The Third International Symposium of Molecular Pathology

2004.8.16-17,Kunming, the People's Republic of China

Supported by
Japanese-Chinese Medical Association
China Medical University

Organizing Committee
Department of Pathology, China Medical University

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The Third International Symposium of Molecular Pathology

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Organizing and Scientific Committee

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	Jin Cui	(KunMing Med. Univ.)

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General Information of The Third International Symposium of Molecular Pathology

Date: August 16-17,2004

Venue: The symposium will be held in the meeting room of Kunming Medical University in Kunming.

All the participants must arrive Kunming Medical University in Kunming by August 16,2004 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.

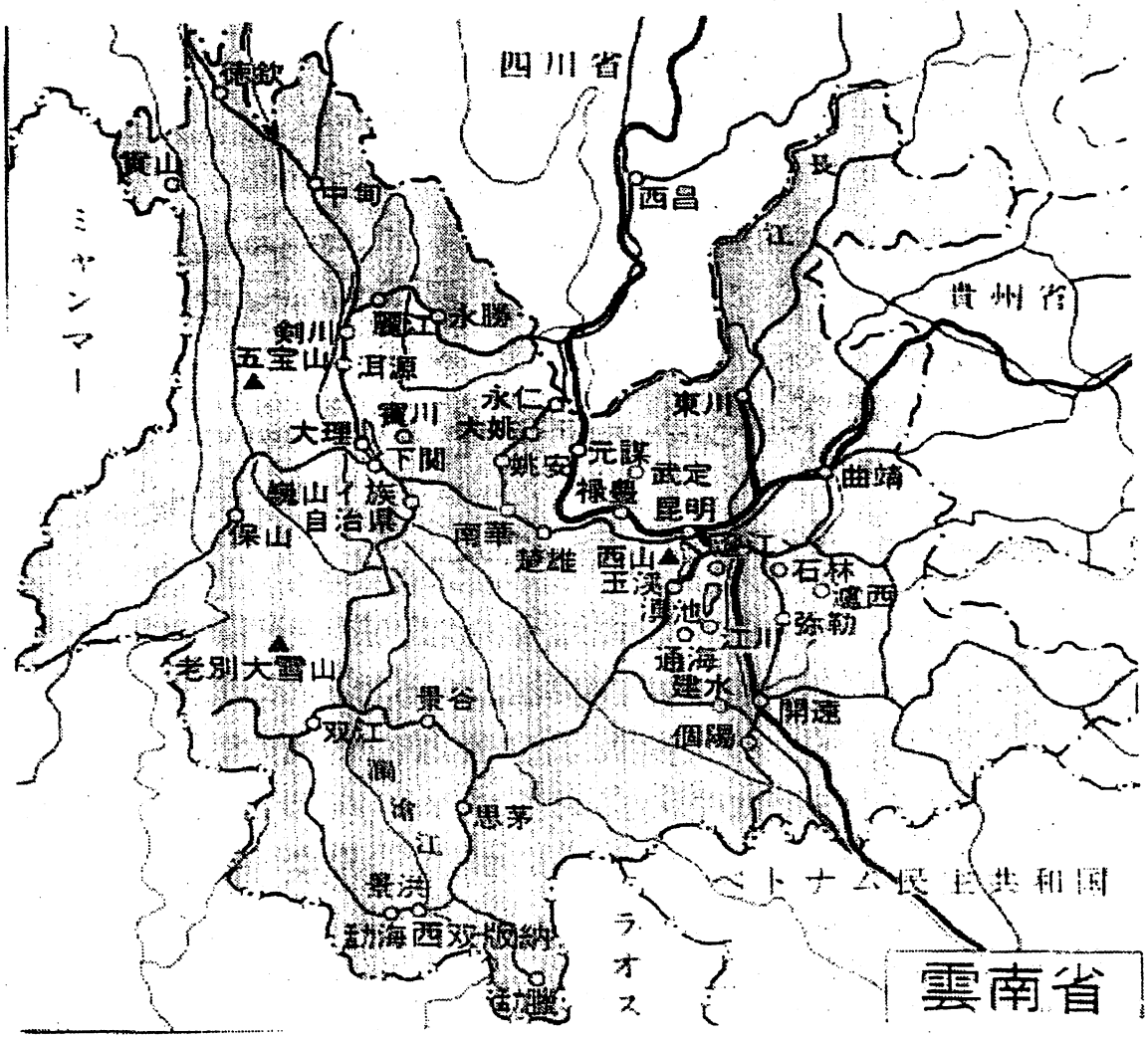
Wear: Informal. Considering the climate in Kunming is very comfortable in the morning and night, but hot in the noon. Please all the participants prepare suit wears.

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. Xinshan Jia at the meeting room in Kunming Medical University in Kunming.

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)×120(H)cm on the plate where will be indicated before the opening of the symposium (in the morning August 17, 2004). 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 Kunming, Dali and Lijiang natural legacy of world's) for all participants and social and associating persons to attending The Third 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 16,2004	Registration of participants
	**********
August 17,2004	International Symposium of Molecular Pathology in Kunming Medical University in Kunming
August 18,2004	Returning of all participants



Greeting

It is my great pleasure to declare the opening the 3rd International Symposium of Molecular Pathology to all participants from China and Japan. The first meeting was held in Dunhuang on August, 26, 1998 and the second one was done in Chengdu, on August, 18, 2001. Each of them was well organized by Prof. Xin Shan Jia, China Medical University, Prof. Eiichi Sato, Kagoshima University and Prof. Hiroshi Nagura, Tohoku University.

Nowadays, outstanding development of molecular pathology is seen and translational research on the basis of knowledge of basic sciences to clinical medicine is planned. All of us must be always intend to acquire technical application and new informations. Molecular pathology will give us good understanding of the basis of diseases. including infections. metabolic and neoplastic disorders.

I hope all of the participants will enjoy the discusssion in this symposium, and also this oppotunity promote collaborative investigations among the participants from China and Japan.

President of the symposium
Kouki Inai. M.D., & Ph.D.
Professor and Dean
Hiroshima University
Graduate School of Biomedical Sciences

****

Ladies and gentlemen:

At first, thank you for attending the meeting toilfully. This Meeting should be held in 2003, because of SARS it is delayed to today. Six years ago, we held the first International Symposium of Molecular Pathology on Aug 26, 1998 in Dunhuang. Six 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, RNAi and the research about embryonic and adult 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 Dali, Lijiang and some showplaces in Kunming.

At last, I want to express a lot thanks to Prof. Jin Cui, Kunming Medical University 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:

Xin Shan Jia. M. D.

(Professor, China Medical University)

2004. 8.17

List of Chinese participants

Academic part

Prof.	XinshanJia	China Medical University
Prof.	Yujie Zhao	China Medical University
Prof.	Xianghong Yang	China Medical University
Acce. Prof.	Jianhua Li	China Medical University
Prof.	MinSong	China Medical University
Acce. Prof.	Xiaoyi Mi	China Medical University
Miss.	Yan Wang	China Medical University
Miss.	Qiang Ding	China Medical University
Miss.	Ying Zhou	China Medical University
Miss.	Yanjiao He	China Medical University
Miss.	Yanmei Zhu	China Medical University
Miss.	Xiumei Zhang	China Medical University
Miss.	Rui Shi	China Medical University
Miss.	Ping Hou	China Medical University
Miss.	Jie Sun	China Medical University
Mr.	ChengboHan	China Medical University
Mr.	Jianjun Li	China Medical University
Mr.	Yuanhang Li	China Medical University
Miss.	Xiaoyun Mao	China Medical University
Miss.	Xuefei Yang	China Medical University
Miss.	Mingxi Jing	China Medical University
Miss.	Yujia Gao	China Medical University
Miss.	Lihui Meng	China Medical University
Miss.	Xiaoxiang Liu	China Medical University
Acce. Prof.	Guangping Wu	China Medical University
Prof.	Dongping Tian	Shantou University
Prof.	Runqing Zhu	Wuhan University
Miss.	ZhijiaoTang	Wuhan University
Mr.	Yong Wang	Wuhan University
Mr.	Qiaoyang Xian	Wuhan University
Prof.	Qiang Wu	Anhui University
Miss.	Lifeng Yan	Enliang hospital of Tai'an county
Mr.	Hailin Wang	The Center Hospital of Dengta
Miss.	Xiufeng Wang	Liuhe Hospital of Dashiqiao

List of Japanese participants

Academic part

Prof.	Hiroshi Nagura	(Tohoku Univ.)
Prof.	Takashi Saku	(Tsaku Univ.)
Prof.	Yoshimichi Ueda	(Kanazawa Medical Univ.)
Prof.	Mikihiro Shamoto	(Fujita Health Univ.)
Prof.	Tsutsumi Yutaka	(Fujita Health Univ.)
Prof.	Kouki Inai	(Hilocima Univ.)
Prof.	Masahiro Kikuchi	(Fukuoka Univ.)
Prof.	Motohiro Takeya	(Kumamoto Univ.)
Prof.	Kazuhisa Hasui	(Kagoshima Univ.)
Miss.	Hui Qin Xing	(Kagoshima Univ.)
Mr.	Junkon Nakayama	(Jikei Univ.)
Prof.	Eiichi Sato	(Kagoshima Univ.)

