in nearly all low grade ones(5.2%)(p=0.01). **Conclusion:** There are several types in gastric lymphoma, among them diffuse large B-cell was the main one. There were co-expression of CD_3 and CD_{79a} in some lymphomas. In B-cell lymphoma, there are intermingling lymphocytes among lymphoma cells that are positive for the antibody such as CD_3 or CD_5. There were a lot of macrophages and dendritic cells stained by CD_{20}, S_{100} and CD_{38} in stroma. Evidence suggests that lymphocyte homing receptors are important not only in the trafficking of normal lymphocytes, but also in dissemination of malignant lymphoma. Thus the analysis of CD_{44S} and CD_{44v6} could be a useful diagnostic parameter to differentiate between low grade and high grade gastric lymphoma.

【**Key Words**】 Gastric Lymphoma MALT CD_{44S} CD_{44v6}

vMIP α ENCODED BY HUMAN HERPESVIRUS 8 BLOCKING HIV CO-RECEPTOR CCR5 OF PERIPHERAL BLOOD MONOCYTES

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Since the co-receptors infective mechanism being recovered, which HIV binding and fusing to the membrane of $CD4^+$ target cells is mediated by co-receptor and $CD4^+$ molecular together, scientist have a great attention in study of HIV co-receptor and its ligands. Mutators or variants of the co-receptors or its ligands can obviously block or delay incidence or progression of AIDS among HIV positive bodies. However, HIV co-receptors and chemokines as its ligands have been investigated as the attractive targets for new antiviral agents of HIV infection. The function and usage of vMIP α encoded by K6 gene of herpesvirus 8 (HHV8) which has homology with human macrophage protein (MIP) have been not clearly known. In present report the K6 gene of HHV8 was cloned and transfected into NIH3T3 cells and *E.coli.* cells. Conditional media from the 3T3-transfected cells and K6 product vMIP α from *E. coli.* cells was used to perform the experiments of ligand-receptor binding and cellular adhesion with peripheral blood macrophages. The conditional media and the purified vMIP α from *E. coli.* could compete to bind CCR5 located on the surfase of macrophages from peripheral blood with I^{125}-hMIP-1 α chemokine of human. Furthermore, cellular adhesion showed that the conditional media and the purified vMIP α did not induced the adhesion of the peripheral blood macrophages to ICAM-1. In conclusion vMIP α encoded by K6 gene of HHV8 can bind to CCR5 of peripheral blood macrophage cells and doesn't induced their adhesion. This suggested that vMIP α can seal off the CCR5. CCR5 is a major co-receptor of HIV infection. To seal the receptor can block HIV to enter into target cells. So, vMIP α sealing CCR5 but not promoting adhesion reaction of MDMs may be a recombinant protein from natural source to inhibit HIV into the target cells. This offers vMIP α a possibility of preventing HIV infection and treating AIDS.

Abstract

THE EXPRESSION OF APOPTOSIS-RELATED GENE CASPASE-4 IN HD ANDALCL

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Objective:
This study is based on the result of the study in HD and ALCL employing gene chip technique. In the latter study, we found that there were distinctly different expression of apoptosis-related gene Caspase-4 between HD and ALCL cell lines at the level of mRNA. From the point of view, we try to identify at the level of protein whether there are different expressions of this gene in HD and ALCL tissues as well.

Methods:
H-E staining, the monoclonal and polyclonal antibodies CD30(BerH2), CD15(C3D-1), CD20(L26) and CD45RO(UCHL1) were used for selecting the cases of HD and ALCL. Specific high affinitive anti-caspase-4 polyclonal antibodies were used immunohistochemically to analyze the expression of Caspase-4 in 18 cases of HD and 15 cases of ALCL.

Results:
The expression of caspase-4 demonstrated a strong positive staining in all ALCL cases, 15 in 15 cases were stained +++, whereas was negative in 16 HD cases (88.8%), while other two cases were + and ++ stained (11.2%) respectively, showing a distinct difference ($p < 0.01$) between two groups.

Conclusions:
The different expression of Caspase-4 in HD and ALCL cases implies a different mechanism of oncogenesis and the different defects of signal transduction pathway of apoptosis in these two entities of lymphomas. (2) Pathohistological features, combined expressions of some cellular markers and Caspase-4 protein in neoplastic cells in few cases showed an overlap and a likely transitional state between HD and ALCL. (3) The expression manner of Caspase-4 is useful for the differential diagnosis of HD and ALCL.

Key words: apoptosis, caspase-4, hodgkin's disease, anaplastic large cell lymphoma, immunohistochemistry

RESEARCH OF THE PROLIFERATION CHARACTERISTICS IN H PYLORI GASTRIC DISEASES

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Objective: to measure telomerase activity and detect the expression of PCNA, P53protein and P16protein in H.pylori associated gastric diseases. To find out the proliferation characteristics of H.pylori gastric diseases and its relationship with gastric cancer.

Methods: 248 samples of different gastric mucosa biopsy were selected. Of them 94 samples had been measured for telomerase activity while 154 samples had been detected for PCNA, P16 and P53 protein. Telomerase activity was measures by TRAP—ELISA protocol. The expressions of PCNA, P16 and P53 protein were detected by SP protocol. H.pylori infection was confirmed or excluded by histological examination hematoxylin—eosin(HE), urease of H.pylori-DNA PCR and enzyme—linked immunosorbent assay(ELISA).

Results: The research are composed of two parts as follows:

1. Telomerase activity and its clinical significance in *H.pylori* associated gastric diseases. There was difference of telomerase activity between atrophic gastritis , dysplasia, gastric cancer and normal gastric mucosa (respectively $P<0.05$, $P<0.01$ and $P<0.01$). Compared different gastric diseases mucosa with normal one, the difference was significant ($P<0.05$). The difference between gastric cancer and different gastric diseases mucosa was significant ($P<0.01$); compared *H.pylori* positive with *H.pylori* negative groups of different gastric mucosa, the difference was significant ($P<0.01$). In the *H.pylori* positive group, there was no difference between the cagA positive samples and cagA negatives ($P>0.05$).

2. Expression of PCNA, p16 and p53 protein in *H.pylori* associated gastric diseases. The index of PCNA was higher in *H.pylori* positive than in *H.pylori* negative group. The expression of p53 protein was the same as PCNA, when it came to p16 protein; the result was just the opposite. There was significant difference between *H.pylori* positive and negative samples in benign gastric lesion group ($P<0.01$). The positive rate of the index of PCNA and p53 protein dropped distinctly after the Eradication therapy of in *H.pylori* positive group. After *H.pylori* eradication therapy, there was significant difference of the index of PCNA and p53 protein between *H.pylori* turned to negative and *H.pylori* still positive groups ($P<0.01$)

Conclusions: 1.The expression of telomeras activity in gastric cancer and *H.pylori* associated gastric diseases, including atrophic gastritis and dysplasia, is higher than in normal gastric mucosa and superficial gastritis. The expression in gastric cancer is highest. Through the detection of the telomerase activity in *H.pylori* positive gastric disease, we found that the expression of telomerase activity in *H.pylori* positive gastric disease was higher than in *H.pylori* negative ones. It is probably that *H.pylori* infection may lead to telomerase activity and then cells' proliferations are promoted.

2.The index of PCNA and p53 protein was higher in *H.pylori* positive gastric mucosa than in *H.pylori* negative group. But when it came to p16 protein, the conclusion was just the opposite. There was significant difference of PCNA and p53 protein between the *H.pylori* negative and positive groups after the antibacterial therapy. The above-mentioned results were consistent of the expression of PCNA, p16 and p53 protein. The conclusion indicates that *H.pylori* infection had promoted the expression of mutant p53 gene and inhibited the activity of p16 gene. Those could lead to the cells' proliferation.

3. *H.pylori* associated gastric diseases, the important precancerous diseases, its proliferation characteristics are similar to gastric cancer.

DIAGNOSIS SIGNIFICANCE OF SERUM β-GLUCURONIDASE DETECTION IN PATIENTS WITH EARLY GASTRIC CARCINOMA

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To study the clinical significance of serum β - glucuronidase (β -G) detected in patients with early gastric carcinoma, the ELISA method was used to detect the activity of serum β -G and the regularity of the change of serum β -G in patients with early gastric carcinoma before and after treatment. Results showed that serum activity of β -G is higher in the group of early gastric carcinoma than that of normal control ($P<0.01$). The serum activity of β -G is decreased after operation or interventional therapy ($P>0.05$). The findings showed that detection of serum β -G is significant in dignosis and judgement of treatment efficiency of early gastric crcinoma.

Key words β -glucuronidase; ELISA;
early gstric earcinoma.

EXPRESSION AND SIGNIFICANCE OF β-GLUCURONIDASE In HEPATOCELLUAR CARCINOMA

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The localization and quantitation of β-Glucuronidase(β-G) in 58 cases of hepatocellular carcinoma were studied wich immunohistochemical and immunoelectron microscopic techiques. The results showed that β-G expressions were identical in cell and sub-cell levels, and both shoned higher than those in normal liver tissue. β-G expression rose along with the increase in tumor of malignancy ($P< 0.01$). Authors suggested that higher β-G was suit the needs of substance metablism in cancer cells, and was regulated by itself. As a marker of enzyme, β-G may be useful in auxillary diagnosis of hepatocellular carcinoma.

Key words β-Glucuronidase Hepatocellular carcinoma
Immunohistochemistry Immunoelectron microscopy

RELATIONSHIP BETWEEN CD44V6 EXPRESSION AND METASTASIS AND PROGNOSIS IN GASTRIC CANCERS

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The aim of this study is to investigate the expression of CD44 splice variant 6(CD44v6) in gastric carcinomas and its significance in the tumour metastatic potential and prognosis of the patients with gastric carcinoma. A hundred and seventy gastric carcinomas with at least 5 year follow-up, including 45 early (EGC), 103 advanced (AGC) and 22 cases of intermediate (MGC) Chinese gastric carcinoma specimens were studied immunohistochemically using a monoclonal antibody against CD44v6 (from R&D System). An antigen retrieval method followed by a routine ABC process was employed. Results showed that in the gastric mucosa adjacent to tumour, only weak and focal CD44v6 expression was detected within the foveolar proliferation zone but not on mucinous surface epithelium. A also focally positive reaction was found in the intestinal metaplasia adjacent to the tumor. CD44v6 was detected in the membrane of cancer cells in 38.8% (66/170) of gastric carcinomas investigated, which is in 26.7%(12/45) of EGC, 27.3%(6/22) of MGC, and 46.6%(48/103) of AGC respectively. CD44v6 was revealed in 48.2% (53/110) of gastric carcinomas with lymph node metastasis and in 71.4%(5/7) of those with liver metastasis; while only in 18.6%(11/59) of gastric carcinomas without any metastasis ($p<0.01$). Furthermore, CD44v6 was detected in 47 out of 107(43.9%) intestinal-type gastric carcinoma, comparing with in 17 out of 62(27.4%) diffuse-type tumor ($p<0.05$). The strongly positive reaction to mAb-CD44v6 was detected only in gastric carcinomas with lymph node or liver metastasis but not in those without any metastasis ($p<0.05$). Patients with CD44v6 positive gastric carcinoma showed a significantly shorter survival period after the surgical operation than those with CD44v6 negative tumor ($p=0.0002$). The findings in this study suggest that the expression of CD44v6 was positively correlated with the progression and metastasis of gastric carcinoma, and correlated with worse prognosis of patients with gastric carcinoma.

Clinicopathological and Molecular Genetic Analysis of 4 Chinese Typical Hereditary Nonpolyposis Colorectal Cancer Families.

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Abstract

Backgroud Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common genetic diseases comprising at least 5-6% of all colorectal cancers. It is characterized by early onset and mostly right-sided tumors. It is known that HNPCC is caused by germline defects in one of five human mismatch repair genes (hMSH2, hMLH1, hMSH6, PMS1, PMS2). and the former two genes account for the large majority of mutations found in families with HNPCC . Until now there have only been some case reports of HNPCC in China and no systemic study of molecular genetic aspects of HNPCC had been done.

Objective To investigate the clinicopathological and molecular genetic characteristics of Chinese typical HNPCC families.

Methods 4 Chinese typical HNPCC families were studied using microdissection, microsatellite instability analysis, immunostaining of hMSH2 and hMLH1 proteins and direct DNA sequencing of hMSH2 and hMLH1 genes.

Results All five tumor tissues of 4 probands from the 4 Chinese typical HNPCC families showed microsatellite instability at more than two loci(MSI-H or called RER+ phenotype).Three out of the 4 cases lost hMSH2 protein expression and one case showed no hMLH1 protein expression. Three pathological germline mutations (2 in hMSH2 and 1 in hMLH1) , which hadn't not been reported previously , were identified.

Conclusions Chinese typical HNPCC families showed relatively frequent germline mutation of mismatch repair genes. High level microsatellite instability and loss of expression of mismatch repair genes correlated closely with germline mutation of mismatch repair genes. Microsatellite instability analysis and immunostaining of mismatch repair gene might be effective screening methods before direct DNA sequencing. It is necessary to establish clinical criteria and molecular diagnostic strategies more suitable for Chinese HNPCC families.