Mr.	Tarohisa Ocumura	(Kagoshima people's Hospital)

Social part

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

Associating persons

Mrs.	Yuriko Sato	Mrs.	Keiko Nozoe
Mrs.	Hideko Hirayama	Mrs.	Hisako Nagara
Mrs.	Mihoko Shamato	Mrs.	Yoko Nagara
Mrs.	Michiyo Mise		

Scientific Program

2004.8.17

Am 9:00 Opening

Greeting Japanese-side President: **Konki Inai**

Introduce the participants Chinese-side President : **Xin Shan Jia**

Chinese-side vice-president: **Jin Cui**

Photography of the symposium (All the participants attending the symposium)

Am 9:30 SPECIAL LECTURE 1 Chairmen: **Kouki Inai, Xin Shan Jia**

Hiroshi Nagura[1,2], Osamu Hotta[3], Yoshio Taguma[3] and Koji Hozawa[4]

[1]Department of Athletics and Nutrition, Sendai College, Sendai, and [2]Divisions of Pathology, [3]Nephrology and [4]Otolaryngology, Sendai Shakaihoken Hospital, Sendai, Japan

Novel features of the palatine tonsil in IgA nephropathy

Am 10:00 SPECIAL LECTURE 2 Chairmen: **Hiroshi Nagura, Jin Cui**

Yujie Zhao

Center of biochip, China Medical University

Bioinformatics applied in medicine

Am 10:30 **Coffee break**

Am 10:40 LECTURE 1 Chairmen: **Motohiro Takeya, Yujie Zhao**

Yoshimichi Ueda, Miyako Shimasaki, Ming Gong, Katsuaki Sato, Shogo Katsuda, Makoto Sugita*, Motoyasu Sagawa*, Tsutomu Sakuma*

Department of Pathology and Thoracic Surgery*, Kanazawa Medical University, Japan

Expressions of aquaporin-1, -3, -5 and alfa-epithelial sodium channel in human lung carcinomas

Am 10:55 LECTURE 2 Chairmen: **Yoshimichi Ueda, Xianghong Yang**

Kazuhisa Hasui, Atae Utsunomiya*, Fusayoshi Murata, Shuji Izumo, Suguru Yonezawa, Takami Matsuyama, Takashi Hayata**, Eiichi Sato

Kagoshima University and *Imamura-Bunin Hospital and **Kagoshima Women's Junior College, Kagoshima, Japan

Supersensitive immunostain of p53 protein in peripheral blood tissue specimens of ATL and HTLV-1 carriers

Am 11:10 LECTURE 3 Chairmen: **Eiichi Sato, Xiaoyi Mi**

Motohiro Takeya,1 Koichi Kaikita,1,2 Takanori Hayasaki,1,2 Toshiyuki Okuma1

Departments of Cell Pathology1 and Cardiovascular Medicine2, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan

Deficiency of CC chemokine receptor 2 attenuates left ventricular remodeling after experimental myocardial infarction

Am 11:25 LECTURE 4 Chairman: **Takashi Saku, Kazuhisa Hasui**

DongPing Tian , Min Su , Rui-Juan Zhang, Xiong Guo, Yukai Wang , Yan Yu, Yan Wang

Department of Pathology, Medical College of Shantou University, Shantou, Guangdong

The Experimental Study of Morphological Development and differentiated Proteins NSE GFAP CNPase and BDNF Expression at Hippocampus of Prolonged Selenium-Deficiency F3 Rats

Am 11:40 **Rest**

pm 1:30 SPECIAL LECTURE 3 Chairman: **Masahiro Kikuchi, Yujie Zhao**

Takashi Saku
Division of Oral Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

EBV-related lymphoepithelial carcinomas of the salivary gland: a molecular pathologic analysis of 181 cases mainly from the Chinese population

Pm 2:00 LECTURE 5 Chairman: **Dongping Tian**

Kazuhisa Hasui, Toryu Hirayama, Fusayoshi Murata, Suguru Yonezawa, Shuji Izumo, Takami Matsuyama and Eiichi Sato
Kagoshima University, Kagoshima, Japan

Immunohistochemical detection of apoptosis in non-neoplastic lymphatic tissues

Pm 2:15 LECTURE 6 Chairman: **Kouki Inai**

Hui Qin Xing[1], Takashi Moritoyo[2], Kazuyasu Mori[3,4], Kei Tadakuma[4], Chie Sugimoto[4], Fumiko Ono[5], Hitoshi Hayakawa[2], and Shuji Izumo[1]
[1]Division of Molecular Pathology, Center for Chronic Viral Diseases. [2]Department of Neurology. Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan;
[3]AIDS Research Center, [4]Tsukuba Primate Center for Medical Sciences. National Institute of Infectious Diseases. Tokyo. Japan;
[5]Corporation for Production and Research of Laboratory Primates. Tsukuba. Japan

Virus tropism dependent brain pathology in simian immunodeficiency virus infected rhesus monkey; an animal model of AIDS dementia complex

Pm 2:30 LECTURE 7 **Chairman: Xinshan Jia**

Qiang Wu, Jun Zhou, Feng Yang
Department of Pathology, Anhui Medical University, 69 Meishan Road, 230032 Hefei, Anhui, PR China

Clinicopathological Study on Alteration of PTEN Gene and its Protein Expression in Human Breast Cancer

Pm 2:45 **Coffee break**

Pm 3:00 **Poster and Disscusion**

Poster 1

Qiang Ding[1] Xinshan Jia[2] Ying Zhou[2] Aijun Liao[2] Lingling Wang[2]
[1] Sun Yat-Sen Unversity;Department of Pathphysiology. [2]China Medical University, Department of pathology

Study the tracheal stem cells during the regeneration after injure induced by 5-FU

Poster 2

Yanjiao He [1], Xinshan Jia [1], Kazuhisa Hasui[2].
1 Department of Pathology, China Medical University, Shenyang 110001, China.
2 Second Department of Anatomy,Kagoshima University Faculty of Medicine, Kagoshima 890-8520.Japan
Nasopharyngeal Non-Hodgkin's lymphomas in Shenyang and their relationship with Epstein-barr virus

Poster 3
Yanmei Zhu [1], Xinshan Jia[1], Yanjiao He [1],Kazuhisa Hasui[2].
1 Department of Pathology, China Medical University, Shenyang 110001, China.
2 Second Department of Anatomy,Kagoshima University Faculty of Medicine, Kagoshima 890-8520.Japan
Expression and significance of CD204 in nasal NK/T cell lymphoma

Poster 4
Xiaoyun Mao, Chengbo Han, Yan Xin, Dongying Wu, Sumin Zhang
*The Fourth Laboratory of Cancer Institute,the First Affiliated Hospital of China Medical University, Shenyang 110001
Mutation of mitochondrial 12S rRNA in gastric carcinoma and their significance.

Poster 5
Yan Wang , En-hua Wang, Guangping Wu
Department of Pathology , China Medical University , Shenyang 110001 , China
Immunohistochemical and ultrastructual study of so-called sclerosing hemangioma of the lung suggests different origins of cells

Poster 6
Runqing Zhu [1], Zhijiao Tang[2], Yong Wang [2], Qiaoyang Xian [2], Jili Zhu [3], Zunfu Ke [1], Xiaoping Meng [4], Lihua Sun [2]
Department of Pathology, Wuhan university,Wuhan.China
The laboratorial pathology study of SARS

Poster 7
Ying Zhou [1], Xinshan Jia [2]
1Emergency Department of the First Affiliated Hospital of China Medical University.
2Department of pathology of China Medical University .Shenyang 110001.China
Dynamics Analysis of Rhodamine123 Staining of Human Bronchial Epithelium During the Wound-repair Process Induced by Fluorouracil.

Poster 8
Rui Shi, Daorong Zhang, Xiao Fang, Hui Yu, Xueshan Qiu, Enhua Wang
Department of Pathology , China Medical University, Shenyang 110001 , China
Expression of Integrin-Linked Kinase(ILK) and E-cadherin in non-Small Cell Lung Cancer

Poster 9
Ping Hou, Zhiguo Song, Min Yu, Shuyu Li, Enhua Wang, Xueshan Qiu.
*Department of Pathology, China Medical University, Shenyang, 110001,P.R.China
**Department of Pharmary, China Medical University.
The clinical significance of thymosinβ$_4$ expression in human non-small cell lung cancer.

Poster 10

Ping Hou, Zhi-guo Song, Min Yu, Shuyu Li, Enhua Wang, Xueshan Qiu.

*Department of Pathology, China Medical University, Shenyang, 110001,P.R.China

**Department of Pharmary, China Medical University.

The clinical significance of thymosinβ_4 and thymosinβ_{10} expression in human non-small cell lung cancer.

Poster 11

Xiumei Zhang, Min Song

Department of Pathology, China Medical University, Shenyang, 110001,P.R.China

Expression and Significance of ERK Protein in Human Breast Carcinoma

Poster 12

Jie Sun, Jianhua Li

Department of Pathology, China Medical University, Shenyang, 110001,P.R.China

Induction of apoptosis in osteogenic sarcoma cells by combination of Trail and chemotherapeutic agents

Poster 13

Xiaoxiang Liu, Deshou Cao, Xiubin Fang

Department of Neurobiology, Basic Medical College, China Medical University, Shenyang 110001, P.R.China

The upregulatory effect of NGF on IL-1β in the lower respiratory tract and viscerosensory afferent sites of the asthmatic guinea pig

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Xiaoxiang Liu, Xiubin Fang

Department of Neurobiology, Basic Medical College, China Medical University, Shenyang 110001, P.R.China

Upregulation of calcitonin gene related peptide levels by nerve growth factor in the experimental asthmatic guinea pig

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*ChengBo Han *, XiaoYun Mao, DongYing Wu, SuMin Zhang, Yan Xin*

*The Fourth Laboratory of Cancer Institute,the First Affiliated Hospital of China Medical University, Shenyang 110001

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Poster 16

JianJun Li, SuMin Zhang, DongYing Wu , Yan Xin

*The Fourth Laboratory of Cancer Institute, the First Affiliated Hospital of China Medical University, Shenyang 110001

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YuanHang Li, Yan Xin, DongYing Wu, SuMin Zhang

*The Fourth Laboratory of Cancer Institute,the First Affiliated Hospital of China Medical University, Shenyang 110001

Expression of Kai1 and FasL in non-small cell lung cancer and their clinicopathological significance

Poster 18

LiHui Meng, DongYing Wu, SuMin Zhang, Yan Xin

*The Fourth Laboratory of Cancer Institute.the First Affiliated Hospital of China Medical University, Shenyang 110001

Protein Expression of KAI1 and nm23-H_1 in Gastric Cancer and Their Clinicopathological Significance

Poster 19

YuJia Gao, XiaoYun Mao, DongYing Wu, SuMin Zhang, Yan Xin

*The Fourth Laboratory of Cancer Institute,the First Affiliated Hospital of China Medical University, Shenyang 110001

Protein Expression of FHIT and nm23-H_1 and their clinicopathological significance in gastric cancer

Poster 20

XueFei Yang, DongYing Wu, SuMin Zhang, Yan Xin

*The Fourth Laboratory of Cancer Institute.the First Affiliated Hospital of China Medical University, Shenyang 110001

Clinicopathological Significance of PTEN and Caspase-3 Expression in Breast Cancer

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Mingxi Jing, Dongying Wu, Sumin Zhang, Yan Xin

The Fourth laboratory, Cancer Institute, The first affiliated Hospital, China Medical University, Shenyang 110001, China

Clinicopathological significance of maspin and mutant p53 protein expression in gastric cancer

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Tong Wang; Yujie Zhao

Center of biochip, China Medical University

The study of HLA-DQA1 genotyping by oligonucleotide microarry

Poster 23

Hailin Wang [1], Zhonggang Chang [1], Shulin Yang [2], Yanjiao He [3]

1 Department of Pathology, The Center Hospital of Dengta, 111300

2 Department of Pathology, The Center Hospital of Zhuanghe, 116400

3 Department of Pathology, China Medical University, Shenyang, 110001

Pathological features of endometrial stromal sarcomas

Poster 24

Hailin Wang, Haixia Liu

Department of Pathology, The Center Hospital of Dengta, 111300

1 case uterine adenomoid tumor

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Lifeng Yan

Department of pathology of enliang hospital,Tai'an county

A case report of testicular malignant lymphoma

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Xiufeng Wang [1], *Shulin Yang* [2]

1Department of Pathology, Liuhe Hospital of Dashiqiao, 115103, Yinkou

2Department of Pathology, Zhuanghe People Hospital, 116400, Dalian

One case report of appendix duplication

Poster 27

*Guangping Wu *,-Enhua Wang , Weijian Hou, Yujie Zhao , Jianhua Li, Changqing Fang, Shuli Liu.*

*Pathological Diagnosis Center, The First Affiliated Hospital, China Medical University, Shenyang 110001, China

Applied value in clinic of Captured by Cytochip for Cancer cells in pleural effusion of patients with lung cancer

Pm 5:30	**Closing**
Pm 7:00	**Welcome Party**

Abstracts

3 Special lectures
7 Lectures
27 Posters

CATALOG

IMMUNOHISTOCHEMICAL DETECTION OF APOPTOSIS IN NON-NEOPLASTIC LYMPHATIC TISSUES

Kazuhisa Hasui, Toryu Hirayama, Fusayoshi Murata, Suguru Yonezawa, Shuji Izumo, Takami Matsuyama and Eiichi Sato
Kagoshima University, Kagoshima, Japan

Apoptosis has been detected by means of TUNEL (TdT-mediated dUTP-biotin nick end labeling) method or ISNT (in-situ nick translation) method. Recently it was introduced that single-stranded DNA (ssDNA) is detected by immunohisto-chemistry (IHC). On the other hand, a rabbit monoclonal antibody was raised against cleaved casparse-3 that is a product in apoptosis and has been supplied commercially. This study aimed to see which IHC of the antibodies against ssDNA, cleaved casparse-3, Bcl-X and Bax is the most useful in the practical staining of apoptotic cells in the routinely processed tissue specimens.

Paraffin sections of human lymph nodes and thymus and mouse gastrointestinal (GI) tract were used in this study under the consent of Medical ethical committee in Kagoshima University Hospital. Primary antibodies used were anti-ssDNA rabbit polyclonal antibody (4B013A, DakoCytomation, 1:100), anti-cleaved casparese-3 rabbit monoclonal antibody (Asp175, 5A1, Cell Signaling, 1:250), anti-Bcl X rabbit polyclonal antibody (A3533, DakoCytomation, 1:1000) and anti-Bax rabbit antibody (A3535, DakoCytomation, 1:1000). Antigen retrieval was performed. The reacted primary antibodies were labeled by ChemMate EnVision system (DakoCytomation) or our supersensitive IHC and were colored by DAB and hydrogen peroxide reaction.

The EnVison system with pretreatment of proteinase K detected ssDNA in tingible macrophages of the germinal centers, macrophages in the nodal medulla, degenerative mitoic cells in the nodal sinus histiocytosis, and many lymphocytes in the thymus, but was not seen in the epithelial cells in the mouse GI tract. The EnVison system with antigen retrieval in EDTA solution by an autoclave visualized cleaved casparse-3 clearly in apoptotic cells in the human lymph nodes and thymus and in the mouse GI tract. Our supersensitive method with antigen retrieval in citrate buffer pH 8 by an autoclave showed obviously positive cellular stain of Bcl X and Bax and their granular stain in cytoplasm of many cells.

It was shown that the IHC of the cleaved casparse-3 is the best one to detect apoptotic cells. In spite of difficult detection of ssDNA in apoptotic nuclei, accumulated ssDNA in macrophages suggested apoptosis or cellular degeneration in their areas. The supersensitive IHC of Bax might label the cells before apoptosis

Bioinformatics applied in medicine

Yujie Zhao
Center of biochip, China Medical University

What is bioinformatics ? Bioinformatics tasks concern the creation and maintenance of databases of biological information. Nucleic acid sequences (and the protein sequences derived from them) comprise the majority of such databases. While the storage and or ganization of millions of nucleotides is far from trivial, designing a database and developing an interface whereby researchers can both access existing information and submit new entries is only the beginning. Bioinformatics tasks involve the analysis of sequence information. **Computational Biology** is the name given to this process, and it involves the following:

- Finding the genes in the DNA sequences of various organisms
- Developing methods to predict the structure and/or function of newly discovered proteins and structural RNA sequences.
- Clustering protein sequences into families of related sequences and the development of protein models.
- Aligning similar proteins and generating phylogenetic trees to examine evolutionary relationships.

The process of evolution has produced DNA sequences that encode proteins with very specific functions. It is possible to predict the three-dimensional structure of a protein using algorithms that have been derived from our knowledge of physics, chemistry and most importantly, from the analysis of other proteins with similar amino acid sequences. The diagram below summarizes the process by which DNA sequences are used to model protein structure.

Biochip, widely recognized as the next revolution in molecular biology, enable scientists to analyze genes, proteins and other biological molecules on a genomic scale. This emerging technology, used in more than 10,000 laboratories already, is having an enormous impact in all areas of biomedicine and across the agricultural, biotechnology, and pharmaceutical industries.

Bioinformatics and biochip applied in medicine can help us to diagnose diseases in gene level, analyse the mutation of gene(as well as SNP's), observe genome expression profile to differentiate some disease subtypes, identify pathogenic microbes, research target of druggery and investigate pharmacological mechanism.

Key Words: Bioinformatics; Biochip

Novel features of the palatine tonsil in IgA nephropathy

Hiroshi Nagura[1,2], Osamu Hotta[3], Yoshio Taguma[3] and Koji Hozawa[4]
[1]Department of Athletics and Nutrition, Sendai College, Sendai, and [2]Divisions of Pathology, [3]Nephrology and [4]Otolaryngology, Sendai Shakaihoken Hospital, Sendai, Japan

The upper aerodigestive tract possesses lymphoid tissues, the palatine tonsils and adenoids, which have a characteristic lymphoid architecture consisting of primary and secondary follicles with a germinal center surrounded by extrafollicular regions and reticular crypt epithelium. They play the role as parts of an integrated mucosal immune system.

IgA nephropathy (IgAN) is defined as a form of glomerulonephritis in which immunoglobulins of IgA isotype, particularly J-chain-linked IgA1 in an immune complex form, dominate or co-dominate within the glomerular deposits. Upper respiratory infection and inflammation including tonsillitis often precede IgAN, and tonsillectomy is effective for the treatment of this disease. In these aspects, the mucosal immune system contributes IgAN pathogenesis, and palatine tonsils and adenoids seem to play a role to generate nephritogenic immune complexes in IgAN. In the present study, palatine tonsils removed from IgAN patients with or without high dose steroid therapy were immunohistochemically analyzed, and compared with those from recurrent tonsillitis.

Palatine tonsils from patients with IgAN are rich in germinal centers, and their size and distribution are in irregular and the outline of the follicular area is hazy. The interfollicular area corresponding to T cell area is much broader and is infiltrated by more B cells than those from recurrent tonsillitis. The reticulated sponge-like epithelial layer on the outer surface of tonsillar crypts represents a specialized compartment important in the immunologic function of tonsils, and that in palatine tonsils from patients with IgAN contains many $CD20^+$ B cells and immature plasma cells. These cells of the B cell lineage are proliferating and labeled with Ki67. Although tonsillar B cells can mature to produce all five major immunoglobulins, percentage and number of IgA-positive tonsillar cells are significantly increased in IgAN.

By high dose steroid therapy, germinal centers and follicular areas become smaller or disappear, and B cells and immature plasma cells almost disappear in the reticulated sponge-like epithelial layer.

From the present study, we strongly favor the view that the tonsil seems to be a unique organ causing initial and/or progressive events to generate nephritogenic immune complexes on IgAN, and there appears to be a dysregulation of IgA production in the aerodigestine tracts probably by a functional abnormality of T cells and its clearance of such patients. Thus tonsillectomy may be a worthwhile procedure to consider in the treatment for IgAN.

"Expressions of aquaporin-1, -3, -5 and alfa-epithelial sodium channel in human lung carcinomas"

Yoshimichi Ueda, Miyako Shimasaki, Ming Gong, Katsuaki Sato, Shogo Katsuda, Makoto Sugita*, Motoyasu Sagawa*, Tsutomu Sakuma*
Department of Pathology and Thoracic Surgery*, Kanazawa Medical University, Japan

[Background] Water is one of the essential molecules for life and dynamic water transport is performed through cell membrane. Since Agre P. (1993) found a transmembrane water channel molecule, aquaporin (AQP)-1, there have been established at least 13 different subtypes and their physiologic and pathologic functions are intensely investigated. Lung is one of organs with active water metabolism and physiologically expresses AQP-1, -3, -4, and -5. Recently, involvements of AQPs in tumorigenesis has been reported in brain tumors and colon carcinomas.