GASTRIC TUMOR-ASSOCIATED ANTIGEN MG7 EXPRESSION IN THE GASTRIC CANCER, PRECANCEROUS LESIONS AND H.PYLORI-RELATED GASTRIC BENIGN LESIONS

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Objective: To investigate the relationship between the antigen MG7 expression and gastric cancer and precancerous conditions; to study the relationship between the antigen MG7 expression and Hp in gastric benign lesions to find the effect of Hp in the process of gastric cancer development.
Methods: The Hp infection was determined by HE stain, PCR and ELISA. The expression level of antigen MG7 was determined by immunohistochemistry method. The classification of intestinal metaplasia of gastric mucosa was determined by histochemistry method.
Results: The positive rate of MG7 expression in normal gastric mucosa, intestinal metaplasia and dysplasia of gastric mucosa and gastric cancer were increased gradually ($P<0.01$) .The positive rate of MG7 expression in superficial gastritis, atrophic gastritis and gastric cancer were increased on sequence ($P<0.01$) . The positive rate of antigen MG7 expression in III intestinal metaplasia of gastric mucosa had significant difference, compared with I and II intestinal metaplasia ($P<0.05$) .There was no expression of antigen MG7 in H.pylori-negative superficial gastritis. The 8 cases of superficial gastritis with antigen MG7 expression were all H.pylori-positive, of which only one case with antigen MG7 expression (++) was found reduced Mg7 expression accompany H.pylori eradication after treatment.
Conclusions: MG7 was distinctive in gastric cancer and it can be a good index in the screening of gastric cancer; III intestinal metaplasia of gastric mucosa, atrophic gastritis and dysplasia of gastric mucosa should be followed up and the detection rate of early gastric cancer could be improved, MG7 had great clinical value in the dynamic follow-up of gastric precursors; although the MG7-positive cases with H.pylori-positive gastritis showed benign in morphology, they also had the potential risk of developing gastric cancer, so the MG7-positive cases with H.pylori-positive gastric lesions should be paid attention to and be followed up.

EXPRESSION OF PI GLUTATHION S-TRANSFERASE IN INTESTINAL METAPLASIA OF GASTRIC MUCOSA AND ITS RELATIONSHIP WITH H.PYLORI INFECTION

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Objective: To study dynamic changing of GST- π in normal gastric mucous ,various type IM, and gastric cancer ; to investigate the relationship between human carcinogen detoxification system and the virulence of H.pylori in IM.

Methods: Detecting the expression of GST- π in 30 cases with normal gastric mucosa,171cases with IM and 18 cases with gastric cancer by S-P immunohistochemical method; the types of IM were classified by high-iron diamine /alcian blue pH2.5/periodic acid –Schiff (HID-ABpH 2.5-PAS) method; Hp infection was confirmed or excluded in all patients by histological examination hematoxylin-eosin (HE), urease of Hp-DNA PCR and enzyme-linked immunosorbent assay(ELISA); 80 H.pylori-positive cases were treated.

Results: The positive rates of GST- π expression in IM and gastric cancer were significantly higher than that in normal gastric mucosa ,where were all negative($P<0.01$).The positive rates of GST- π expression of gastric cancer were lower than that of IM($P<0.01$). The positive rates of GST- π expression in I subtype IM, II subtype IM and III subtype IM were decreased in sequence (83.3%,71.1%,48.9%). The positive rates of GST- π in Hp-positive groups with IM were higher than Hp-negative groups with IM ($P<0.05$) After Hp treatment, the positive rate of GST- π in Hp-eradicated group were significantly higher than that of Hp-positive group before the Hp treatment ($P<0.01$). The positive rate of GST- π expression in Hp-eradicated group were significantly higher than gastric cancer group($P<0.01$).

Conclusions: From normal gastric mucous, IM to gastric cancer , the expression rate of GST- π is from no expression to high and to low again. From I→II→III IM, the expression rate of GST- π is from high to low .We concluded that III type IM had a high risk with gastric cancer if there was low or no expression of GST- π in it. Our findings demonstrated that risk of developing gastric cancer could be increased if there was low or no expression of GST- π in Hp-infection patients, as well as demonstrate d that the human carcinogen detoxification system and the virulence of Hp might interact with each other in IM.

TELOMERASE ACTIVITY IN NORMAL GASTRIC MUCOSA, H.PYLORI ASSOCIATED GASTRIC DISEASES AND GASTRIC CANCER

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Objective: To measure the telomerase activity in normal gastric mucosa, H.pylori associated diseases and gastric cancer. To examine the relation between telomerase activity and H.pylori.
Methods: Measure the telomerase activity in 10 cases of normal gastric mucosa, 67 cases of H.pylori positive and 27 cases of H.pylori negative diseases and 23 cases of gastric cancer. Also telomerase activity was measured in 10 cases of H.pylori infection before and after H.pylori eradication. Telomerase activity was detected by TRAP-ELISA protocol. H.pylori infection was confirmed or excluded in all patients by histological examination hematoxylin-eosin (HE), urease of H.pylori-DNA PCR and enzyme-linked immunosorbent assay (ELISA).
Results: There was no expression of telomerase activity in normal gastric mucosa. The positive rate of telomerase activity in H.pylori positive group was significantly higher than that in H.pylori negative group ($P<0.01$). Telomerase activity of gastric cancer was significantly higher than that of normal gastric mucosa and H.pylori associated diseases ($P<0.01$). After H.pylori eradication treatment, the positive rate of telomerase activity was significantly lower compared with itself before H.pylori eradication ($P<0.05$).
Conclusions: From normal gastric mucosa to H.pylori associated gastric diseases and to gastric cancer, telomerase activity increased gradually. Our results indicated that telomerase activity has positive relation with H.pylori infection.

Biological background for differential diagnosis of salivary pleomorphic adenoma from myoepithelioma by CT images and characteristic histopathology of paucivascular stroma

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Salivary pleomorphic adenomas are characterized by its colorful and abundant stroma, which has been the reason for another term "mixed tumor." The stroma has fibrous, hyaline, myxoid, and cartilaginous appearances and the proportion of each type of stroma varies with individual case. However, their paucivascularity in the stromal space is common to all cases. In order to understand the biological background of their characteristic vascularity in the stroma, we have studied pleomorphic adenomas and myoepitheliomas comparatively, in terms of vascular channel distribution by immunohistochemistry for endothelial cell markers, and of expressions of anti-angiogenic factors, such as chondromodulin-I (ChM-I) or ED-B region of fibronectin, and of delayed contrast CT images. For this end, we used surgical specimens of 104 pleomorphic adenomas and 72 myoepitheliomas from Niigata and Chengdu as well as 10 primary cultures of pleomorphic adenoma cells and computed tomography (CT) images from 5 patients with pleomorphic adenoma and 2 patients with myoepitheliomas. There were quite a small number of blood vessels, especially capillaries, in the stromal space of pleomorphic adenoma, irrespective of its histological variation, although myxochondroid matrices were the poorest in vascularity. In contrast, the parenchyma of myoepithelioma tended to be divided by vascular stromal septa, and capillaries were scattered evenly over the tumor tissue. Immunohistochemically, ChM-I was strongly localized in stellate cells in the chondromyxoid stroma of pleomorphic adenoma and plasmacytoid cells floating in the myxoid stroma of myoepithelioma. ChM-I gene was also expressed in the tumor cells mentioned above. In pleomorphic adenoma cells of primary culture, ChM-I expression at both protein and mRNA levels was confirmed by in-situ hybridization and RT-PCR. In these cells, fibronectin ED-B region was also frequently spliced out. These results indicated that the stroma of pleomorphic adenoma contained anti-angiogenic factors which are released by the tumor cells. CT images of pleomorphic adenoma was characterized by more delayed and mottled enhancement which lasted longer. In contrast, myoepitheliomas, especially ones of spindle cell type showed quick and even enhancement and clearance. These characteristic CT images were explainable by their paucivascular stromal architectures which might be maintained by the parencymal biosynthesis of angiogenic suppressors.

ANALYSIS OF 72-kDa AND 92 -kDa GELATINASES ACTIVITY IN CHRONIC ADULT PERIODONTITIS

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Background: Periodontitis is characterized by extensive destruction of the gingival tissues and associated supporting structures of the teeth. Matrix metalloproteinases (MMPs), a hos cell-derived proteolytic enzyme family are believed to have an important role in the pathogenesis of periodontal diseases.

Methods: In 12 samples of inflamed periodontal tissues from adult periodontitis and 4 samples of periodontally healthy controls were obtained in our dental hospital. Tissue extracts were prepared and analyzed for 72-kDa gelatinases (MMP-2), 92-kDa gelatinases (MMP-9) activity by Gelatin Zymogrphy.

Results: Latent matrix metalloproteinase (pro-MMP-2) and (pro-MMP-9) were expressed both in inflamed periodontal tissues and in clinically healthy tissues, while active MMP-2 not active MMP-9 was detected only in tissues obtained from patients with adult periodontal diseases.

Conclusions: Our results revealed that these host-derived proteases were involved in the pathogenesis of chronic adult periodontitis. MMP-2 is a very important proteases and activated MMP-2 may contribute to tissue destruction in periodontal disease.

Key words: matrix metalloproteinase. Gelatin zymogrphy. periodontitis.

INTESTINAL T-CELL LYMPHOMA: A STUDY OF ITS NEOPLASTIC CELL LINEAGE AND RELATIONSHIP TO EPSTEIN-BARR VIRUS

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ABSTRACT

Background and Objective: Intestinal T-cell lymphoma(ITCL) has been known as a heterogeneous lymphoid neoplastic group, which is characterized with different clinicopathologic features. Except enteropathy-type intestinal T-cell lymphoma(ETCL), the other ITCL entities are still inconclusive. As to the etiology and pathogenesis of ITCL, there is higher frequency of Epstein-Barr virus(EBV) infection in the east than that in American and Europe, but it is still a matter of controversy whether EBV infection is an etiological event or a secondary one in the pathogenesis of ITCL. In this study, forty-two cases of ITCLs were studied for their clinicopathologic features, phenotypes, genotypes and the state of EBV latent infection, the subtypes of EBV, the route of EBV infection, the expression of EBV genome and its significance. Therefore, the aim of present study was to investigate the cell origin of ITCL, as well as to ascertain EBV status of the tumor and the role of EBV infection in the pathogenesis of ITCL.

Methods: Forty-two cases of ITCLs were classified according to the updated Keil classification. All clinical data were reviewed and all patients were followed-up. For immunohistochemical staining a panel of monoclonal and polyclonal antibodies were applied: T-cell makers(CD45RO, CD3ε, CD3, CD4, CD5, CD8), B-cell maker (CD20), histiocyte maker (CD68), NK-cell-associated antigen (CD56), cytotoxic granular protein(TIA-1), CD21, bcl-2, and the products of EBV genome(EBNA-2, LMP-1). In situ hybridization for EBER1/2 and polymerase chain reaction for antigen receptor(TCR-γ、IgH) gene rearrangement and for EBV nuclear antigen gene (EBNA-3C) of two EBV subtypes were performed. Part cases which were positive in both in situ hybridization and immunohistochemistry were detected by double staining. Amplifi-cation product of EBNA-3C gene was analyzed by DNA sequencing.

Results: Histologically, 42 ITCLs were classified into pleomorphic medium and large cell (n=38, 90.48%), monomorphic medium-sized (n=2, 4.76%), pleomorphic small cell (n=2, 4.76%). The lesions showed ucler appearances, with frequent angiocentricity and angioinvasion, associated with zonal necrosis. Features of lympho-epithelial lesion(LEL) and reactive histiocytosis with phago-cytosis were observed in most cases. Immunohistochemically, all 42 cases of ITCL revealed CD45RO positivity, in which 10 (23.81%) expressed CD8, 7 (16.67%) expressed CD4, 12 (28.57%) expressed CD56, and 39 (92.86%) expressed TIA-1. None expressed CD20 and CD68. Genetically, rearrangements of TCR-γ gene were demonstrated in 31 cases(73.81%), however none IgH gene rearrangement was detected. The immunophenotypes and

genotypes of 42 cases could be delineated into three categories: α/β or γ/δ T cell type(30, 71.43%), natural killer (NK) –like T cell type(11, 26.19%), and NK cell type(1, 2.38%). Most $CD56^+$ ITCLs were NK-like T-cell lymphomas, but one was NK-cell lymphoma. Clinically, most patients with ITCLs were young males with abdominal pain, hematochezia, fever and weightloss. Multiple lesions and spontaneous perforations were frequently present. Generally, $CD56^+$ ITCLs more frequently showed single lesion than $CD56^-$ ITCLs, the patients with $CD4^-$ ITCLs preferred fever than those with $CD4^+$ ITCLs, and there were more spontaneous perforations occurred in $CD8^+$ ITCLs than that in $CD8^-$ ITCLs. All types of ITCL have an extremely poor prognosis with a survival median of 3.0 months, which one year survival rate and two year survival rate are 30% and 22% respectively. The patients without TCR-γ gene rearrangements showed poorer prognosis than those with TCR-γ gene rearrangements($p<0.05$). None significant prognostic factor for ITCLs was determined.

On the whole, EBV infection were detected at DNA, mRNA, and expressive protein levels in 41 of the 42 cases(97.62%). 38 cases (90.48%) exhibited specific bands by polymerase chain reaction, in which 32(84.21%) were EBV type A, 2(5.26%) were type B and 4(10.53%) were mixtures of type A and B. There were seldom base-insertion and base-deletion in EBNA-3C gene. In 36(85.71%) cases, the tumor cells showed positive in EBER1/2-in situ hybridization, as well as were demonstrated to express CD45RO, CD4, CD8, CD56, or TIA-1 by in situ hybridization-immunohistochemistry double staining. The expression frequency of LMP-1 was 38.10%(16/42), revealing a direct correlation to CD56 expression

($p<0.05$). There was no valuable correlation between LMP-1 expression and prognosis. None case presented CD21, bcl-2 and EBNA-2 positive reaction. The biologic role of LMP-1 was not associated with bcl-2 expression. EBV infection in ITCLs would not be mediated via CD21. There were two patterns of EBV latent infection in ITCLs, the more common one was type I and the another was type II.

Conclusions: ITCLs are characterized with unusual clinico-pathological and immunohistochemical features in China. The immunophenotypes and genotypes of their neoplastic cells could be delineated into α/β or γ/δ T cell type, natural killer (NK) –like T cell type, and NK cell type. Most ITCLs are of different T-cell subpopulations or different T-cell differentiation stages, especially of cytotoxic T-cell lineage. A high level EBV infection , most of which are EBV type A, is presented in Chinese ITCLs. The patterns of EBV latent infection in ITCL are type I and type II . The special clinico-pathological features of ITCL would due to the cytotoxic function and the role EBV infection plays in the pathogenesis of ITCL. ITCL and nasal NK/T-cell lymphoma might belong to the same spectrum.