[Purpose] Expressions of AQP-1, -3, -5 and alfa-epthelial sodium channel (alfa-ENaC), another cell membrane channel for water metabolism, were studied both at protein and mRNA levels in lung carcinomas of different subtypes and biological aggressiveness, to elucidate the biological and clinicopathological significance of AQPs and alfa-ENaC in lung carcinoma.

[Materials and Methods] 45 fresh-frozen lung carcinoma tissues (27 adenocarcinomas; 10 squamous cell carcinomas; each 4 large and small cell carcinomas) and three nonneoplastic lung tissues resected surgically and embedded in OCT-compound were immunostained with polyclonal antibodies against AQP-1, -3, -5 and alfa-ENaC. Their mRNAs in tumor cells of representative cases were quantitatively analysed by laser-captured microdissection / real time RT-PCR.

[Results] AQP-3 was exclusively expressed in tumor cells of nonmucinous bronchioloalveolar carcinoma (BAC), while AQP-1 and -5 were positive in invasive tumor cells of Noguchi type C lesions and well-differentiated adenocarcinomas. Tumor cells of mucinous BAC overexpressed not only AQP-1, -5 and also alfa-ENaC. AQPs and alfa-ENaC were rarely detected in other subtypes, except for AQP-3 in a few cases of poorly differentiated adenocarcinomas. Expressions of those genes in the carcinoma cells were upregulated at the message level.

[Conclusion] The results showed distinct expression patterns of AQPs and alfa-ENaC in lung carcinomas, some being related to cellular differentiation and others being tumor-related abnormal gene expressions. AQPs and alfa-ENaC genes overexpressed in mucinous BAC may become one of target genes for intractable endobronchial spreading mucinous BACs.

Supersensitive immunostain of p53 protein in peripheral blood tissue specimens of ATL and HTLV-1 carriers

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It is important clinically to predict the occurrence of adult T-cell leukemia (ATL)/lymphoma (ATLL) in human T-cell lymphotropic virus type 1 (HTLV-1) carriers and the transformation to acute or lymphoma type in chronic type ATL. Developing supersensitive immunohistochemistry (ssIHC) of HTLV-1-related protein, p40Tax we found that p40Tax is expressed in ATLL cells and leukemic cells, whereas p40Tax is also detected in mononuclear cells in HTLV-1 carriers. This study aimed to see whether expression of p53 protein is an index to predict the occurrence of ATLL in HTLV-1 carriers. Peripheral blood tissue specimens (PBTS, Acta Histochem. Cytochem. 36: 345.2003) of 5 cases of chronic type ATL, 5 cases of acute type ATL, 8 cases of HTLV-1 carriers, 2 cases of lymphocytosis and 5 cases of the other leukemia were used. Employing anti-Ki67 antigen antibody (MIB-1, DakoCytomation), anti-p53 protein antibody (1801, Novo Castra) labeling ser46, and anti-p53 protein with phosphorylated ser392 antibody (p53-Phos, Novo Castra), reacted primary antibodies were visualized by means of ssIHC (nsCSA system developing that in Histochem J. 34:215.2002), comparing the detection by means of ChemMate EnVision system. The IHC was performed by Dako autostainer. In ATL there were many Ki67 antigen-positive cells, relatively many p53-Phos-positive cells and some p53-1801-positive cells, when the other leukemia revealed some Ki67 antigen-positive cells and rare cells positive for p53-Phios or p53-1801. The lymphocytosis showed the same pattern with ATL. In HTLV-1 carries, cells positive for p53-Phos or p53-1801 increased significantly ($p<0.05$) according to the age, although they were much less than those in ATL. It was suggested that expression of p53-1801 and p53-Phos detected by ssIHC could be an index to predict the occurrence of ATL in HTLV-1 carriers, considering the age.

Deficiency of CC chemokine receptor 2 attenuates left ventricular remodeling after experimental myocardial infarction

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Left ventricular remodeling after acute myocardial infarction is dependent on the extent of the initial ischemic damage and the subsequent healing of the infarct and surrounding tissues. A key component of cardiac remodeling following acute myocardial infarction is the inflammatory response, which modulates cardiac tissue repair. To investigate the relationship between the monocytic inflammatory response and left ventricular remodeling after myocardial infarction we incorporated the mice deficient in CC chemokine receptor 2 (CCR2), the primary receptor for monocyte chemoattractant protein-1 (MCP-1), which is one of the most potent monocyte chemotactic chemokines.

Immunohistochemical analysis revealed rapid infiltration of macrophages into infarcted tissue within 7 days in wild type mice. However, this process was greatly impaired in CCR2 deficient mice. Echocardiography demonstrated beneficial effects of CCR2 deficiency on left ventricular remodeling at 7 and 28 days after myocardial infarction. *In situ* zymography showed augmented gelatinolytic activity in wild type mice within 7 days after myocardial infarction, while gelatinolytic activity was barely detectable in $CCR2^{-/-}$ mice. Moreover, the distribution of gelatinolytic activity in serial sections was very similar to the distribution of macrophages rather than neutrophils. Expression of matrix metalloproteinases and tumor necrosis factor-α mRNAs was upregulated in infarcted regions from WT mice compared to $CCR2^{-/-}$ mice at 3 days after myocardial infarction. These findings indicate that direct inhibition of CCR2 functional pathway might contribute to the attenuation of left ventricular remodeling after myocardial infarction.

EBV-related lymphoepithelial carcinomas of the salivary gland: a molecular pathologic analysis of 181 cases mainly from the Chinese population

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In the last ten years, we have collected a total of 181 cases of lymphoepithelial carcinomas (LEC) of the salivary gland from the mainland of China, Taiwan, and Russia (63 cases from Shanghai, 42 from Guangzhou, 40 from Chengdu, 9 from Beijing, 4 from Wuhan, 3 from Changchun, 3 from Kunming, 3 from Xi'an, 2 from Urumqi, 6 from Taipei, 3 from Taichung, 1 from Moscow). Their clinicopathologic features are as follows: no sex predilection, a mean age of 43.5 years, most common in the parotid gland followed by the palatal minor gland. Histopathologically, salivary LEC (SLECs) cases were classified into two subtypes: large and small cell nest types. EBV infections were confirmed by in-situ hybridization (ISH) and PCR amplifications in all of the cases examined.

The characteristic lymphoid stroma of SLECs were examined by immunohistochemistry and ISH for extracellular matrix (ECM) molecules, lymphocytes and macrophages. A basic unit structure of SLECs was composed of centrally-located undifferentiated carcinoma cell nests and surrounding lymphoid cells. Such unit structures were separated by fibrous septum-like stromata. The lymphoid cells were mainly CD45RO+ T cells, which were CD8+ predominant, mixed with B cells and macrophages. Immunohistochemically, each unit structure was surrounded by perlecan+ and tenascin+ fine fibrous elements, and the boundary between carcinoma cells and lymphoid cells was widely perlecan+/tenascin+, suggesting that the fibrous stroma of SLECs were produced by both carcinoma cells and lymphoid cells.

To clarify the significance of EBV in SLECs, we analysed the *LMP1* gene, whose gene products are transported in the cell membrane to function as a TNF receptor triggering a multitude of signaling pathways including NfkB, MAPKs, and others. EBV-infection was confirmed by PCR and ISH in 62 cases of SLECs. LMP1 protein was immunohistochemically demonstrated in 31 cases (50%). The characteristic 30-bp deletion in the carboxyl (C)-terminus region of the LMP-1 gene was found in 20 cases (32%). Most of them were from Guanzhou, Chengdu and Taiwan, while most of the cases from Shanghai and other areas showed no 30 bp deletion. In addition, several point mutations including codon 338 were commonly shared by the cases with or without the 30 bp deletion. These results indicate that

there are two major genomic variations in SLEC EBV. The frequent mutations in the C-terminus indicate that SLEC may have the same behavior as nasopharyngeal carcinomas.

In the next step, we isolated the complete EBV LMP1 genes from 12 samples, and compared these LMP1 genes with those of nasopharyngeal carcinoma (NPC, CAO) and the prototype B95-8 EBV. Salivary LEC LMP1 genes were more similar to CAO genes than to prototype B95-8 genes. The analysis also identified several conserved (67-100%) variations in SLEC-LMP1 and CAO-LMP1 distinct from B95-8-LMP1. These included 10-amino acid deletion, 5-amino acid deletion and 12-single amino acid variations. In addition, the promoter region of the LMP1 gene was also examined in 20 samples of SLECs, and all of them had 37 common point mutations, 92% of which were similar to those of CAO. One of the SLEC LMP1 genes with the aforementioned conserved variations inhibited the growth of an embryonic kidney cell line, 293T, and at the same time highly activated the NF-κB pathway in expressed protein amount-dependent manners. And, these activities were equivalent to those of B95-8 and CAO. These findings suggest that the biological functions of SLEC-LMP1 are significant but that the mutational events in the SLEC LMP1 sample including the well-known 30-bp deletion did not affect these two prominent activities.

In order to examine possible routes of EBV entry, paraffin sections from 50 cases of LECs were analyzed by immunohistochemistry for IgA, secretory components (SC), CD21, and LMP1. CD68-immunopositive macrophages were selectively recovered by laser capture microdissection, and extracted DNA samples were also analyzed by PCR. Immunohistochemically, there was no CD21 expression in the carcinoma cells, however, IgA and SC were focally observed in the carcinoma cells which were LMP1-positive. In addition, these three molecules were demonstrated in macrophages immunohistochemically and by PCR. The results indicate that CD21 is not likely to function as a cell surface receptor for EBV in these carcinoma cells, but that transcytosis via SC-anti-EBV IgA cannot be completely ruled out. In addition, it is obvious that macrophages are at least involved in the processes of infection and metabolism of EBV in LEC cells.