KEY WORDS Intestinal T-cell lymphoma; Epstein-Barr virus; clinicopathology; phenotype; gene rearrangement; latent infection

STUDY ON EXPRESSION OF TELOMERASE GENES AND APOPTOSIS RELATED GENES IN MAMMARY ATYPICAL DUCTAL HYPERPLASIA

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Objective: Expression of telomerase genes (hTR, hTRT) and apoptosis related genes (p53, bcl-2) was investigated in mammary atypical ductal hyperplasia. **Methods:** Expression of telomerase genes (hTR, hTRT) and apoptosis related genes (p53, bcl-2) by in situ hybridization and expression of the mutant p53 protein by immunohistochemistry were detected in 44 cases of patients with mammary atypical ductal hyperplasia and compared with that of the 6 benign hyperplasia and 26 carcinoma of breast. **Results:** Hyperexpression of telomerase genes in severe atypical ductal hyperplasia is of significant difference from that in mild-medium atypical ductal hyperplasia and breast cancer. The upgrading of atypia was in line with decreased expression of wild p53 mRNA and increased expression of the mutant p53 protein.As for bcl-2 mRNA, it shows moderate expression, especially in severe atypical ductal hyperplasia. **Conclusions:** Our study reveals significant correlation between expression of telomerase genes (hTR, hTRT) and the state of malignant transformation in mammary atypical ductal hyperplasia. Decreased expression of wild p53 gene, increased expression of mutant p53 protein and overexpression of bcl-2 gene were associated with telomerase.

Activation of extracellular signal-regulated kinase and phosophatidylinositol 3-kinase in human breast tumors

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Abstract

Objective To investigate the expression and activity of extracellular signal-regulated kinase (Erk, p42 MAP kinase) and phosophatidylinositol 3-kinase (PI3-kinase) in human breast tumors.

Methods Western blotting, immunoprecipitation and kinase activity assay were used in 37 cases of breast tumors.

Results Significantly increased expression of Erk and p85 subunit of PI3-kinase were found in all and 32 out of the 37 breast tumors compared with adjacent normal tissues (100% and 86.49%, respectively). Significantly higher level of the activity of Erk and PI3-kinase were observed in 28 and 25 out of the 37 cases (75.68% and 67.57%, respectively). There was no more correlation between the increase in expression and activity of Erk than in the activity of Erk and PI3-kinase.

Conclusions In this study breast tumors exhibited overexpression and enhanced activity of both Erk and PI3-kinase compared with adjacent normal tissues, raising the possibility that Erk and PI3-kinase may be potential targets for new strategies for the treatment of breast cancer.

Keywords: *breast neoplasms, p42 MAP kinase, 1-phosphatidylinositol 3-kinase, signal transduction*

The research of transforming growth factor's effect on the disease of prostate hypertrophy

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The natural or abnormal growth of human prostate is affected by male hormone which also lives on the prostate. Polypeptide growth factors have been found in prostate tissue, which shows that male hormone is not the only substance affecting the growth of prostate.

Recently research of the effect of growth genes has been conducted on the emergence and development of benign prostatia hyperplasia and prostate cancer. Though great progress has been made recently in the research concerning the affect of cell growth genes upon prostate diseases in benign prostatia hyperplasia. It is concluded that TGF-α is able to promote the breakin of prostate epidermis cells and basement membranes cells. Whereas TGF-β is proved to have restraining effect on the growth of prostate epidermis cells after removal of drugs in the aspect of cells breeding. Transmit program death of cells is not yet proved on human tissues.

The research is in an attempt to focus on the symbolizing of TGF-α and TGF-β base on human benign prostatia hyperplasia tissues, to further discuss the effect of TGF-α and TGF-β on human benign prostatia hyperplasia, and in the end to provide effective method to prevent and treat benign prostatia hyperplasia. Based on that and with the adoption of the world's current advanced method of research on benign prostatia hyperplasia, combined with research method of the traditional pathology and the modern immunological method, used with streptoly digin-peroxidase enzymatic tissue immunochemical method (SP) in 40 cases of BPH tissues and 5 cases of normal prostate to take TGF-α and TGF-β immunity orientation, half ration expression, this makes a further step to expatiate on the affect of the polypeptide growth factors in benign prostatia hyperplasia and tries to find effective ways to prevent and treat current growing disease of benign prostasia hyperplasia in the world.

On the slices, expanse staining of TGF-α of 40 BPH can be seen in the cytoplasm of antrum epithelium, whereas TGF-β is broadly stained in basement membrane and cytoplasm stained in cells. TGF-α and TGF-β showed no pigmentation in the epidermis of 5 normal prostate tissues, pigmentation only. The pigmentation intensity and area are much smaller than that in benign prostasia hyperplasia. Statistic analysis shows that the positive rate of expression of TGF-α in BPH tissue epidermis and it is

higher than normal prostate tissues ($P<0.01$). Besides the expression of TGF-α in BPH tissue epidermis is evidently higher than in it ($P<0.01$). The positive rate of expression of TGF-β in BPH tissue epidermis and it is higher than normal prostate tissues ($P<0.01$). And the expression of TGF-β in BPH tissue, it is evidently higher than that in epidermis tissues.

The above experiment shows that the excessive express of TGF- in epithelius and TGF-α in BPH sow discord pawn are most likely the key factors of BPH emergence and development. TGF-α has no effect on sow discord pawn cells but has evident stimulating effect on epithelius cells, which shows that it plays important role in epithelius benign prostatia hyperplasia. The strong positive staining rate of TGF-β in BPH tissues is obviously higher than prostate tissues. And the positive express of TGF-β in BPH is also obviously higher than BPH epithelius tissues, which shows that it likely takes part directly or indirectly in the breaking and proliferation. From the above, the effect of multi growth genes on prostate cells is quite complicated, and the unequilibrium caused upon benign prostatia hyperplasia cells by multi- growth factors maybe have some connection with the emergence of BPH.

Analysis of Genetic Heterogeneity in Prostate Cancer

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Background. Prostate cancer (PCa) is heterogeneous and multifocal, with diverse clinical and morphological manifestations. In addition, recent molecular studies have shown that there is a genetic heterogeneity. This is one of reasons why there has been a wide discrepancy in the reported detection frequency of p53 mutations in PCa. In the present study, we attempted to determine the genetic characterization among PCa foci in the same specimen using analysis of ras, p53 mutations and p16, glutathione S-transferase pi (GST π) methylation.

Methods. Three PCa specimens obtained from radical prostectomy were examined. Three specimens were shown as follows; the A sample (75 yrs, stage C) is a moderate differentiated adenocarcinoma, the B sample (64 yrs, stage C) a moderate , and the C sample (77 yrs, stage B2) a well. Each specimen was processed by the whole-mount technique. Using Laser-capture microdissection system, cancer foci were obtained from the frozen-70% ethanol fixed sections. DNA was extracted from each cancer focus according to the standard protocol. Mutations of the ras and p53 genes in each foci were analyzed by PCR-SSCP and sequencing analysis, methylation of the p16 and GST π analyzed by methylation-specific PCR (MSP).

Results. Three foci were microdaissected from the A sample, five foci from the B, and two foci from the C. In the A sample, A/1 and A/2 foci had a mutation of the p53 (CGC to CAC at codon 181) and methylation of the p16 and GST π while A/3 focus had no p53 mutation and p16 and GST π methylation. In the B sample, B/1, B/2 and B/3 foci had a mutation of the p53 (ATG to ATA at codon 169) and a methylation of the GST π while B/4 and B/5 had a methylation of the p16. Both of C/1 and C/2 foci had a mutation of the ras and a methylation of the p16.

Conclusions. Our findings indicate that there is genetic and epigenetic heterogeneity in human prostate cancer. Multiple foci of PCa appear to arise independently within the same prostate. It is necessary to examine multiple foci of PCa for genetic or epigenetic characterization.

INTERNATIONAL SYMPOSIUM OF MOLECULAR PATHOLOGY

THE STUDY OF CELL CYCLE REGULATORY RELEVANT GENES AND THE MECHANISMS OF CARCINOGENESIS IN BREAST CANCER

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In order to explore the effects of G_1-S cell cycle regulating proteins on mammary carcinogenesis and progression, cyclin D1, cyclin E, P16, P21 ^{waf1} and PRb expressions were detected immunohistochemically in 36 benign breast tissues, including 17 cases without epithelial hyperplasia and 19 with hyperplasia, and 59 breast carcinomas. Cyclin D1 DNA and mRNA expression were also detected by in situ hybridization in 25 breast carcinoma and 15 benign breast tissues. The results showed that no overexpression for cyclin D1 and Cyclin E was detected in duct epithelial without hyperplasia but one case with cyclin E overexpression. The positive rates of cyclin D1 and cyclin E in breast cancer were significantly higher than those in benign tissues ($P<0.05$). Cyclin D1 overexpression was related to its gene amplification and mRNA expression. There was a positive relation between cyclin D1 overexpression and lymph node involvement ($P<0.05$), and there was a trend for tumors larger than 5cm to overexpress cyclin E more frequently. Positive relation was evident between overexpression of cyclin D1 and cyclin E ($P<0.05$). The ratios between P16, P21 and cyclin D1, cyclin E contents decreased from normal breast epithelia, hyperplasia to carcinomas. Tumors with higher P21 content than cyclin D1 were smaller in size and had lower frequency in node involvement ($P<0.05$). P21 positivity was correlated with cyclin D1 overexpression ($P<0.01$), and there was a trend to more cyclin E overexpression with the increasing expression of P21. Strong expression of PRb was positively associated with cyclin D1 overexpression ($P<0.01$). The results suggested that cyclin D1 and cyclin E overexpressions occur mainly in early stage of breast carcinoma, which may play an important role in the transformation and development of breast cancer cooperating with genes of p16, p21^{waf1} and Rb.

Key Words Breast neoplasms; Oncogene; Antioncogene; Gene expression; Cell cycle; Immunohitochemistry; In situ hybridization

TELOMERASE ACTIVITY IN BLADDER CANCER WITH REFERENCE TO THEIR FEATURES

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Telomerase are the specific structure which stabilise the ends of eukaryotic chromosomes and prevent the loss of genetic information. In humans, They show a specific of 500-3000 repeats of TTAGGG. In malignant tumors, telomerase reactivation plays an important role in the acquisition of cellular immortality. But the molecular mechanism of the telomerase reactivation in human carcinogenesis has not been clarified. To evaluated the telomerase activity in bladder cancer ,We examined the tolemerase activity in exfoliated cells in urine of 74 patients who have a bladder cancer and 40 normal persons.

As a result, There were approximately 83% (62/74) showed telomerase activity which comparing with 49% (32/ 66) telomerase positive of patients who were examined their exfoliated cells in urine by clinicopathologic parameter. It wes significantly high. In histological grades ,there are 77% in G_1 ,83% in G_2 , 88%in G_3 telomerase positive respectively in exfoliated cells in urine. Whereas all 40 normal exfoliated cells in urine were no telomerase activity .

In this study, We found an obvious association bewteen telomerase activity and clinicopathologic features of bladder cancer. It is suggested that telomerase would be useful in the diagnosis and follow-up of bladder cancer. It is indicated that telomerase might be a potencial prognostic indicator for bladder cancer. In addition , this enzyme fulfills many of the cfiteria for an ideal cancer target. Since the molecular mechanism of telomerase reactivation is not understood, further studies need to be done.

EXPRESSION OF P53, C-erbB-2, EGFR PROTEINS IN BENIGN AND MALIGNANT PROSTATE AND THE RELATIONSHIP WITH CLINICALFEATURES

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Prostate cancer(Pca) and benign prostate hyperplasia(BPH) are the most common prostatic disorders. But their pathological mechanism is still unknown. This study undertaken to investigate the expression of p53,C-rebB-2,epidermal growth factor receptor(EGFR) in Pca and BPH by detecting their proteins. Formaline-fixed specimens of 67 cases of Pca, 85 cases of BPH and 8 cases of normal prostate were stained using immunohistochemical method to examine the expression of p53, C-erbB-2, EGFR. After observing the characteristics of the sections and comparing with the clinical features , It is concluded that:

1. The expression rate of p53 is 46..3% which located in nuclei. C-erbB-2 oncoprotein is located in cytoplasm and its expression rate is 41.8%. EGFR which is situated on cell memberans is 19.4 %positive. This suggested that the etiology of Pca have different mechanism and associated with multiple oncogenes.

2. It is noted that the expression of p53,C-erbB-2 oncoprotein varied significantly among different grades of Pca.

3. There is a significant relationship between coexpression of p53, C-erbB-2 and the survival period of Pca. With this finding, we can conclude that the p53 and C-erbB-2 oncoprotein is a good indicator of grading, staging and prognosis of Pca. However, No relationship was found between the EGFR expression and clinical characteristic of Pca.

4. There is seldom expression of p53, C-erbB-2 in normal prostate. the BPH is not a pre-Pca in our study.

Molecular Cytogenetic Changes in Soft Tissue Leiomyosarcoma

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ABSTRACT

Activation of oncogenes and loss of functions of tumor suppressor genes (TSG) resulting in instability of human genome is one of the mechanisms in tumorigenesis. Study genomic changes of specific tumor type is important to our further understanding on tumor origin, progression, biological behaviour as well as finding related abnormal genes.

Leiomyosarcona of soft tissue (LMS) is a relatively rare malignant tumor occurring predominantly in adults, Up to now, our knowledges on its pathogenesis, especially on cytogenetics and molecular genetics are limited for the difficulty of acquiring the research material. Previous cytogenetic studies revealed that leiomyosarcomas are a heterogeneous tumor entity which lack representative chromosomal aberrations, These studies were based on conventional cytogenetic karyotyping which needs metaphases of tumor cells for analyses through successful cell culture. The difficulty of acquiring high quality tumor cell metaphases impedes finding characteristic chromosomal abnormalities and analyzing

relationship between genetic changes and tumor clinical pathological features. Besides, the understanding on molecular biology of LMS are also limited, tumor suppressor genes p53, pRB and p16 were abnormal in only partial LMS, other genes related to LMS pathogenesis remain unknown.

Because of the reasons mentioned above, we chose LMS of soft tissue as the study material. Using comparative genomic hybridization (CGH) technique instead of G band karyotyping as well as loss of heterozygosity (LOH) analysis to study the molecular cytogenetic changes of LMS.

The experimental results were summarized as follows:

1.Chromosomal imbalances were detected in 30 cases of LMS by comparative genomic hybridization. Complex changes in DNA copy numbers involving various chromosomal regions were detected in 25 cases of LMS. A total of 201 gains and 151 losses were detected, The most frequent gains were detected in 1q, 5p, 8q(10 cases each, 33%) and 16p, 17p, 5q,15q(9 cases each, 30%) with the minimal involving regions of 1q31-32, 5p14-pter, 16p13-peter, 17p11-12 and 8q24-qter, implying these chromosomal regions may harbor oncogenes related to LMS pathogenesis, The most frequent losses were detected in 6p,10q(53% and 57%), 3p(47%), 22q(43%), 11q(23.3%) and 13q(20%), with the minimal involving regions of 6p21.2-22.3, 10q 22-23, 3p21-24 and 22q12-qter, implicating these chromosome regions may harbor tumor suppressor genes related to LMS tumorigenesis. Analyzing relation between copy number changes and histological gradings of LMS revealed that copy

number changes were significantly different among grade Ⅰ, Ⅱ and Ⅲ LMS ($P<0.05$), copy number increases in grade Ⅲ LMS were significantly higher than in grade Ⅰ LMS ($P<0.05$), whereas copy number losses among grade Ⅰ, Ⅱ and Ⅲ LMS had no significant differences ($P<0.05$), implying activation of oncogenes is related to LMS differentiation and malignant potential, whereas inactivation of tumor suppressor gene seems to be not related to LMS differentiation.

2. LOH status on chromosome 3p14.2-pter were studied in 22 cases of LMS by microsatellite DNA-polymerase chain reaction-silver staining technique, The results showed among 5 microsatellite loci on chromosome 3p, LOH occurred most frequently on D3s1295(36.8%) and D3s1289(10.5%), whereas D3S1293 had no LOH occurred in this group of LMS studied, 10 cases of LMS showed at least one loci occurring LOH(10/22,45.4%), 1 case showed LOH occurred on 2 loci of chromosome 3p, implying LOH on chromosome 3p14.2-23 region is relatively frequent, 3p21.1 region around D3s1295 and D3s1289 may harbor tumor suppressor gene related to LMS.

Taken together, chromsomal imbalances of LMS is complex and involving various chromosomal regions, implying multiple oncogenes activation and tumor suppressor genes inactivation involve in the pathogenesis and progression of LMS, 3p21.1 may harbor tumor suppressor genes related to LMS.

Key words Leiomyosarcoma Comparative genomic hybridization

A case of rich-cell neurilemoma easily made mistaken for maligant tumor

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Patient: Liang XX male: 40 year-old

Patient felt numb in left leg. Clinical diagmosis: occupying disease in vertebral canal. Operation field: Tumor developed in vertebral canal, dumbell shape with completed dura, few blood.

Gross: A grey irregular form, $4\times3\times2cm^3$, with membrane fragile. A few blood on section.

Histologhcally: Tumor tissue consist of shuttle cells arranged in whirlpool shape, with weak stained plasma and oval nuclei granular chroma some small nucleolus, cellular some nucleis were arranged in fence-shape, with some blank band cells scattered irregularly in reticular region. The nucleis can not be observed, the results of immunohistologichemisty indicate: S-100(++) Vimentin(++) Ki-67(++).

Pathological diagnosis: neuricemoma, rich-celltype.

Discussion: Although it is common, the size of nudeus is increase, malform ation, deep stain. So it's easily for us to make mistake for maligrant tumor.This kind of expression cells degeneration need to distinguish from leiomysma and neurofibroma. The leiomyomas have redcyto plausma have blunt and round hucleus and show knit shape under microscope. volope can be neurofibroma, waving Outlook cell body could be seen, the terminathon of nucleus are more acute, meanwhile immunohistologi chemistry present: S-100(++) Vimentin(++) Ki-67(++). This result supported the diagnosis of neurofibroma.

Clinicopathological and Immunohistochemical Study of 30 Cases of Rhabdomyosarcoma

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Object: Rhabdomyosarcoma (RMS) is too much common manlignant tumor of soft tissue. Its diaynosis and differential diaynosis with other sarcoma are stiil difficult to all of us. 30 cases of rhabdomyosarcoma are reported: Among 30 cases RMS. Male 17 cases. Female 13 cases,30 cases were studied immunohistochemically by S-P method.Specific antibodies against for myoglobin and desmin Was found in 71.8% and 56.4% of the Cases studied respectively. The positivity Was dependent on the degree of cell differentiated, Results Suggest that immunohistochemistry is a useful tool for the diagnosis of poorly differentiated rhabdomyosarcomas.Cross-striations Were found in only 6 of the thirty Case (20%). It is now generally accepted that demonstration of cross-striations is not essential for the diagnosis, neverthelessness, the characteristic features of fibrillary material arranged in whorls around the nucleus are of diagnostic significance. Histologically, it is also believed that searching for early differentiated rhabdomyoblasts Combined with the hostological pattern is of vital importance for an accurate diagnosis.

References:

①Enzinger FM. Weiss SW. Soft tissue tumours st Lowis:CV mosby CO 1983:338-370.

②Kahn HJ, et al. Immunchistochemical and electromicroscopic assessment of childhood rhabdomyosarcoma;increased fregehcy of diagnostic over routine hislological methods cancer 1983;51:1897.

③Altmannsberger M, et al. Desmin is a specific marker for rhabdomyosarrcoma of human and rat origin, Am J Jathol 1985, 118:85.

The correlation between expression of oncogene protein products p53, p21, p185 and cell differentiation and prognosis in rhabdomyosarcoma

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Abstract Objective To study the correlation between expression of oncogene protein products p53, p21, p185 and histological type, cell differentiation and prognosis in rhabdomyosarcoma (RMS).

Methods 41 RMS cases which had follow-up material were selected for this study. Expression of protein products of oncogene p53 ,p21and p185 were synchronously detected and compared by immunohistochemical ABC method.

Results The positive rates for p53, p21 ras and p185 c-erbB-2 were 72%, 68% and 60% respectively. Positive expression did not relate to age, sex or RMS histological type, but related to the degree of RMS differentiation. The positive rate of p53 ad p21 ras in well differentiated cases were 42.9% and 28.6% while that of the poorly differentiated group was 85% and 80% respectively ($P<0.05$). The positive rate of p53 in the RMS group with metastasis was 86.6%, significantly higher than that of the non-metastasized group, which was 66.7% ($P<0.05$). There was a significant difference between those with one year survival, whose p52 positive rate was 86.7% and those who survived for more than 3 years, whose p53 positive rate was 47.1% ($P<0.05$).

Conclusion The results suggest that the irregular expressions of p53 and p21 were related to tumor differentiation and the degree of malignancy. p53 positivity may indicate a poor prognosis.

Key words Rhabdomyosarcoma Prognosis
Oncogene products

MACROPHAGE SCAVENGER RECEPTOR (CD204), A NEW DIFFERENTIATION MARKER FOR MACROPHAGES

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Introduction

Macrophage scavenger receptor (MSR) is one of the major receptors of macrophages. MSR plays an important role in the pathological deposition of cholesterol during atherogenesis through receptor-mediated uptake of modified low density lipoproteins. It is also important for macrophages to recognize and eliminate pathogenic microorganisms. Since MSR molecule is highly conserved at the amino acid level among animal species, it has been difficult to elicit strong immune responses in recipient animals to generate high affinity antibodies. To avoid such immunological tolerance, we utilized MSR-knockout mice as an immunization animal.

Production of Monoclonal Antibodies against Human MSR

Five monoclonal antibodies (MH1, SRA-C6, SRA-D10, SRA-E5, SRA-F8) were generated by immunizing MSR-knockout mice with recombinant human type I MSR protein (amino acid residues 156-450) as immunogen [1-2]. These antibodies were confirmed to be specific for MSR protein by Western blot analysis. Immunoglobulin subclass of all antibodies was IgG1, kappa.

Tissue Distribution of Human MSR

Immunohistochemistry using these monoclonal antibodies disclosed the distribution of MSR-positive cells in various organs and tissues [1-2]. All antibodies recognized tissue macrophages such as alveolar macrophages, Kupffer cells of the liver, splenic red pulp macrophages, sinus macrophages in lymph nodes, and interstitial macrophages in various organs. Perivascular macrophages in brain (Mato cells) were also positive for these antibodies. In atherosclerotic plaques, macrophage-derived foam cells were clearly stained by these antibodies. Freshly isolated blood monocytes were negative; however, they became positive for these antibodies after 3 days in culture. Dendritic cells such as interdigitating cells of lymphoid tissues and epidermal Langerhans cells were invariably negative.

Reactivity in Animal Tissues

To clarify whether any of the antibodies recognize animal MSR molecules, we performed immunohistochemical staining of the livers, spleens, and/or lymph nodes from various animal species [2]. Each antibody showed a unique reaction pattern in the animal tissues. SRA-C6 showed the narrowest interspecies reactivity; reaction was restricted to humans and cats. Three antibodies recognized macrophages in monkeys, cows, horses, beagles, and cats. SRA-E5 recognized macrophages in all animal species examined including monkeys, cows, horses, beagles, cats, rabbits, guinea pigs, hamsters, rats and mice. In similar manner as in human tissues, reactions were restricted in macrophages and macrophage-related cells in most animals except for hamsters, rats, and mice. In these animals, hepatic sinusoidal endothelial cells were also positive as well as tissue macrophages.

Conclusion

Five monoclonal antibodies were generated by immunizing MSR-knockout mice with recombinant human type I MSR protein. All antibodies recognized tissue macrophages, but not freshly isolated blood monocytes. Each antibody showed a unique reaction pattern against animal species. Especially, SRA-E5 recognized MSR molecules of various animal species including rats and mice. From these observation, these antibodies are considered to be useful not only for studying the role of MSRs but also for analyzing macrophage differentiation in human and animals. Through these evaluations, MSR has been assigned to be a new CD molecule, CD204, at 7th Workshop and Conference on Human Leucocyte Differentiation Antigens.

References

1. Honda M, Akiyama H, Yamada Y, Kondo H, Kawabe Y, Takeya M, Takahashi K, Suzuki H, Doi T, Sakamoto A, Ookawara S, Mato M, Gough PJ, Greaves DR, Gordon S, Kodama T, Matsushita M. Immunohistochemical evidence for a macrophage scavenger receptor in Mato cells and reactive microglia of ischemia and Alzheimer's disease. Biochemical and Biophysical Research Communications 245: 734-740 (1998).

2. Tomokiyo R, Jinnouchi K, Honda M, Wada Y, Hanada N, Hiraoka T, Suzuki H, Kodama T, Takahashi K, Takeya, M. Production, characterization, and interspecies reactivities of monoclonal antibodies against human class A scavenger receptors. Atherosclerosis, in press (2001).

Diagnosis of Emerging and Re-emerging Infectious Diseases

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Emerging and re-emerging infectious diseases become worldwide problems to be solved appropriately. It is easily assumed that pathologists have a chance to make an appropriate diagnosis and judgment of such cases. Immunohistochemistry and *in situ* hybridization technique can be powerful tools for the solution of the diagnostic requests.

Here I would present several informative cases of emerging and re-emerging infectious diseases, in which histochemical approach was useful in making histopathological diagnosis.

1) Cryptosporidiosis: A young boy had a close contact with a cow in Hokkaido during summer vacation. A week later, severe diarrhea occurred, and endoscopic biopsy was performed from the colon and terminal ileum. In formalin-fixed paraffin sections, a few basophilic and PAS-positive small particles are seen along the brush border of the small intestinal epithelium. Indirect immunoperoxidase staining using a 1:1000 diluted serum from a patient suffering from known *Cryptosporidium parvum*-induced diarrhea showed positive signals in the particulated pathogens. Immunostaining using a monoclonal antibody also confirmed the diagnosis of zoonotic cryptosporidiosis.

2) Viscral leishmaniasis: A 30 year-old Japanese man who had stayed in India and Australia for years complained of persistent high fever, anemia and liver dysfunction. The liver biopsy disclosed microgranulomas without caseous necrosis. The diagnosis was also suggested by indirect immunoperoxidase staining using the diluted patient's own serum to show positively labeled small particles in the cytoplasm of epithelioid macrophages or Kupffer cells. Elevation of the serum antibody titer against *Leishmania donovanii*, endemic in the Indian continent, was demonstrated *in vitro*.

3) Acanthoamebic encephalitis: A 67-year-old HIV-negative man suffering from liver cirrhosis suddenly complained of left hemiparesis and rapidly progressive consciousness disturbance. CT scan demonstrated marked brain swelling, and brain biopsy was performed during decompression surgery. Macrophage-like organisms having round nuclei with centrally clustered

chromatin were observed around small vessels, and cyst-like thick-walled round bodies were intermingled with inflammatory and glial reaction. Again, the patient's own serum diluted at 1:500 clearly labeled the pathogens. Opportunistic infection by *Acanthoamoeba culbertsoni* was confirmed by heat-retrieved immunoperoxidase staining using a panel of mouse antisera.