Since there have been no established cell systems originating from SLECs, we tried to isolate carcinoma cells from fresh surgical samples to study modes of EBV infection and its role in LEC cell proliferation. After many trials, we were successful in growing epithelial cell fractions from primary cultures of a parotid LEC of a 16 year old boy. After 7 passages, the cells started to grow stably and showed short-spindle shapes. They were immunohistochemically positive for keratin, CK14, CK19, vimentin, MUC1, S-100 protein and LMP-1 protein, suggesting that they were of salivary duct epithelial origin. PCR and RT-PCR showed the presence of LMP1 and LMP2A in the cells. Immunoblot analyses demonstrated the biosynthesis of

LMP-1 protein, indicating that EBV infection was maintained in the cells. Their LMP1 had no characteristic 30-bp deletion.

These salivary LEC projects have been carried out in collaboration with many Chinese pathologists. I would appreciate their kind and sincere cooperation.

Virus tropism dependent brain pathology in simian immunodeficiency virus infected rhesus monkey; an animal model of AIDS dementia complex

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OBJECTIVES: Highly active antiretroviral therapy (HAART) has been successful to reduce progression of acquired immunodeficiency syndrome (AIDS). Nevertheless, recent autopsy analysis of the brain from patients with human immunodeficiency virus (HIV)-1 infection reported same or even increasing numbers of AIDS encephalopathy. To investigate the reason for this insufficient effect of HAART for central nervous system (CNS) complication, we used simian immunodeficiency virus (SIV) inoculated macaques, an animal model of human AIDS, and investigated relationship between degree of the lymph node pathology and that of AIDS-related brain pathology.

METHODS: Nine rhesus monkeys were examined. Three macaques were infected with T-cell tropic SIVmac239, two macaques with macrophage tropic SIV 239/envMERT, and the other two with T-cell tropic SHIV-RT. Another two uninfected macaques were used as the controls. Paraffin-embedded formalin-fixed lymph nodes and brain tissues from each animal were examined using routine hematoxylin-eosin staining, various immunohistochemistry, and in situ hybridization. Electron microscopy of the cerebral cortex was also performed.

RESULTS: Animals infected with T-cell tropic SIVmac239 developed AIDS more than 150 weeks after infection and showed typical AIDS pathology in the lymph node. The cerebral cortex showed focal gliosis and EM analysis demonstrated mild changes such as accumulation of lamellar bodies in dendrites and swelling of astrocytic processes. However, there was no evidence of microglial nodules nor multinucleated giant cells in the white mater. The animals infected with macrophage-tropic SIV239env/MERT did not develop AIDS in the same period of infection. The white mater of the animal, however, showed microglial nodules with multinucleated giant cells. In situ hybridization demonstrated expression of viral mRNA in these giant cells. There was no abnormality in the cortex.

The Experimental Study of Morphological Development and differentiated Proteins NSE GFAP CNPase and BDNF Expression at Hippocampus of Prolonged Selenium-Deficiency F3 Rats

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Objective: The purpose of study is to know the Se-deficiency was whether or another significant factors for the brain development. The scholar had payed more and more attention to the relationship between Se and neural differentiation and growth. There had some report about Se affected the neural cell development in vitro. But how Se affects the brain morphological development in vivo, and how the Se& I combined deficiency? There is a litter report. In our study. We utilized the P4 , P21 rats brain samples from Se-deficient and I-deficient F3 rats to investigated the morphological changes, neural mature differentiated protein-GFAP、NSE、CNPase and neurotrophic factors BDNF expression at hippocampus(Hp).

Material and Method: The experimented animals were from Se-deficient, I-deficient F3 rat. The animals were divided four groups: Se-deficient; I-deficient; Se & I-deficient and controlled (supplement Se & I) groups. We respectively took P4(4 per group) and P21(6 per group) brain samples→fixed→embedded→sliced→made HE、Nissl、PAS stain, GFAP 、NSE、CNPase、BDNF immunohistochemistry. In addition, we extracted total protein from the P21 fresh hippocampus tissue(4 per group) to detect the GFAP、NSE、CNPase、BDNF protein expression by Western Blot technique.

Results:

1、**Morphological changed**: Contrasted to the controlled group, cortex cellular migrative speed and dentate gyrus (DG) of hippocampus were slow-moving at the Se-, I- and Se-I- , delamination of cortex was unclear; at P21, Se-, I- and Se-I- groups had higher cell density at cortex and polarity of arrangement was get -behind than the controlled group. The same changes were at I- and Se-I- than Se- groups .

2、**Neural mature differentiated protein expression:** The positive cell of GFAP and gray intensity of control group were higher than others at CA1 and DG regions of hippocampus ($P<0.01$), at CA3 regions are higher than Se-, Se-I- groups ($P<0.01$). Western Blot reveals the GFAP protein concentration was highest at control group, the difference among other groups were no significant. The gray intensity of NSE at control group was higher than I- and SeI-($P<0.01$), there are no difference with Se-. Western Blot showed there were differences among the groups, consistent with immunohistochemistry results. CNPase positve cell express at Hp and cortex. The results of accounting positive cell at CA3 were different. It shows I- and Se-I- had

more positive cell than control and Se- group. And the gray of positive fibers were more intensive at cortex of I- and Se-I- group, the projecting areas were wider.

3、**Neurotrophic factor BDNF express:** The immunohistochemistry showed BDNF commonly expressed at Hp、cortex and midbrain. The controlled group had more gray intensity than Se- and SeI- group at hippocampal proper but there was no difference at DG regions by immunohistochemical results; the I- had higher gray intensity than Se-. Western Blot revealed the BDNF protein concentration of Hp at I- was higher than others.

Conclusions:

(1)、Se and I are very important elements for rat brain development , especially Hp and cortex development. Se-, I- or Se-I- can cause cortex、Hp CA1、CA3 pyramidal cell and granual cell of DG regions over neurogenesis and slow-moving at different level.

(2)、The neural mature differentiated protein expression decreases respectively at Se-、I-、Se-I-. Se- mainly affects the AS. and that I- affects the neuron. Moreover I-、Se-I- could induce the oilodendrocyte proliferation , Se- only effect was less

(3)Neurotrophic factor BDNF expressed less at Se-group, but more at I-group. There are maybe some different mechanism for Se-、I- affect the production of BDNF at Hp.

Key words: Selenium , Iodine. rat hippocampus NSE GFAP CNPase BDNF

Clinicopathological Study on Alteration of PTEN Gene and its Protein Expression in Human Breast Cancer

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Abstract

Purpose To investigate the alteration of PTEN gene and its protein expression in breast cancer so as to evaluate its clinicopathological significance. **Methods** Immunohistochemical staining, S-P method, was used to detect the positivity of PTEN in 23 cases of normal breast tissues, 28 cases of proliferative breast disease including 8 cases of atypical hyperplasia (ADH), 8 cases of ductal carcinoma in situ (DCIS) and 98 cases of breast cancer. Meanwhile, cyclinD1 and microvessel density(MVD) marked with Factor Ⅷ-related antigen were also investigated in 98 cases of breast cancer. PCR-PAUGE was used to detect loss of heterozygosity (LOH) and microsatellite instability (MSI) near PTEN loci in 98 breast cancer. **Results 1.** PTEN was weakly expressed in normal breast tissues. Atypical hyperplasia and DCIS expressed higher levels of PTEN than normal tissues($P<0.05$), but had no remarkable distinction with invasive breast cancer. The expression of PTEN was negatively correlated to histological grading, axillary lymph node metastasis, clinical staging and MVD. **2.** The frequency of loss of flanking marker D10S215 around PTEN was 32.7%(32/98). In the cases with PTEN-LOH samples, 5 of 32 were PTEN-negative. PTEN-LOH was positive correlated to clinical staging, axillary lymph node metastasis. But microsatellite analysis showed that there was no microsatellite instability about D10S215 of PTEN gene existed in all these samples. **3.** A negative relationship existed between the expression of PTEN and cyclinD1. **4.** With weak and no expression of PTEN, there was a positive correlation between the expression of cyclinD1 and histological grading, axillary lymph node metastasis. **5.** With strong expression of PTEN, there was no correlations between cyclinD1 expression and

axillary lymph node metastasis. **Conclusion 1.** The change of PTEN expression is an early event in malignant transformation of breast epithelium. Significant correlation between the expression of PTEN and cyclinD1 also suggested that the down regulation of PTEN expression in breast cancer correlating with histological grading, axillary lymph node metastasis, clinical stage and MVD may be considered as a potential indicator to judge the differentiation and metastasis of breast cancer. **2.** LOH of PTEN gene may be a relatively late event in breast cancer development, which may play important roles on invasion and metastasis in breast cancer. And microsatellite analysis shows no microsatellite instability about D10S215 of PTEN gene existed. Microsatellite instability of PTEN might not be the main change in breast cancer. **3.** Immunohistochemistry is useful for the detection of LOH of PTEN gene on D10S215 in breast cancer. **4.** The effect of cyclinD1 on axillary lymph node metastasis of breast cancer may partly be under the regulation of PTEN.

Study the tracheal stem cells during the regeneration after injure induced by 5-FU

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Objective

Stem cells have been regarded as undifferentiated cells capable of proliferation, self-renewal, production of a large number of differentiated progeny, and regeneration of tissue. An alternative method for identifying both murine and human hematopoietic stem cells was recently developed and is based on the efflux of the DNA binding dye,Hoechst33342. the SP protocol can be used to identify stem cells in other tissues including skeletal muscle and epidermis. The ATP-binding cassette transporter ABCG2/Bcrp1 mediates the SP phenotype. We constructed a tracheal injury model in Wister rats treated with 5-FU. Using light microscope, electronic microscope, immunohistochemistry, Hoechst3332 staining and RT-PCR methods investigated the process of regeneration to localize the tracheal stem cells.To locate and observe the tracheal stem cells in situ

Methods

We constructed a tracheal injury model in Wister rats treated with 5-FU. Using light microscope, electronic microscope, immunohistochemistry, Hoechst3332 staining and RT-PCR methods investigated the process of regeneration to localize the tracheal stem cells

Results

1.5-FU treatment and the recovery progress: Three hours after 5-FU treatment, tracheal epithelium began to shed, pseudostratified columnar ciliated epithelium desquamated partly and exposed the basement membrane, Twelve hours the whole trachea epithelium desquamated with trifle naked- nucleus-like cells nailing just above the basement membrane alternation. 3hours after removing 5-FU, these cells disappeared instead by extreme squamous epithelium; the nucleus became flat with plasma almost covering the whole basement membrane like a membrane. at this time the PCNA staining show negative. Six hours later, extreme flat epithelium gradually differentiated into flat epithelium with a densely staining nucleus. Using electronic microscopy, these negative cells are poorly-differentiated: characterized by a large nucleus, scanty cytoplasm, undeveloped organelles, and no mucus granule and cilia. PCNA immunohistochemischy showed that negative staining cells located sparsely among the positive staining cells.Nine hours later, flat epithelium gradually differentiated into cuboidal epithelium.It could be seen that there were small mucus granule on the top of some cells and cilia on other cells under electron microscopic.12hours these cells became more cuboidal. After 24hours there could been seen the cilia under light microscope.(figure1-F), Forty-eight hours later, we observed that the tracheal rings were completely covered with pseudostratified columnar ciliated epithelium.