4) Diphtherial laryngitis: A 76 year-old man suddenly suffered from severe and fatal dyspnea. In an emergency room, a laryngeal crust was obtained during intubation. Numbers of Gram-positive rods were seen in the thick membranous material covering the intact squamous mucosa. Non-flaggelated rods with a unique thickening at the end of bacteria were observed ultrastructurally. Immunostaining for diphtherial toxin and PCR identification confirmed the diagnosis of lethal diphtheria.

5) Enterohemorrhagic *E. coli* (EHEC) infection (O-157, H7): A 20-year-old man complained of hematochezia and severe abdominal pain. Emergency ileocecal resection was performed. Histology showed severe, multifocal hemorrhagic necrosis of the colonic mucosa with attachment of Gram-negative rods on the eroded surface. Immunostaining using DAKO's antiserum against *E. coli* revealed dense bacterial infection at the site of mucosal damage. EHEC, O-157, H7, was cultured from the feces.

6) Methicillin-resistant *Staphylococcus aureus* (MRSA) septicemia: A 70-year-old man who had undergone aortic replacement surgery complained of high fever and dyspnea. His general condition was rapidly deteriorated. The autopsy confirmed systemic dissemination of microabscesses and formation of multifocal cavitating pneumonia, in which Gram-positive cocci were densely colonized. Heat-retrieved immunoperoxidase staining using monoclonal antibodies against Penicillin-binding protein 2' indicated the systemic infection of MRSA. Microbial culture study also confirmed the hospital-acquired infection of MRSA.

Pathologists must be aware of the sociomedical importance of the prompt diagnosis of infectious diseases, in order not only for appropriately treating the patients, but also for preventing unnecessary transmission of contagious pathogens in the human society and in the hospital. Histologic morphology is full of information, when suitably combined with the clinical and laboratory studies.

Differentiation between malignant mesothelioma and metastatic carcinoma cells in human serous cavity effusion by monoclonal antibodies

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Diagnosis scope of exfoliative cytology of serous lacuma effusion includes the exfoliative cells from effusions of pleural, pericardial and peritoneal cavities. The overwhelming majority of tumor cells in effusion specimens comes from the creep and metastasis of malignant tumors. The chief task of effusion cytological diagnosis is to distinguish malignant mesothelioma from metastatic carcinoma, especially from adenocarcinoma and to determine the primary locus of them that the primary locus is unknown. This article summarized currently 40 cases of malignant effusion from pleural and peritoned cavities that were collected by the pathological diagnostic center of the First Affiliated Hospital of China Medical University. The method of the expression of monoclone antibody was used to distinguish malignant mesotheliom from metastatic carcinoma.

Materials and methods: The samples of malignant effusion from effusions of pleural cavity were 19 cases and those from peritoned cavity were 21 cases. Monoclone antibodies used were CK(L), CEA, MC, CR(reagents were purchased from Fuzhou Maixim Biological Technology Development Company, China).

Results: On the basis of high-grade suspicious malignant tumor cells selected by light microscope, the stain of monoclone antibodies was used to confirm the clinical diagnosis: There were 5 cases malignant mesothelioma in 19 case effusions from pleura cavity. Their CK(L) expressions were strong positive, MC ones were positive, CK ones were weak positive and CEA ones were negative. 1 case was the breast carcinoma, and its CK(L) expression was weak positive, MC one was negative, CR one was weak positive, CEA one was strong positive. 9 cases were lung adeocarcinoma: CK(L) expressions were positive, MC ones were negative, CEA ones were strong positive , CK ones were negative. 4 cases lung squamous cell carcinoma: CK(L) expressions were positive, MC, CR and CEA ones were all negative. There were 15 cases intensinal carcinoma, 1 case malignant mesothelioma, 3 cases adenocarcinoma of stomach, 1 case pancreas carcinoma and 1 case overy carcinoma. The expression of monoclone antibodies was the same to those of malignant tumor cells from pleura cavity. The materials mentioned above were mostly confirmed by paraffin section.

Discussion: Significance was indicated by practice that the monoclone antibodies could be used to distinguish malignant mesothelioma from metastatic carcinoma. But the premise was under the condition of papanicolaoustain method and after the high-grade suspicious malignant tumor cell selected by light microscope. Then the immunohistochemistry method could be used. It was benefit to determine the classification of malignant tumor cells.

It was also more important for metastatic adenocarcinoma especially papillary adenocarcinoma that cytokeratin(CK) epithelial membrane antigen(EMA) and protein associated basement membrane were positive expression CEA was negative expression in malignant mesothelioma.

Gene mapping of IgG hypergammaglobulinemia and sequence analysis in SLE model-New Zealand mice

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Systemic lupus erythematosus (SLE) is a multigenic autoimmune disease associated with IgG hypergammaglobulinemia, IgG anti-DNA antibodies and immune complex-type glomerulonephritis. F_1 hybrid between New Zealand Black (NZB) and New Zealand White (NZW) mice were a spontaneous SLE model which were used widely at present. In order to map the susceptibility allele of IgG hypergammaglobulinemia derived NZB and detect the effects upon the production of IgG antibodies, We set up the (NZB×NZW)F_1 ×NZW backcross mice model and used polymorphism microsatellite markers, quantitative trait locus (QTL) analysis, nucleotide sequence analysis, RT-PCR, flow cytometry analysis and enzyme linked immunosorbent assay (ELISA).

Experimental results: (1)Susceptibility allele of IgG hypergammaglobulinemia for SLE in (NZB×NZW)F_1 mice was localized the telomeric region on NZB chromosome 1 which is linked to Fcgr2b gene according to the QTL analysis. (2)Sequence analysis revealed for the first time that NZB had two deletion sites in promotor region including transcription factor-binding consensus sequences, i.e.AP-4-binding site and S-box. (3) We examined levels of Fo γ RⅡB_1 expression on splenic germinal center B cells from NZB, NZW and NZB/W F1 mice using flow cytometric analysis with a monoclonal anti-Fc γ RⅡ antibodies and found that expression of Fc γ RⅡB_1 was lower in NZB

and NZB/W F_1 than found in NZW. Under the condition of immunzing NZB and NZW mice with foreign antigens, the expression of Fc γ R II B_1 was also lower in NZB than in NZW. (4)RT-PCR analysis showed that there was a lower expression in Fcgr2b mRNA of NZB splenic germinal center B cells than in normal Balb/c mice.(5) ELISA and analysis of variance were used to determine the combinatorial effects of Fcgr2b allele and H-2 complex (MHC) in IgG anti-DNA atibodies production, the results indicated that the high IgG anti-DNA antibody titers seen in NZB/W F_1 mice are regulated by a combinatorial effect of $H\text{-}2^{d/z}$ and the NZB Fcgr2b allele.

According to all results, we concluded that high level of serum IgG in NZB/W F_1 mice with spontaneous SLE is controled by Fcgr2b allele, with IgG anti-DNA antibodies are regulated by Fcgr2b allele and H-2 comples. We speculated that B cell hypereactivity associated with IgG hypergam-maglobulinemia observed in SLE would be related to dysfunction of Fc γ R II B_1 induced by polymorphism of NZB Fcgr2b promotor region. The abnormal of NZB Fcgr2b allele may play a crucial role in the development of SLE.

SCREECING OF GENES WHICH INTERACTION WITH NEUCLEOCAPSIDE PROTEIN OF HANTAVIRUS IN VERO E6 CELLS WITH YEAST TWO-HYBRID SYSTEM

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The neucleaocapsid protein of hatavirus is encoded by S gene, which might be associated with viral uncoating , assembly ,budding ,and release. The virus replicate in vero E6 Cell after Hantanvirus infection, and cytopathogenic effect did not appear. It suggested that interaction between virus and host cell did not kill cells, virus replication may be completed by using some composition of host cells. For the purpose of study structure and function of S gene and N protein of hantavirus, and exploring the interaction mechanism of hantavirus and host cell, in this paper, we screened the host protein which have assisting affect on N protein. by yeast two-hybrid system.

The gene bank of vero E6 cell DNA was constructed by PCR method. Vero E6 cell gene was inserted into activation site of the PGBT9 plasmid as prey plasmid; and S gene of Hantavirus was inserted into activation site containing report gene plasmid (PATC2) as bait plasmid .the two plasmid were transfer into the yeast strain, and selected colonis containing either prey pasmid (PATC2) and bait plasmid (PGBT9) on selective medium. and the pGBT9 plasmid of contain N protein was determined according β -galactosidase activity and VroE6 gene fragment was amplificated with specific primer .Cloning strain was classified according to the length of inserted fragment and restriction endonuclease map and was Sequenced. Related sequence was compared and analysis using gene bank and searched homology sequence. Inferring the function of related proteins.

1.material and method

1.1Construction of bait plasmid containing S gene

The Sotkamozhu strain S gene of Puumala virus was obtained by RT-PCR method, and ligated S gene into CAL4DNA combined region of PGBT9 plasmid, and then transform them into yeast HF7C strain.37^{0}Ccultuer for 6 day, on tryptophan selective medium, Screening None color colonis that contain inserted sequencing of S gene.

1.2 Construction of prey plasmid containing random gene bank of vero E6 cell.

Extrated and purified mRNA with poly(A) from vero E6 cell. The double DNA were synthesized by cDNA synthesized reagent box of random primer. The match oligpolymer of EcoRI and XhoI ligated respectively into 3`end and 5`end of double cDNA, and then insert cDNA gene bank(2×10^7 random alone clones) into Cal4 activation site of PACT2 prey plasmid .

1. 3 Interaction of yeast two-hybrid system

The prey plasmid (PACT2) were transformed into PGBT9N strain, transforming clones were screened on selective medium. The HF7c yeast clone can live and reproduce only when either protein expressed by plasmid PACT2 and PGBT9 specific recombined. β -lactorase activation were detected with colony lift assay (CLONTECH LABORATORY) for ensuring screened clones contain PGBT9 plasmid indeed. Purify plasmid from HIS$^+$/ β -GAL$^+$ clone, and then retransformed Into HF7c strain.

1.4 Analysis of selected plasmid

Plasmid were Extracted from positive clones, and vero E6gene sequence inserting on PACT2 plasmid were amplificated by PCR method with specific primer, and then the length of PCR products and restricted endonuclease map were compared by argrose gel electeophoresis.

Clone strains were grouped according to length of inserted fragment and endonuclease map. The three clones were selected random from each group and were analysis by autosequencer. The known related sequences were researched from gene bank and compared.

2 Results

2.1 grouping according to length of insert fragment and restricted endonuclease map

All 68 strains were divided to 8 group (A-H) according to length of insert fragment on PACT2 carrier. Length of insert fragments is between 0.4-2.3Kb.the clone number of each group is from 2 to 21

Table 1 grouping of clones containing prey plasmid according to length of insert fragment

Group	A	B	C	D	E	F	G	H
Length (kb)	4	0.53	0.81	1	1.2	1.4	1.8	2.3
Number of clone	8	16	14	3	21	4	3	2

The PCR products of each clone was digested with EcoRI and HaeIII respectively, comparing endonuclease map after argrose gel electrophoresis. all 68 clones can be divided into 11 group ,except group B and E The same length of insert sequence, whose endoneuclease map also Identical . group B further divided into B1 and B2, group E divided into E1, E2 and E3.

Table 2 grouping of group B and group E according to endonuclease map

group	B1	B2	E1	E2	E3
Length of EcoRI digested (bp)	530	530	500, 700	300, 900	1200
Length of Hae III digesteve(bp)	230, 300	530	100, 300, 800	400, 800	500, 700
Number of clones	15	1	18	2	1

1.2 Comparing of sequences analysis

Table 3.The homology of amino acid sequences encoded by inserted sequence compared with known sequence of gene bank

Known matched protein in related Gene Bank	group (number of clones)	Homologous (%)	Percentage in total clones
Ubiquitin -like protein	B1 (15)	99%	73.5%
	C (14)	100%	
	E1 (18)	99%	
	G (3)	99%	
Mouse protein kinase	A (8)	72.3	16.2%
	D (3)		

The three clones of each group were sequenced for determine common conserve sequence of each group. Common conserve sequence of every group were degenerated into amino acid sequences and compared with Known gene sequences of gene bank. Result showed that 73.5% selected plasmids which specifically bind to N protein contain the insert fragment which is genetically homogeneous to Ubiquitin protein, homology is more then 99%; 16% of selected clones, contain the inserting which is relevant to a mouse protein kinase, homology is about 72%. About 10%clone contain different sequence respectively. But it is insignificant in statistics.

3.Discussion

Ubiquitin have been found in HIV-1 by highly purifing from lymphocyte and inside of lukemia virus (ALV) and retrovirus C. It has been defined that ubiquitin covalence combined with Gag protein of HIV, this covalence combined protein mimesis ubiquitinized cell protein administrating viral assembly.

N protein of hantavirus is protein associated with viral assembly. Our result showed that ubiquitin has liability of interaction with N protein of hantavirus, and suggested that this interaction might be correlative with viral assembly. That is hantavirus might undertake assembly function by combined N protein and utilized ubiquitin. Whether or not they covalence combined with Gag protein of HIV as ubiquitin would be studied further.

Ubiquitin decorate play many function in cell. This most general function is that the combination of poly-ubiquitin chain with arm protein is marker of degradation. And it also is necessary stage for pinocytosis process of cytoplasmic membrane receptor and activation of IKBa proteinase complex. Histone 2A and 2B are main protein combined only one ubiquitin. they are expression Relative transcription activation. In addition, some skeleton proteins also combined single ubiquitin molecular. Virus needs skeleton protein of host cell in the process of assembling and budding. From the result we can think that N protein of hantavirus might play the function of assembling and budding by combining skeleton protein of ubiquitinized cell.

This experiment showed that N protein of hantavirus might have certain affinity with some protein kinase of vero E6 cell, It suggested that N protein might participate in some reaction of enzyme chain, and cause relative signal conduction, and active host protein related viral production, or make beneficial environment for virus replication.

THE EFFECT OF NERVE GROWTH FACTOR IN THE REGULATION OF NEUROPEPTIDES IN THE PATHOGENESIS OF THE ASTHMATIC GUINEA PIG

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We confirmed previously that substance P (SP), neurokinin A (NKA) and calcitonin gene related peptide (CGRP) were involved in the pathogenesis of the experimental asthma. And it was also proved that nerve growth factor (NGF) could promote the production of SP, NKA and CGRP in the cells of the dorsal root ganglia. However, it was still not known if NGF was involved in the regulation of SP, NKA and CGRP in the asthma. The 48 guinea pigs were divided into 4 groups: ① normal group, without any treatment; ② control group, injected with 10mg of 1% albumin in normal saline into abdominal cavity, a week later inspired with the instilled normal saline into the respiratory tract; ③ asthmatic group, same as the control group except the inspiration of the instilled 1% albumin in normal saline; ④ asthmatic group with application of NGF antibody, 50 μl of NGF antibody (1:400) was inspired into the nasal cavity before the induction of the asthma. The animals were decapitated and the blood, trachea, bronchus and the lung were taken out and processed for radioimmunoassay. The results showed that the levels of SP, NKA and CGRP in the blood, trachea, bronchus and lung of the asthmatic group were much higher than those in the normal control and control group ($P<0.01$), and the levels of SP, NKA and CGRP in the blood, trachea, bronchus and the lung in ④ group were decreased compared with the asthmatic group. Conclusion: NGF might be involved in the regulation of SP, NKA and CGRP in the pathogenesis of the experimental asthma.

FASTING LIMITS NOREPINEPHRINE RELEASE ON REPERFUSION FOLLOWING ISCHEMIA: A MECHANISM OF REDUCED INJURY?

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Introduction: Fasting for 24 hrs has been shown to limit injury due to global ischemia and reperfusion. Fasting has also been shown to blunt some physiologic responses to catecholamines and reduce norepinephrine(NE) turnover rates. Since NE release has been associated with ischemia/ reperfusion(I/R) injury, we hypothesized that fasting would protect the heart from I/R injury by blunting the hemodynamic response to catecholamine stimulation and limiting NE release on reperfusion. Methods: Isolated rat hearts were exposed to graded concentrations($10^{-10}$to 10^{-6}) of both NE and isoproterenol(ISO) with measurement of heart rate(HR) and developed pressure (LVDP). Tissue NE concentrations prior to ischemia and effluent NE release on reperfusion were measured by HPLC. Results: Fasted animals had blunted hemodynamic sensitivity to ISO. The dose-response curve of ISO, but not NE, was shifted to the right significantly in fasted hearts (EC_{50}7.74vs 8.29).While tissue stores of both NE and epinephrine were unchanged by fasting, the release of NE on reperfusion after 20 min of ischemia was markedly reduced by fasting (0.068 vs 0.234 μ M, $P<0.05$). Conclusions: fasting reduces NE release following I/R despite unchanged tissue stores. Thus, the lower effluent concentration of NE is likely due to an active mechanism inhibiting NE release during ischemia and reperfusion.

Key words:Norepinephrine.Fasting.ischemia

Experiment Study For The Effect of Qing Shen Tiao Zhi Tablet on MDA and SOD in Hyperlipoidemia Animal

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ABSTRACT **Objective:** Our purpose was to study the effect of qing shen tiao zhi tablet (qing) on malondialdehyde (MDA) and superoxide dismutase (SOD) in hyperlipoidemia animal. **Methods:** thirty-five wistar rats were randomly divided into 4 groups, **I:** normal control group; **II:** hyperlipoidemia model group; **III:** small dose prevention and cure guoup; **IV:** high dose therapy group. Blood lipid were detected by auto biochemical analysis instrument; MDA by thiobarbituric acid (TBA) reaction; SOD by the method of adrenaline. **Results:** on 20 th day after rats were given hyperlipoid feed, the blood ChoL and MDA in group **II** was higher than in normal control ($P<0.01$). Blood MDA could be decreased by small dose prevention and cure ($P<0.05$), blood lipoid was decreased slowly, and was recovered into normal level until the experiment ended. On 55 th day of the experiment, MDA of liver in group **II** was still higher than in control ($P<0.05$). SOD did not change during all course of the experiment. **Conclusion:** Taking small dose of Qing and hyperlipoid feed at the same time can prevent and cure the rising of blood lipid, inhibit the increasing of MDA in plasma and liver. High dose of Qing can decrease blood lipid and MDA in liver. No effect was found in small or high dose group on SOD of red blood cell and liver in the experiment.

KEY WORDS hyperlipoidemia; malondialdehyde; superoxide dismutase

The Protect Effect of K^+ Channel Openers On Ischemic Brain Injury in Rat

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ABSTRCT **Aim :** To investigate the of K^+ channel openersin ischemic brain injury. **Methods:** K^+ channel openers were administered intraventricularly 30 min before the occlusion of right middle cerebral artery, the ischemic damage of brain was measured by histopathological examination. **Results:** There was a significantly higher MDA concentration ($P<0.05$), a significantly lower SOD concentration ($p<0.05$), and water content ($P<0.05$) 4 h after the occlusion in animals receiving K^+ channel openers. Electronic microscopy study showed a fewer mitochondria degeneration ($P<0.01$) on day 3 in animals receiving. K^+ channel openers. **Conclusion:** K^+ channel openers can prevent neurological damage from ischemic brain injury.

KEY WORDS K^+ chnnel openers; superoxide dismutase (SOD); malondialdehyde (MDA)

INVESTIGATION OF THE MELTING CURVE OF IMMOBILIZED OLIGONUCLEOTIDE

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Abstract: In this paper, we investigated the melting curve of perfect and single center base mismatchedimmobilized 20-mer DNA duplex by collecting the fluorescence intensity image at different temperature. Solution phase melting studies were performed by measuring the absorption hypochromism of the same sequences. The decrease in melting temperature was attributed to the lower local ionic strength at the solidliquid interface .

Keywords: Hybridization, Melting curve, Melting temperature, Confocal microscope

1. Introduction

DNA hybridization with immobilized probes on the glass is finding increasingly applications in a varietyof areas, including single nucleotide polymophisms (SNPs) detection. sequencing by hybridization (SBH) andmore recently DNA computing (1 -3). Thermodynamic investigation of solid-phase hybridization is importantin these applications but little published data are available.(4,5) Unlike the homogeneous liquid phasehybridization, heterogeneous solid phase hybridization of immobilized oligonucleotides was affect by the bulkdiffusion, surface diffusion, dielectric constant and local ionic strength. Melting temperature is often used as anindicator of the thermal stability.

2. Experimental

Perfect and one base mismatched probe NH2-5'-AG GAG GCT AG(C/A/T)T TCT CTC AGG-3' andfluorescence labeled target FAM-5'-CC TGA GAG ACT TAG CCT CCT-3' used in this study were synthesized and purified by Tacoro (Dalian. China). This sequence was carefully chosen to minimize thechance of intra- and intermolecular secondary structure. Hybridization buffer HyperHybTM is purchased fromResearch Genetics. All other reagents were of analytical grade.

Melting experiments in solution(6) were performed using Shimadzu 2100 UV-VIS spetrophotometer.Absorbance versus temperature was measured at 260nm every l C from 26 to 90 "C. The heating ratewas 0.5 'C/min. The concentration of each unlabelled strand was 1 u M. The melting profiles were smoothedwith 7 point Savitzky-Golay Filter and normalized.

The probe array was spotted on the glutaraldehyde-activated glass slide by Pix3500 System (CartesianTech.). The 4X4-array layout was illustrated in Fig 2-f.

All experiments of immobilized oligonucleotide were performed in real time on an experimental setup(Fig.l) consisting of Leica TCN SP confocal microscope, 25 u L hybridization chamber and a water bathcontrolling the temperature of the hybridization chamber.

Melting experiment was carried out with the temperature increasing from 25'C to 69"C at a rate of0.5 'C /min. The nuorescence images collected e\rery 2'C by confocal microscope were the average of fourscans. The images were processed and the intensity data were extracted by Leica Qwin sofiware.

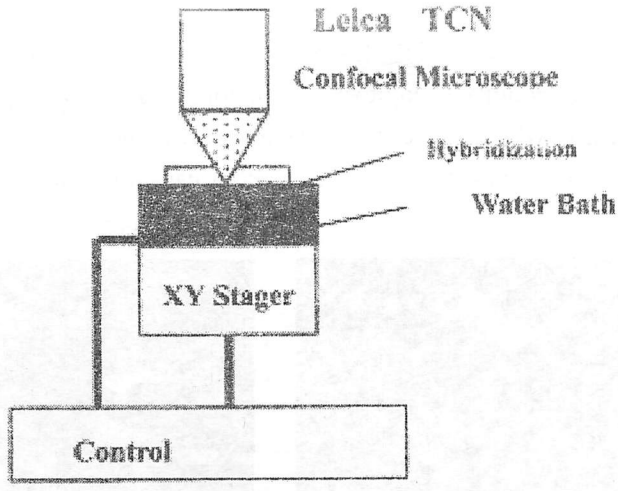


Fig.1. Experiment Setup

3. Result and Discussion

Fig.2 displays the normalized melting curves of perfect and one base mismatched duplex in solution.

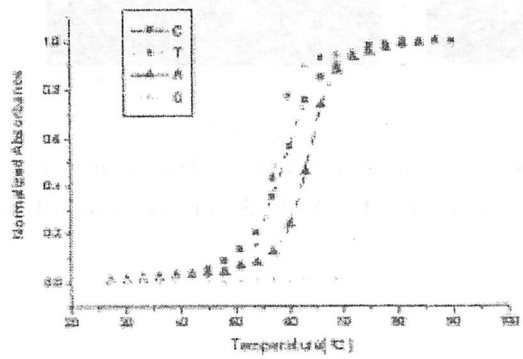


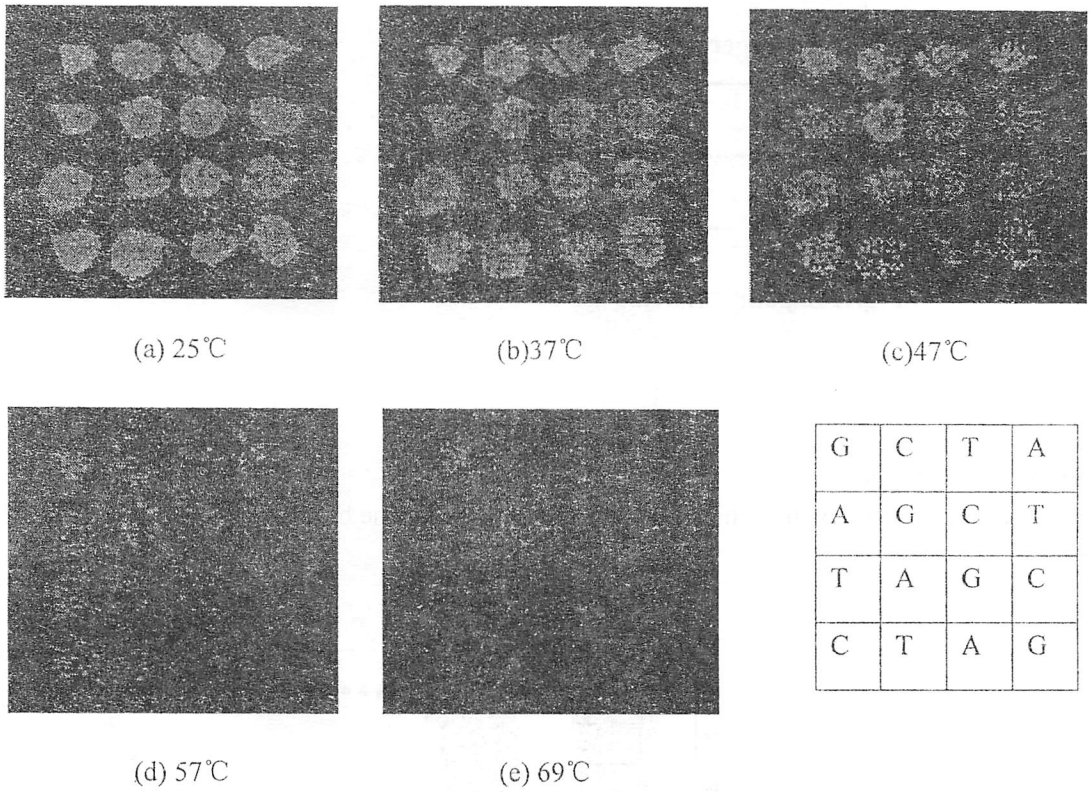
Fig. 2. Normalized absorbance of perfect and one base mismatched duplex in solution

Fig. 3 shows the 1mm X 1mm probe array with cells of about 200 u m diameter at different temperature as indicated. It can be seen from the images the fluorescence from bulk solution was apparently rejected by the confocal system.

Fluorescence intensity versus temperature was plotted in Fig. 4.

The melting temperature of perfect and one base mismatched duplexes, both in solution and on chip were summarized in Table 1 . The observed decrease in melting temperature of duplex on chip may be attributed to following two aspects. Firstly, stability of the duplex is strongly dependent on the local ionic strength. The shift of the melting temperature may reflect the lower ionic strength in the solid interface comparing with that in solution. The low ionic strength was

induced by the low dielectric constant of the thin siloxane layer to which the probes were covalently attached. Secondly, the immobilized single-strand oligonucleotide with phosphate backbones carring a high charge density will also alter the dielectric environment and the melting behavior. Using the uncharged probe or/and target species such as PNA can carry out further study of this effect.