2.Hoechst 33342 effluxing population seen under the Fluorescence microscope: smear of epithelial cells were dissociated from tracheal treated by 5-FU for 12hours.Isolated cells were incubated in the presence of 5μ g/ml Hoechst33342 for 30 minutes, washed and observed with reflected Fluorescence light and contrast phase. Under contrast phase cells were seen with Integra outline ,meanwhile with UV excited most cells seen the blue nucleus, then open the light vision ,some cells with no Hoechst33342 dye combination among the positive cells.

3. **PCR Analysis.** RNA was prepared according to the manufacturer's instructionsfrom tracheal epithelial cells after treated by 5-FU. RT-PCR analyses were performed as described previously, using ABCG2 5' end primer CCA AAG CGT AGT GTC TGT AGC A and 3' end primer TGC CCT GAT TTA TCA AGT AGT C. The PCR conditions for the ABCG2 reaction were 95°C for 4 min, followed by 30 cycles at 95°C for 45 s, 55°C for 50 s, 72°C for 50 s, and a final extension of 6 min at 72°C. Actin PCR used the same conditions as the initial ABCG2 PCR reaction.

Conclusion

Rat tracheal stem cells exist the pollutant resistance G_0 cells which can efflux Hoechst33342 and express the gene ABCG2/Bcrp1.

[key word]:Fliorouracil; tracheal stem cell; injure; regeneration; Hoechst33342; ABCG2/Bcrp1

Nasopharyngeal Non-Hodgkin's lymphomas in Shenyang and their relationship with Epstein-barr virus

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【Abstract】 **Objective:** To study the clinical features, immunophenotypes and the significance of Epstein-Barr virus infection of nasopharyngeal non-Hodgkin's lymphomas in Shenyang and discuss whether CD57 can be used to diagnose NK/T cell lymphoma or not. **Methods:** Nasopharyngeal non-Hodgkin's lymphomas (158 cases) in Shenyang were studied. The samples were stained with haematoxylin and eosin for histological examination. Immunohistochemistry studies were performed using monoclonal antibodies, against CD3 for T-lymphocytes, CD20 for B-lymphocytes, and CD56 and CD57 for NK cells, and compared CD56 with CD57. In situ hybridization for EBV-encoded small nuclear RNA (EBER-1) was performed in 38 cases. **Results:** 101 cases (63.92%) were NK/T cell lymphomas, and 72.28% (73/101) of the initial sites were in the nasal cavity, 9.90% (10/101) in the tonsils and 17.82% (18/101) in the pharynx. 23 (14.56%) cases were true T cell lymphomas, with 26.09% (6/23) of the initial sites occurring in the nasal cavity, 30.43% (7/13) in the tonsils and 43.48% (10/23) in the pharynx. 21.52%(34/158) of cases were B cell lymphomas, and 14.70% (5/34) of the initial sites were in the nasal cavity, 64.71% (22/34) in the tonsils and 20.59% (7/34) in the pharynx. Correlation between lymphomas and initial sites was examined by Chi-square test and significant correlation was found (P <0.001 $\chi^2=55.831$). Comparing CD56 with CD57 was performed in 78 cases. NK/T cell lymphomas (73 cases) were positive for CD56 but negative for CD57.Few T cell lymphomas (5 cases) were positive for CD3, negative for CD56 and scattered positive for CD57. Using EBER-1 in situ hybridization, the samples were positive in 34 of 38 cases (89.5%), including 29/29 cases (100%) of NK/T cell lymphoma, 2/2 cases (100%) of T cell lymphoma and 3/7 cases (42.9%) of B cell lymphoma. **Conclusions:** NK/T cell lymphoma is the predominant type of lymphoma among nasopharyngeal non-Hodgkin's lymphomas in patients from Shenyang; The distribution of NHL may be related to the initial sites at which NHL occurs: CD56 is the marker to diagnose NK/T cell lymphoma. Although CD57 is also the marker of NK cell, it can't be used to diagnose NK/T cell lymphoma; The NK/T cell lymphomas we studied were associated strongly with EBV (100%).

【Key words】 Nasopharyngeal; Lymphoma; NK/T cell lymphoma; CD56; CD57; Epstein-Barr virus; In situ hybridization

Expression and significance of CD204 in nasal NK/T cell lymphoma

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【**Abstrsct**】 **Objective** To study the expression of CD204 in nasal NK/T cell lymphoma and analyze the pathology diagnostic significance of CD204. **Methods** Samples of 65 paraffin-embedded specimens were studied,including 51 nasal NK/T cell lymphoma, 6 nasal B cell lymphoma, 4 nasal T cell lymphoma and 4 nasal inflammation. The samples were stained with haematoxylin and eosin for histological examination. S-P immunohistochemistry studies were performed using monoclonal antibodies against CD204. Immunoelectron microscop was used to detect the distribution of CD204. **Results** Using immunohistochemistry. CD204 was expressed mainly in cytomembrane and cytoplasm. samples are positive in 49/51(96.1%) nasal NK/T cell lymphoma, 2/6(33.3%) nasal B cell lymphoma, 2/4(50%) nasal T cell lymphoma and 0/4(0%) nasal inflammation. The expression of CD204 in nasal NK/T and B cell lymphoma has distinct difference (P=0.001), NK/T and T cell lymphoma has difference(P=0.023).Immunoelectron microscopy study suggest that CD204 antigen distributes mainly in cytomembrane and cytoplasm. **Conclusions** CD204 can be used to diagnose NK/T lymphoma, and differential diagnose of nasal NK/T cell lymphoma with nasal inflammation,B and T cell lymphoma.

【Key words】 CD204 antigen; nasal NK/T cell lymphoma; immunohistochemistry; imunoelectron microscopy

Applied value in clinic of Captured by Cytochip for Cancer cells in pleural effusion of patients with lung cancer

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【**Abstract**】 **Objective** To evaluate the clinical applied value of automatic recognition and captured by the antibody cell microarray-cytochip for cancer cells in pleural effusion of patients with lung cancer. **Methods** The cytochip that is not reported in the whole world at present was developed to immunize hybridization the cells in pleural effusions of 42 patients with lung carcinomas and of 20 ones with lung benign lesions. **Results** The point of positive hybridization showed round, border clear, and the shape of cell displayed well, the cells of no special combination except point of positive hybridization was hardly found by the times of 40, 100, 200 and 400 of the ordinary microscope progressively. The positive hybridization numbers of ESA、CD44V6、LEA were 35, 30, 38, respectively in 42 adenocarcinoma cells: The positive hybridization numbers of them were only 3 and a fewer lymphocytes were found on the CD44V6 in 20 ones with lung benign lesions through the cell microarray; the other 7 cluster of differentiation antibodies did not captured carcinoma cells except lymphocytes neutrophils and macrophages from the two pleura effusions. **Conclusion** Cytochip showed advantages as follows: small-sized and information rich; high-throughput: automatic recognition: obviously comparable with other similar matter. low cost. simple operation and need a little sample. appears to be an important applied value for diagnosing carcinoma cells in pleural effusion of patients with lung carcinoma cells.

【**Key words**】 cytochip; microarray; glass slide

Dynamics Analysis of Rhodamine123 Staining of Human Bronchial Epithelium During the Wound-repair Process Induced by Fluorouracil.

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[Abstract]

Objective To analyze the condition of Rhodamine123 staining of human bronchial epithelium during the wound-repair process induced by fluorouracil(5-FU).

Methods Stem cells show reduced staining with Rhodamine123.We developed an in vitro injury model of human bronchial epithelium induced by 5-FU to find bronchial stem cell. The normal and injurious bronchial epithelial cells were obtained by enzymatic digestion (Type XIV protease .Sigma)and analysed by flow cytometry. 1)PI staining to identify the percentage of cells in different cell phase . 2) Rhodamine123 staining in live cells were to contrast the percentage of negative cells in two groups .

Results 1.Analysis of cell cycle: The percentage of G_0/G_1 of live cells in normal group was 86.54±4.50%, $S+G_2/M$ phase cells occupied 13.14±4.39% of normal bronchial epithelium; necrosis and apoptotic cells in injurious group increased .occupied 13.62±4.90%,the percentage of G_0/G_1 phase cells in remain live cells of injurious group increased , 93.33±4.95%,cells in $S+G_2/M$ phase almost disappeared;with the recover of bronchial epithelium, cells in $S+G_2/M$ phase increased.

2.The percentage of Rhodamine123 negative staining live cells in normal group was 10.46±2.17%, and in injurious group was 17.82±3.16%. with the recover of bronchial epithelium, the percentage of Rhodamine123 negative staining live cells reduced to nearly normal level.

Conclusions

5-FU can make the cycling cells degeneration and necrosis and reserve G_0 phase cells. There are bronchial stem cells which have the capacity to efflux Rhodamine123 among these G_0 phase cells. Just the proliferation and differentiation of these stem cells regenerated bronchial epithelium.