(a) 25℃ (b)37℃ (c)47℃

(d) 57℃ (e) 69℃

G	C	T	A
A	G	C	T
T	A	G	C
C	T	A	G

Fig. 3. (a-e) Fluorescence Image at Different Tempenure. (f) Probe Layout. NH_-5'-AG GAG GCT AG(C/A/T)T TCT CTC AGG-3', Perfect and l -base mismatch at position 10 is indicated

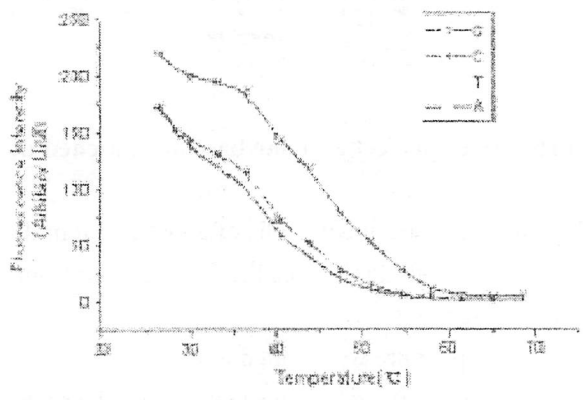


Fig. 4. Melting Curve of perfect (G) and one-base mismatched(A/T/C) duplex

Table 1. Melting temperature of perfect and one base mismatched dupiexes

	In solution	Immobilized
G	74.2	43.7
C	59.0	39.2
T	57.5	38.1
A	63.4	38.8

In conclusion, we presented the preliminary investigation of melting behavior of the immobilizedoligonucleotide and compared with that in solution. This work will help us to optimize the hybridizationcondition in microarray applications.

References

[l] S.P.A. Fodor, J. L. Read, M.C. Pirrung, L. Stryer, A. T. Lu and D. Solas, Science, 251 (1991) 767-773.

[2] J.G. Hacia, L.C. Brody, M. S. Chee, S. P. A. Fodor and F. S. Collins, Nature Genet., 14 (1996) 441-447.

[3] G. Yershov, V. Barsky, A. Betgovskiy, E. Kirillov, E. Kreindlin, I. Ivanov, S. Parinov, D. Guschin, A. Drobishev, S. Dubiley and A. Mirzabekov, Proc. Natl. Acad. Sci. USA, 93 (1996) 4913-4918.

[4] A. V. Fotin, A. L. Drobyshev, D. Y. Proudnikov, A. N. Perov and A. D. Mirzabekov, Nucleic Acids Res., 26 (1998) 1515- 21.

[5] E. Southern and K. Mir, M. Shchepinov, Nature Genet., 21 (I Suppl) (1999) 5-9.[6] M. J. Doktycz, M. D. Mouis, S. J. Dormady, K. L. Beattie and K. B. Jacobson, J. Biol. Chem., 270 (1995) 8439-45.

STUDY ON CLONING OF P1 ATTAVHMENT PROTEIN GENEOF MYCOPLASMA PNEUMONIAE

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Mycoplasma pneumoniae(MP) is one of most common causes of human infection of respiratory tract and is the principal etiological agent of atypical Pneumoniae. M. pneumoniae infection has a increasing trend in recent year, especially on children. The infection has a serious symptom, long process, and various complication. So, it is noticed worldwide. The pathogenic theory of MP infections still unclear.

P1 attachment protein is the most important pathogenic agent of M. pneumoniae, it can attach firmly to epithelium cells of host, This attachment has been recognized as a Prerequisite for colonization and subsequent development of disease。Mature P1attachment protein contains l 568aa and its MW is 169758 dal. It is a major immunogen of M pneumoniae, which can cause humoral immune response and produce lgG and lgA antibody. So it has been suggested as possible vaccine candidates and may be developed into a effective diagnose agent.

In our study, the P1 structure gene was obtained by PCR method. PCR primer of P1 structure gene as follow: 5´-GCT GAA TTC CAG TTC TTA AAA GCA AGC-3´; 5´-GT GAA TTC GGA GGTAGG TGT TGG CTA-3´, PCR product was Recovered and Ligated it with pUC19 plasmid vector together after digested with EcoR Ⅰ, then transforming it into E.coli DH5a strain. The recombinants were screened on X-Gal plat, and recombinant plasmids were identifyed by PCR method and restricted endonuclease map.

The results are as follow:

Selecting recombinants: The number of white colonies which contain insert target gene was selected on X-gal plate.

Identifying recombinant plasmid: Plasmid was extracted and purified. The two band was found on 1% agarose gel electroghoresis after digested with EcoR Ⅰ, The one is 5. o Kb and another is 2. 7Kb which similar with pUC19 plasmid carrier(fig 1). This result is identical with theoretical value.

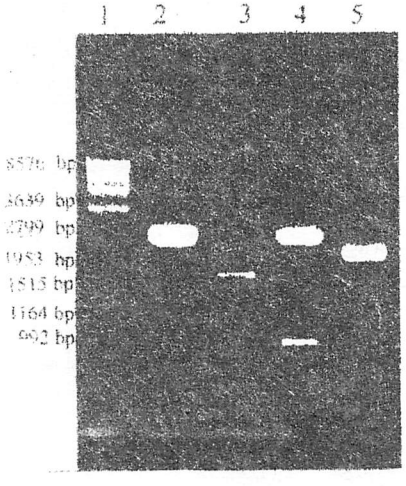

Fig 1.Restriction endonuclease map of recombination plasmid
1. pUC19 plasmid DNA digested with EcoR1
2. pUC19 plasmid DNA
3. Recombination plasmid DNA digested with EcoR
4. Recombination plasmid DNA
5. Standard (SPP1/EcoR1)

Recombinant plasmid was amplified with P1 Specific primer (RepMP2/3), and PCR products was detected on 1% agarose gel. 2.0 Kb band was found (fig3).These indicate that the recombinant plasmid contain p1 structure gene.

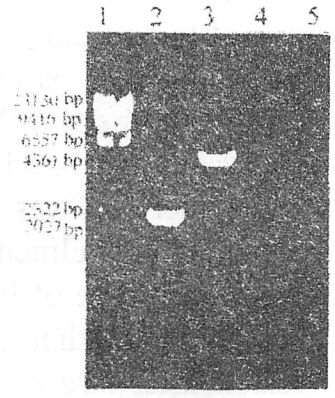

Figure 2. PCR products of Recombinant Plasmid
1.Standard(λ DNA/Hind Ⅲ)
2.PCR products of P1 (Rep MP2/3)primer
3. PCR products of P1 structure gene
4.recombinant plasmid
5.negtive control

P1 protein is a major immunogen of M. pneumoniae, which can cause humoral immune response and produce lgG and lgA antibody. So it has been suggested as possible vaccine candidates and may be developed into a effective diagnosis method. P1 structural gene contains 4881 nucleotides, The codon UGA is read as Tryptophan in M. pneumoniae and otherwise read as terminal signal in Escherichia coli , thus ,complete expression of P1 gene is difficult in Escherichia coli . There is report that the G fragment of P1 gene has successfully translated with SPV1 vector in Spiroplasma, although it has seven UGA codons .

We design primer of Pl structural gene which lies between nt 159-5042 according to the up — down conservation sequence (nt 75~5117,) and add the EcoRI digestion site and protective base simultaneously.

In this test, We have obtained a recombinant strains which contain P1 structure gene. Our result should play a foundation for further research.

The Relationship Between c-fos and Prolactin Gene Expression in the Rat Pituitary after Restraint Stress

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Our purpose was to explore if there is c-fos expression in pituitary and the relationship between c-fos and prolactin (PRL) gene expression after restraint stress. Wistar female rats were exposed to restraint stress for 60 min and killed at 15, 30, 60, 120, 180 min after stress. In situ hybridization was performed to detect the levels of c-fos and PRL mRNA in the anterior pituitary. We found that there was a low basal level of c-fos expression in the anterior pituitary. A rapid increase was noted from 15 to 30 min after restraint stress, then the expression declined. A second peak appeared at 120 to 180 min after stress. The change of PRL mRNA was paralled with the c-fos expression. We included that c-fos might mediate the activation of PRL gene expression after stress. Immediate-early genes expression in pituitry may be a marker of activation of neuroendocrine system.

A STUDY ON MORPHOLOGICAL CHANGES OF THE AORTIC ENDOTHELIAL CELLS IN ARTHEROGEROGENESIS

(Observed by en face, Scanning and Transmission Electron Microscopy)

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66 white male rabbits, the 18 rabbits of control group were fed with common diet, the 48 rabbits of experimental group were fed with hypercholesterol diet. They were killed in different intervals from 1 day to15 weeks. Morphology of endothelial cells in normal and hypercholesteral diet was described with Hautchen technique, SEM and TEM methods.

Application of Hautchen technique of endothelium show normal endothelial cells are usually in simple mosaic layer similar figuration of cells shown by the silver impregnation method, the round or oval nuclei presented by hematoxylin staining. However, there are still some differences among the different sites. For example, the EC in arch of aortae are polygonal, the EC in abdominal and thoracic aortae are teardrop-shaped and the EC at the vascular arborization has a pattern of radiaform. The mitostosis of different stages of EC also have been seen. It showed that the EC possess the function of regeneration and repair. This experiment also directly proved that endothelial lesion might be the initiating event in artherogesis and played very important role in forming and developing of artherosclerotic plaques.

It was suggested that there were two sorts of foam cells in artherosclerotic plaques: one originated from monocytes, the other came from smooth muscle cells of arterial wall. Monocytes and smooth muscle cells reacting to the lesions of endothelial cells, as well as platelet aggregation played an important role in artherogenesis.

It was suggested also that haemodynamic stress factor play a promotional role in artherogenesis.

*Ultrustructural Study of Diffuse Axonal Injury

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2.Shi Yuxiu,Dep.of Histo-Embryology, China Medical University, Shenyang

Through the ultrastructural observation of 15 Diffuse Axonal Injury (DAI) patients, we can find that the ultrastructural changes are already very obvious in 4 hours after trauma.These changes maily manifested as swelling、distortion、surface coarse of the myelinic sheath and the axon、dissociation and degeneration of the myelinic sheeth、disappearance of the mitochondria and disorder of the microfilament within the axon, then , dissolution、liquization、vaculization of the axonal plasma ,at last,vanishing of the axon.The severity of these changes varies.An expansive area can be found at the area where axon disappearance and myelinic sheath dissociation was obvious, at the end of it, the clues of axon disconnection can be found. The "retraction ball"which can be observed within 11 hours after trauma may formed at the expansive area,which implies the importance of early clinical treatment for DAI. On the other hand, the structural disorder of neuronal mitochodria and endoplasmic reticulum,even the vaculization in neuoral plasma,can be observed too.The onset of these changes is related to the severity of the trauma. In the same patient, ultrastructural changes become pronounced as the time of trauma goes by, but there is no significant correlation between them in different patients. The relationship between the image features and the prognosis of 30 cases of DAI is also analysed.It is concluded that CT and MRI examination can improve the clinical diganosis of DAI. It can be divided into two types according to theses findings: the central type (the injury referred to the white substance located within the lateral margin of the basilar nuclei) and the peripheral type (the injury referred to the white substance located beyond the lateral margin of the basilar nuclei only),the mortality of the former is statistically significantly higher than that of the latter.

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EFFECT OF PMA AND MEMBRANE FLUIDITY ON THE NEMATOLYSOSOMES IN RAT NEURON OF SPINAL CORD*

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We have repored that TMPase and ACPase positive nematolysosome (NLY) exist in central neurons of rat and guinea pig. In this study we observed the effect of PMA and membrane fluidity on the shape and distribution of NLY in cultured neuron of spinal cord . The cells were (a)incubated with PMA(50ng/ml) for 1.5 hours,(b)preincubated with PMA(50ng/ml) for 1.5 hours ,then incubated with PMA(50ng/ml) and linoleic acid (30ug/ml)for 1 hour. The control cells were not treated with any reagents. ACPase cytochemistry and electron microscopic enzymocytochemistry were used to show lysosomes. Membrane fluidity was determined by fluorescence polarization technique with the plasma membrane probe DPH. In control cells , lysosomes were clustered around the cell center. In PMA treated cells , lysosomes were distributed into the periphery of the cell. For electron microscopy, most lysosomes were roughly spherical and few NLY were seen in normal cultured neurons without any treatment. When the cells were incubated in the medium containg 50ng/ml PMA, the number of NLY remarkably increased in the cytoplasm . We noticed the number of NLY increaed as the lysosomes moved outward induced by PMA. The plasma membrane fluidity increased in linoleic acid and PMA treated cells compared with that of the control cells treated with PMA only. But no changes in the shape and distribution of NLY were observed in the former compared with that of in the later. The results mentioned above indicate that PMA alter the distribution and shape of the lysosomes. PMA induce lysosome move outward from the cell center to the periphery of the cell and the numbers of NLY increased. It seems the movement outward of lysosomes may be associated with the formation of NLY. In our experiment the increased membrane fluidity has no significant relation to the shape and distribution of NLY.

*Sponsored by China Natural Science Foundation

*The observation of double-labeling lysosome and neurofilament in motor neuron of anterior spinal cord in rat

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Recently ,there has been an increase in research and reports about the different shapes and functions of lysosomes. Especially in neuron of spinal cord , the presence of nematolysosomes might be closely related to the decomposition of neurotransmitters and the processing of metabolites. Some articles reported ,the movement of lysosome and their organization within a cell is associated with the cytoskeleton , but their physiological functions are largely remain unknown .In order to study the relations between the lysosome and the neurofilament , we applied one of lysosome marker enzyme-ACPase cytochemical method and immunohistochemical reaction simultaneously to demonstrate the whole distribution of lysosome and neurofilment .