[Key words] Bronchial epithelium; Stem cells; Fluorouracil; Human; Rhodamine123

Immunohistochemical and ultrastructual study of so-called sclerosing hemangioma of the lung suggests different origins of cells

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[Abstract] Objective To study the morphological characteristics, immunohistochemical stain and histological origin of so-called sclerosing hemangioma of the lung(S-SH), and to investigate the significance and diagnostic value of expressions of surfactant protein B(SP-B), thyroid transcription factor-1(TTF-1) and other markers in S-SH. **Methods** Using transmission electron microscope and immunohistochemistry methods, the expressions of SP-B, TTF-1, mast cell trypsin(MCT), epithelial antigen markers (CK-H, CK-L, EMA, CEA), mesothelial antigen(MC), neuroendocrine markers (NSE, Ch-A, synaptophysin, calcitonin, ACTH, GH), vimentin and CD34 were observed in 30 cases of S-SH. **Results** S-SH demonstrated a mixture of four histological patterns: solid, papillary, hemorrhagic and selerotic pattern, which often showed transitional phenomena. Cuboidal cells on the surface, which contained short microvilli and lamellar bodies in cytoplasm, arranged in one row and sometimes interfused into multinuclear giant cells. Immunohistochemical results showed that there cells demonstrated strongly positive staining to SP-B, TTF-1, CK-L, EMA and CEA. The other major cell component-polygonal stromal cells were strongly positive to vimentin and TTF-1, and positive or weakly positive to 2 or 3 neuroendocrine markers in each case. Sparse neuroendocrine granulae and abundant microtubules were observed in cytoplasm of the cells. Both cuboidal and polygonal cells displayed negative immunohistochemical results to CD34 and MC. Some cell clusters in solid region were positive for SP-B and EMA. Most cells which were positive for MCT existed sparsely in almost full vision field. **Conclusion** Cuboidal cells of S-SH originate from reactive proliferating type II pneumocytes and sometimes interfuse into multinuclear giant cells. The polygonal cells in stroma probably originate from multipotential primitive respiratory epithelium and have multiple differentiating ability. The presence of mast cells is also one of histological characteristics of S-SH.

[Key words] So-called sclerosing hemangioma of the lung Lung neoplasms Immunohistochemistry

Expression and Significance of ERK Protein in Human Breast Carcinoma

Zhang Xiumei, Song Min,

ABSTRACT

Objective: To investigate the expression of ERK and p-ERK protein in human breast cancer and their corresponding tissue ,to access the significance of ERK signal pathway in tumorigenesis and progression of breast carcinoma. **Methods**: 40 breast cancer cases were used in S-P immunohistochemistry technique and Western Blot study. **Results:** The expression of ERK_1, ERK_2 and p-ERK protein levels increased remarkably in breast cancer tissues in comparison to normal tissues($P < 0.01$). These protein expression was upregulated 1.32-, 1.53-and 4.27-fold, respectively. Overexpression of ERK_1, ERK_2 and p-ERK proteins were obviously correlated with clinical stage of breast cancer. Protein levels of ERK and p-ERK were higher in stage III patients than in stage □and stage □patients($P < 0.05$). These proteins were strongly related with axillary lymph node metastasis of breast cancer, but no correlated with histopathological type and status of ER and PR of breast cancer. Expression of ERK_1 and ERK_2 protein showed a positive linear correlation. **Conclusion:** ERK signal transduction pathway is a key factor during human breast tumorigenesis and breast cancer progression.

Key words: Extracellular Signal-Regulated Kinase; Breast Carcinoma; Phosphorylation; Immunohistochemistry; Metastasis

Expression of Integrin-Linked Kinase(ILK) and E-cadherin in non-Small Cell Lung Cancer

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【**Abstract**】 **Objective** To study the relationship between histological type ,differentiation,clinical stage and lymphatic metastasis with the expression of Integrin-Linked Kinase(ILK) in human non-small cell lung cancer(NSCLC),and analyze whether there was relativity between ILK and E-cadherin .**Methods** Immunohistochemical method,S-P method, was adopted to detect the expression of ILK and E-cadherin proteins in 76 NSCLC cases with the neighboring noncancerous tissue. **Result** Immunohistochemically,the overexpression of ILK protein in NSCLC was 53/76 (70%),including 33/44 (75%) squamous carcinoma and 20/32 (62.5%) adenocarcinoma, its expression was not related to the histological type (P=0.247). Expression of ILK was related to differentiaion(P=0. 009), lymphatic metastasis(P =0. 006), and survival time (P=0.0057). Overexpression of ILK in NSCLC was associated with unfavorable prognosis. Our results also indicate an inverse correlation between the level of ILK and that of E-cadherin(P=0.0002).**Conclusions** In NSCLC. ILK can interact with many tumor-associated factors,through which it appeared to be involved in several oncogenesis-related events, including promotion of cell survival, as well as cell migration and invasion. ILK kept significant inverse correlation to E-cadherin, and it would be one of the pathway for ILK to affected differentiation. clinical stage, lymphatic metastasis and prognosis of patients.ILK expression can be a useful predictor of poor prognosis in NSCLC. and it also may be of significant value in tumor therapy.

【**Key words**】 Lung neoplasms ; Carcinoma , non-small-cell lung ; Integrin-Linked Kinase(ILK); E-cadherin

The clinical significance of thymosin β_4 expression in human non-small cell lung cancer.

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This study was supported by the grants from the Scientific Technology Foundation of Liaoning Education Office (to Qiu Xueshan, No. 9910500108)

【 **Abstract** 】 **Objective** To make a thorough study on the clinicopathologic significance of the expression of human thymosin β_4 (T β_4) in human non-small cell lung cancer .**Methods** Samples of 76 paraffin-embedded specimens with the neighboring noncancerous tissue were studied using anti-T $\beta_4$lgYantibody(primary antibody) .Immunohistochemical staining and related software analysis were used. **Results** Both human T β_4 was highly expressed in lung cancer while weak position in non-cancerous tissue. It expresses in cytoplasm associated in immunoreactive cells. Expression of T β_4 was positively associated with TNM stage(P = 0.032) ,lymphatic metastasis (P=0.029) and venous metastasis (P=0.045) .A negative correlation was found between expression of T β_4 and differentiation (P=0.002) .Patients with positive expression of T β_4 had a worse prognosis than those with a negative T β_4 expression (P <0.05) .**Conclusions** T β_4 in NSCLCs , is related to differentiation .TNM stage and metastasis , as well as the occurrence and the development of lung cancers. T β_4 might play an important role in the invasion , metastasis and prognosis of the NSCLC.

【Key words】 NSCLC; T β_4 ; Metastasis; Prognosis.

The clinical significance of thymosin β_4 and thymosin β_{10} expression in human non-small cell lung cancer.

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【 **Abstract** 】 **Objective** To make a thorough study on the clinicopathologic significance of the expression of human thymosin β_4 （Tβ_4）and thymosin β_{10} （Tβ_{10}）in human non-small cell lung cancer. **Methods** A total of 76 paraffin-embedded specimens were enrolled. Immunohistochemical staining and related software analysis were used. **Results** Both human Tβ_4 and Tβ_{10} were highly expressed in lung cancer while weak position in non-cancerous tissue. It expresses in cytoplasm associated in immunoreactive cells. Expression of Tβ_4 and Tβ_{10} was positively associated with TNM stage($r=0.239$, $P=0.032$; $r=0.263$, $P=0.018$ respectively) ,lymphatic metastasis ($r=0.243$, $P=0.029$; $r=0.286$, $P=0.009$ respectively), venous metastasis ($r=0.224$, $P=0.045$; $r=0.257$, $P=0.021$ respectively) .A negative correlation was found between expression of Tβ_4 and Tβ_{10} and differentiation ($r=-0.368$, $P=0.002$; $r=-0.277$, $P=0.046$ respectively) .Patients with positive expression of Tβ_4 and Tβ_{10} had a worse prognosis than those of negative expression ($P<0.05$) .**Conclusions** Both Tβ_4 and Tβ_{10} might play an important role in the invasion , metastasis and prognosis of the NSCLC.

【Key words】 NSCLC; Tβ_4; Tβ_{10}; metastasis; prognosis

INDUCTION OF APOPTOSIS IN OSTEOGENIC SARCOMA CELLS BY COMBYNATION OF TRAIL AND CHEMOTHERAPEUTIC AGENTS

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Abstract: Osteosarcoma is one of the most common primary malignant tumors of bone.It has a bad prognosis.TRAIL is amember of the tumor necrosis factor(TNF)cytokine family.TRAIL induces cell death in a variety of tumor cell lines but doesnot seem to be cytotoxic to many normal cell types in vitro.Its potent apoptotic activity is mediated through its cell surface death domain containing receptors,DR4 and DR5.TRAIL interacts also with 3"decoy"receptors that donot induce apoptosis,DCR1,DCR2.which lack functional death domains.and osteoprotegerin(OPG).The aim of our study was to investigate the cytotoxic activity of TRAIL and chemotherapeutic agents(MTX,DOX,CDDP)on established osteosarcoma cell line—OS-732.Then we combined TRAIL with chemotherapeutic agents to assess the synergistic effects.The rate of killing was 24.44% with TRAIL concentration of 100ng/ml for 24h.The cells were comparatively more responsive to DOX and CDDP with a dose-effect relationship.In OS-732 cells,DOX and CDDP cooperated synergistically with TRAIL,in that the combined treatment resulted in about 58.36% and 54.10%,respectively.With TRAIL alone or drugs alone.the percent apoptosis was less than 25%.However,the combination of TRAIL and MTX did not present synergistic effects on OS-732 cells.Compared with TRAIL alone.we did not see significant difference($P>0.05$).Our results indicate that osteosarcoma OS-732 cells were not responsive to TRAIL-induced apoptosis,DOX and CDDP sensitize osteosarcoma OS-732 cells to TRAIL-induced apoptosis.The combination of TRAIL and MTX presented no synergistic effects on killing OS-732 cells.