Materials and Methods Wister rats were used for the experiments.Spinal cord was perfused with a fixative consisting of 2% PFA and 0.2% GLU in 0.01M PBS buffer, PH 7.4, containing 8% sucrose. The spinal cord was removed and cut into 15 and 40 um sections. For ACPase cytochemistry , the specimens were incubated in a Gomori-type medium for 80 min at room temperature. For light microscopy, 15um sections were treated with 2% ammonium sulfide solution after the incubation .Indirect immunoperoxidase method was carried out on all sections. Applying normal goat serum markedly reduced nonspecific staining especially at relatively low dilutions (1:200) of the primary antiserum(sigma). After these , the specimens were postfixed in 1 % osmic acid for 30 min at 4℃. Then ,the specimens were dehydrated and embedded in Epon .ultrathin sections were cut and observed under a TEM at 65 kv.

Results Light microscopy . ACPase positive reaction which is brown-black granule was found in the cytoplasm, axon and also synapse in the neurons without the nucleal region. Immunohistochemical reaction microscopy has shown that this fibrous material form a network all over the cytoplasm, which is high density in the axon.Electron microscopy . ACPase positive reaction was high density black sediment accompany with other organelles often and was found in the thread-like structures and in the spherical lysosomes. Nematolysosomes is often running parallel to neurofilament.Immunohistochemical reaction microscopy demonstrated nuerofilament is consists of bundles of filaments which in a cage-like network or a radial pattern throughout the cytoplasm.

Discussion One purpose of the present study was to fine out the relations between the lysosome and the cytoskeleton system . It is also unknown whether the cytoskeleton system which has been believed to participate directly in the regulation of intracellular lysosome movement plays any important role in regulating the activity of "lysosome three-dimensional network system".Double-staining experiments with enzyme cytochemical method and immunohistochemical reaction demonstrated coexistence of lysosome and neurofilament at the same section .Lysosome and neurofilament were both observed in the cytoplasm. -----

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The selection of oligonucleotide probe for *in situ* hybrization from already known genes by means of DNA database on the web

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It is possible to detect the suitable sequences mRNA from known genes by means of *in situ* hybridization (ISH), but it needs to prepare a proble for the ISH. Oligonucleotide probe is easily synthesized and its specificity can be ascertained by means of homology analysis on DNA database on the web sit. This study has been undertaken whether a good oligonucleotide probe for the ISH can be selected by means of the DNA database on the web.

The present study, target gene is rat H^+ -K^+ -ATPase beta-subunit one. In order to get cDNA information of rat H^+ -K^+ -ATPase beta-subunit gene, key word analysis program on the web 1) http://www.ncbi.nlm.gov/Entrez/ was used to set the program linked to nucleotide information. The gotten cDNA sequence was checked again by means of homology analysis program on the web, 2) http://blast.genome.ad. jp, using the latest cDNA information. A hundred pairs of primers candidates for polymerase chain reaction (PCR) from the gotten cDNA were selected by means of the program on the web, 3) http://www-genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi. The left-side primers were the most suitable probes for the gotten cDNA. The probes were sorted according to the base number of thec candidates were and the repeated probes were deleted. Final probe (a1, a2... f1, f2...) divided the cDNA sequence in A (probe a1, a2...) to F (probe f1, f2...) regions. The selected probes were examined in the specificity to the cDNA by means of homology analysis on the web program 2). It was shown that the A and C regions were the most specific for H^+ -K^+ -ATPase beta-subunit gene of rat and rattus. B and F regions were for that rat, rattus and mouse. E region was for rat,

rattus and human. And D region was for rattus, O region was for cunicukus, pig, mouse, human and dog.

The study employed e3 probe in the E region to see whether the selected probe can be applied for ISH. The anti-sense probe (RHKATPe3A) was 5'-tta aaa tga aca gga ttg tca agt t, and the sense probe (RHKATPe3s) was 5'-a act tga caa tcc tgt tca ttt taa taa. The both probes were synthesized by a custom survice by Takara Biotechnology Co., Ltd (Kusatsu, Japan). ISH was performed, using DIG REMBRANDT for RNA ISH and Detection kits (Kreatech Diagnostics AT, Amsterdam, The Netherlands) with the synthesized Dig-5'-end-labeled probes (RHKATPe3A and RHKATPe3S).

The DIG REMBRANDT for RNA ISH and detection kits was checked by the poly-A probes to detect the level of the kits, employing paraffin sections of human stomach with a *Helicobacter pylori* related ulcer. High temperature antigen unmasking technique either using autoclave or microwave and catalyzed signal amplification were applied for getting more intense positive signals of poly-A mRNA. In paraffin sections of rat stomach, ISH of the anti-sense probe labeled parietal cells, indicating signals of H^+ -K^+ -ATPase beta-subunit gene. In paraffin sections of human stomach with fundic glands regenerative foveolar epithelial cells and early gasreic adenocarcinoma, singals were detected as the homology of the DNA sequence corresponding the anti-sense probe suggested.

Consequently, we have reported the selection method of oligonucleotide probe for ISH from known genes by means of DNA database on the web. The method is simple, objective and reliable one suitable for beginning of ISH.

An immunohistological study of a lesion similar to human atopic dermatitis in NC mice

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Introduction

The nishikinezumi with cinnamon color (NC mouse) is bred and kept as a pet in Japan. NC / Fujita (NC/F) mice, which are kept in the laboratory animal center of our University, develop dermatitis similar to human atopic dermatitis from 5 ～ 8 weeks of age under conventional circumstances. The dermatitis gradually proceeds and persists during the lifetime of the mouse; thus, the NC/F mouse has been considered as an animal model of atopic dermatitis based on its skin eruption, the histological findings of the eruptive area and its skin barrier function. We previously reported that one of the causes of dermatitis in NC / F mice was the IgE mediated type I allergy to Myocoptes musculinus (*Kekuidani*), which is a kind of parasitic mite found on mice. To investigate the cause of the persistent dermatitis, we attempted patch tests against human atopic dermatitis and also examined the migration of Langerhans cells (LCs), which are antigen presenting cells, in NC/F mice.

Materials and Methods

NC / F mice were prepared for the experiments and BALB/C mice were used for controls.

Results and Discussion

Many lymphocytes, and a few eosinophils and mast cells were observed in the dermis of the eruptive area in NC mice. More IL-4 and IL-5 positive cells were noted in the skin in the eruptive group than in the non-eruptive group. IL-4 and IL-5 positive cells in the eruptive group increased weekly from 5 weeks to 18 weeks (at acute phase). Some IFN-γ positive cells were also observed in the dermis in the eruptive group, especially during the chronic dermatitis phase (after 18 weeks). IFN-γ positive cells were scarce in the skin in the non-eruptive group and eruptive group with acute dermatitis. We confirmed the immunological responses of Th2 in the acute dermatitis, but immunological activation of Th2 transformed to Th1-type activation in the chronic phase dermatitis. Many IgE ε RI-positive cells were noted in the dermis of the eruptive group in the acute phase. Serum IgE was elevated from 7 weeks to 21 weeks, and then it decreased; thus, we considered that dermatitis in the acute phase was due to IgE mediated allergy. Most of the mast cells had IgE ε RI in the dermis of the eruptive group. We speculated that the large number of Ia-positive cells in the dermis of the eruptive group after 21 weeks together with a serum IgE level that was lower than in the acute phase might be more associated with delayed hypersensitivity than IgE

mediated immediate allergy, namely type IV allergy .

We also confirmed that antigen presenting cells, especially Langerhans cells, which were Ia positive cells, migrate from epidermis to dermis. There have been no reports on the correlation between type IV allergy and the migration of LCs in NC mice, although it is well known that one of the causative factors of atopic dermatitis is a mite. The patch test using mite components showed positive reactions in atopic patients (Kumei et al, 1990; Kubota et al, 1994). The positive ratio of patch testing with a mite antigen in atopic dermatitis was higher than that in normal controls (Cadtelain et al, 1993; Suzuki et al, 1994). Therefore, we used Dp extract, a mixture of Dermatophagoides pteronyssinus and Dermatophagoides pteronyssing in this study to attempt patch test. In one vial, 178 mg dried extract of Dermatophagoides pteronyssinus and Dermatophagoides pteronyssing (Greer laboratories INC, NC, USA), of which the amount of antigen protein was 17.33% of the extract. We labeled Dp antigen with PKH26 dye, and performed patch testing in an atopic model mice, NC/Nga mice; a kind of NC mouse to determine whether the atopic dermatitis is type IV allergy or not. Results were judged according to Draize's criteria at 0.5, 1, 3, 6, 9, 24, 48, 72 and 168 hours after patch testing. The migration of LCs at 1-hour was observed by immunohistochemical study of protein kinase C-II (PKC-II) staining as a marker of LCs, and very few LCs were noted in the skin at 48-hour and 72-hours. In addition, we observed LCs labeled with PKH26 dye in the T cell area, sinus and marginal areas of regional lymph nodes at 24, 48, 72 and 168-hours. Electron microscopically, PKC-II- positive cells in the regional lymph nodes had Birbeck Granules (BGs) and atypical granules. Our findings suggest that LCs process antigens, and then migrate to the T cell area of the regional lymph nodes for presenting antigens in delayed hypersensitivity.

It is well known that GM-CSF, which is produced by keratinocytes, induces the maturation and migration of LCs. In our study using the western blotting method, the level of GM-CSF in the patch testing sites increased with time. The number of LCs decreased as the level in GM-CSF in the skin increased and the peak GM-CSF level was noted at 48-hours. At 72-hours, the level of GM-CSF had decreased. We confirmed that LCs migrate from the epidermis to the regional lymph nodes, initiated by GM-CSF, via the dermis.

To our knowledge, this is the first report focusing on elucidating the exact relationships between atopic dermatitis and the migration of LCs in NC mice

ESTROGEN AND PROGESTERONE RECEPTORS P^{53} IN ENDOMETRIAL CARCINOMA

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Abstract **Objective:**To assay the positive expression rate of estrogen receptor (ER) and progesterone receptor (PR) and P^{53} in endometrial carcinoma and clinical significance.**Method**:ER,PR and P^{53} of 44 cases endometrial carcinoma specimens were measured by immunohistochemical method.**Results:**The total positive expression rate of ER,PR and P^{53} are 66.57%,63.64%,27.27% separately in endometrial carcinoma.Positive expression rate of ER,PR and P^{53} are related to the grades of rumor.The positive expression rate of ER,PR drop lower but the positive rate of P^{53} rise higher with grade of tumor in endometrial carcinoma rising higher ($P<0.05$).There is not any significant instatistics about the relationship between the positive expression rate of ER,PR P^{53} and clinical period.**Conclusions:**ER, PR, P^{53} and the grading of the tumor tissue reflect biologic behaviors of en-dometrial carcinoma.ER. PR and P^{53} assays are important in predicting prognosis and selecting clinical en-docrinotherapy.

Key words :endometrial carcinoma; estrogen recrogen receptor; progesterone receptor; P^{53} ; immunohistochemistry

Pathological research on breast cancer with vimentin expression

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ABSTRACT Objective: To investigate vimentin expression in breast cancer epithelia and its relationship with various pathological features of breast cancer.**Methods:**The immunohistochemistry was used to detect vimentin, proliferating cell nuclear antigen (PCNA) and cytokeratin expression in breast cancer epithelia. The DNA dot-blotting hybridization was used to detect the expressing status of c-myc oncogene amplificaton in breast cancer tissues, and the radio-labelling dextran-coated activated charcoal absorption was applied to assay the estrogen receptor(ER) quantity.**Results:**The vim-entin could be expressed in 30% breast cancer tissues,and its expression correlated to histologic grade, ER status and PCNA index,etc. The breast cancer in which the vimentin expressed positively was found to be slightly different from the ordinary breast cancer epithelia in morphology, and the cytokeratin staining was also poor. The proto-oncogene c-myc amplified more frequently.**Conclusion;**The breast cancer with positive vimentin expression is a kind of breast cancer which is more malignant and probably with poor prognosis.

KEY WORDS: breast cancer;vimentin;protooncogenes

The expression and clinical significance of vascular endothelial growth factor in breast cancer

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Abstract: Objective To study the role of vascular endothelial growth factor (VEGF) in development and evaluating prognosis of breast cancer. **Methods** S-P immunohistochemical method was used to detect VEGF expression in breast benign and malignant disease. **Results** :VEGF expression positive rate in breast cancer (75.5%) was higher than that in breast benign disease (3/9), the differecne was significant ($P<0.05$). In 40 cases of breast cancer, the expression of VEGF in lymphatic metastasic group(21/23), was more than that in none lymphatic metastasic group(13/21), the difference was significant ($P<0.05$).**Conclusion** VEGF play an important role in development and evaluating prognosis of breast cancer.

Key words: vascular endotheial growth factor;breast cancer;immunohistochemical method

EXPRESSION AND RESEARCH OF GENE PROTEIN CD44S AND CD44V6 IN GASTRIC CANCE

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KEYWORD:
Gastric cancer · CD44S · CD44V6 Tumour diversion, · Recursion · Expression of gene protein

AIM
Research the gene expression, the catalog of tumour tissue, blood vessel invasion, lymphnode diversion, recursion and prognosis, and so on.
MATERERIALS AND METHODS :
According to the WHO classification, classify clinic of case, pathology and data in 161 curatively resected gastric carcinomas. Pick the faction possessing most tumour, 10.0 percent formalin, olefin slice and ABC immunity dye as the matererials of experiments. In the experiment, use CD34 to mark the cell in the vessel.
RESULT OF EXPERIMENT:
In 161 curatively resected gastric carcinomas, CD44S is expressed in 32 percent, and CD44V6 in 67.0 percent.
CD44S is expressed 0.0 percent, CD44V6 in 72.0 percent and V6 in 100 percent in axilla lymph node.In contrast, the occurenc rate of the vessel invasion and the gastric cancer is scirrhus> canal cancer>gastric cancer. Vessel invasion and lymph node diversion is only expressed ($P<0.001$) and $P<0.005$ in remoteness diversion
CONCLUSION:
The strong expression of CD44S is significantly correlated with vessel invasion.
The strong expression of CD44V6 distinctly shows the diversion and Recursion of gastric cancer.
The expression of CD44 P is direct proportion of recursion, diversion and bad prognosis.

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