Key words:TRAIL,MTX,DOX,CDDP,osteosarcoma.apoptosis

THE UPREGULATORY EFFECT OF NGF ON IL-1 β IN THE LOWER RESPIRATORY TRACT AND VISCEROSENSORY AFFERENT SITES OF THE ASTHMATIC GUINEA PIG

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Objective: To explore the role and neuroimmune mechanism of nerve growth factor (NGF) in the pathogenesis of asthma. **Methods:** By means of immunohistochemistry combined with the micro-image analysis to investigate the changes of interleukin-1 β (IL-1 β) immunoreactivity and the regulatory effect of NGF in lower respiratory tract and viscerosensory afferent sites of the asthmatic guinea pig. The intensity of staining reactivity, determined by means of an image analysis system, was expressed as grey value (0-black to 255-white). **Results:** There was no obvious difference in the mean grey values of IL-1 β positive products between normal saline control group and simple sensitized group ($P > 0.05$). The mean grey values of IL-1 β positive products were significantly decreased in the lower respiratory tract, nerve terminals in vascular smooth muscle, C_7~T_5 spinal ganglia and corresponding spinal dorsal horn of the asthmatic guinea pigs compared with the normal saline control group and the simple sensitized group ($P<0.01$). However, the mean grey values of IL-1 β positive products in the lower respiratory tract, nerve terminals in vascular smooth muscle, C_7~T_5 spinal ganglia and corresponding spinal dorsal horn were much higher in NGF antibody group(inhalation of NGF antibody into nasal cavity) than in asthmatic group ($P<0.01$), indicating that NGF can upregulate the expression of IL-1 β. **Conclusion:** The present results indicate that the overexpression of IL-1 β induced by NGF might be one of the mechanisms for the involvement of NGF in the pathogenesis of asthma, and inhibitory expression of IL-1 β by NGF antibody in the lower respiratory tract and viscerosensory afferent system might be a new route for the prevention and treatment of the bronchial asthma.

[Key words] Interleukin-1 β; Nerve growth factor; Lower respiratory tract; Spinal ganglia; Spinal dorsal horn; Asthma; Guinea pig

UPREGULATION OF CALCITONIN GENE RELATED PEPTIDE LEVELS BY NERVE GROWTH FACTOR IN THE EXPERIMENTAL ASTHMATIC GUINEA PIG

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Objective: To explore the regulatory mechanism of nerve growth factor (NGF) on calcitonin gene related peptide (CGRP) in the lower respiratory tract and viseral sensory system of the experimental asthmatic guinea pig. **Method:** Radioimmunoassay was used to determine the alteration of CGRP levels in the lower respiratory tract and visceral afferent sites while NGF was absent (inhalation of NGF antibody through nasal cavity) in the asthmatic guinea pig. **Results:** The contents (pg/g) of CGRP in the trachea(71.72±17.82) , bronchus(75.56±19.23), lungs(82.85 ±23.33), C_7～T_5 spinal ganglia (83.83±21.29) and correspondent spinal dorsal horn(74.47±22.25), nodose ganglia(77.65±20.21) and solitary nucleus area(69.95± 12.27) in the asthmatic group were much higher than those in the normal saline control group [(48.06±12.32), (53.36±15.22), (62.39±20.25), (67.76±20.29), (60.63 ± 14.45), (65.54 ± 17.27), (59.33 ± 21.22)] ($P < 0.01$). However, the contents(pg/g) of CGRP in the trachea(35.25 ± 8.76), bronchus(42.26 ± 10.96), lungs(58.89±18.83), C_7～T_5 spinal ganglia (62.67±22.24) and correspondent spinal dorsal horn(56.55 ± 16.69), nodose ganglia(60.34 ± 17.97) and solitary nucleus area(54.46±16.62) were much lower in the experimental asthmatic guinea pig with NGF antibody in the respiratory tract than in asthmatic group ($P<0.01$). **Conclusions:** NGF upregulates the contents of calcitonin gene related peptide in the lower respiratory tract and visceral sensory sites of the experimental asthmatic guinea pig, and both might be involved in the pathogenesis of asthma.

【Key words】 Nerve growth factor; Calcitonin gene related peptide; Asthma; Guinea pig

Decreased mtDNA Copy Number of gastric cancer: a new tumor marker?

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Abstract:

Objective: To explore the relationship between mtDNA (mitochondrial DNA) and gastric cancer by comparing the difference of mtDNA copy number in gastric cancers and paracancerous tissues.

Methods: HV_1 (hypervariable region) and HV_2 of mitochondrial D-loop region from 20 cases of gastric cancer and 20 paracancerous tissues were amplified by PCR; meantime β-actin was served as a quantitative standard marker, followed by polyacrylamide gel electrophoresis (PAGE) and silver staining. in which the difference of mtDNA copy number was compared between gastric cancers and paracancerous tissues.

Results: There existed significantly quantitative difference in HV_1, HV_2 (standardized with β-actin) between gastric cancers and paracancerous tissues($P<0.01$). mtDNA copy number was associated with important enzymes in nucleus such as AKP, cAMP-PDE and cGMP-PDE ($P<0.05$), although not with tumor histological type and invasive depth ($P>0.05$).

Conclusion: The occurrence of gastric cancer was closely associated with decreased mtDNA copy number, which may be a new tumor marker.

Key words: gastric carcinoma; mitochondrial DNA; quantitative analysis; marker

Mutation of mitochondrial 12S rRNA in gastric carcinoma and their significance

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AIM: To detect the variations of mitochondrial 12S rRNA in patients with gastric carcinoma, and to study their significance and the relationship between these variations and the genesis of gastric carcinoma. Methods: PCR amplified mitochondrial 12S rRNA of 44 samples including 22 from gastric carcinoma tissue and 22 from adjacent normal tissues, were detected by direct DNA sequencing. Then lasar capture microdissection technique (LCM) was used to separate the cancerous cells and dysplasia cells from the patients with specific mutations. Denaturing high performance liquid chromatography (DHPLC) plus allele-specific PCR (AS-PCR), nest-PCR and polyacrylamide gel electrophoresis (PAGE) were used to further evaluate this mutant property and quantitative difference of mutant type between cancerous and dysplasia cells. Finally, RNAdraw bio-soft was used to analyze the RNA secondary structure of mutant type 12S rRNA. Results: Compared with mitomap database, some new variations were found, among which np652 G insertion and np716 T-G transversion were found only in cancerous tissues. There was a statistic difference in the frequency of 12S rRNA variation between intestinal type (12/17, 70.59%) and diffusive type (5/17, 29.41%) of gastric carcinoma ($P<0.05$). DHPLC analysis showed that 12S rRNA np652 G insertion and np716 T-G transversion were heteroplasmic mutations. The frequency of 12S rRNA variation in cancerous cells was higher than that in dysplasia cells ($P<0.01$). 12S rRNA secondary structure, while others such as T-G transversion did not. Conclusion: The mutations of mitochondrial 12S rRNA may be associated with the occurrence of intestinal gastric carcinoma. Most of variations exist both in gastric carcinomas and in normal tissues, and they might not be the characteristic of tumors. However, np652 G insertion and np716 T-G transversion may possess some molecular significance on gastric carcinogenesis. During the process from normality to dysplasia, then to carcinoma, 12S rRNA tends to transit from homoplasmy (wide type) to heteroplasmy, then to homoplasmy (mutant type, np717 T-G).

Protein Expression of KAI1 and nm23-H_1 in Gastric Cancer and Their Clinicopathological Significance

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Abstract

AIM: To compare the expression of tumor suppressor gene KAI1/CD82 and metastatic suppressor gene nm23 in gastric cancer tissues, and to find a predicted molecular marker for infiltration and metastasis of gastric cancer

METHODS: Rabbit anti-human KAI1 polyclonal antibody (1:30 dilution) and rabbit anti-human nm23-H_1 monoclonal antibody were applied to detect the expression level of KAI1 and nm23 respectively in eighty-seven cases of paraffin-embedded specimens of gastric cancer

RESULTS: Positive expression rate of KAI11 protein was 69% in gastric cancers, significantly lower than in para-cancerous tissues(84%). nm23-H_1 expression rates in gastric cancer and para-cancerous tissues were 33% and 59% respectively, $P<0.05$. KAI1 expression rate in gastric cancers with lymph node metastasis (60%) was significantly lower than in those with no lymph node metastasis(95%), $P<0.05$; KAI1 protein expression was associated closely with survival rate of patients with gastric cancer($P<0.01$), and higher KAI1 expression rate tended to be a longer survival time. Among gastric cancers with positive KAI1 expression, there was 45% of gastric cancers simultaneously with positive nm23-H_1 expression, and in 27 cases without KAI1 expression, only 7% cases with nm23-H_1 expression, there was statistically significant between the two groups, $P<0.05$.

CONCLUSION: KAI1 protein might be served as a new molecular marker to objectively predict prognosis of patients with gastric cancers. Gastric cancers with loss expression of nm23-H_1 posses even more invasive tendency, and more susceptible to lymph node metastasis.

Key words: KAI1 gene; nm23 gene; Gastric carcinoma; protein expression

Protein Expression of FHIT and nm23-H_1 and their clinicopathological significance in gastric cancer

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ABSTRACT

AIM: To explore the protein expression of FHIT and nm23-H_1 and their clinicopathological significance in gastric cancer.

METHODS: PV9000 two-step immunohistochemistry method was employed in this research to detect the expressions of FHIT and nm23-H_1 in 98 cases of gastric cancer.

RESULT: The positive rates of FHIT and nm23-H_1 expression were 38.8% (38/98) and 33%(28/87) respectively. The negative rate of Fhit protein expression in gastric cancer is 61.2%. The expression of FHIT correlated with the histological classification, Lauren classification and lymph node metastasis($P<0.05$). The postive rate of Fhit protein expression was more and more high when the gastric cancer was advanced, but it has no statistical significance($P>0.05$). The correlation analysis revealed a significantly reverse correlation between the expression of nm-23-H_1 and tumor stage ($P<0.05$).

CONCLUSION: □Which Fhit protein has no expression takes place frequently in gastric cancer. FHIT maybe an important candidate tumor suppressor gene in gastric cancer. □The expression of Fhit and nm23-H_1 protein have tight correlation with lymph node metastasis in gastric cancer, and they may work together. It was suggested that the expression of Fhit and nm23-H_1 can be important marker to predict the metastasis and prognosis of gastric cancer.

Expression of Kai1 and FasL in non-small cell lung cancer and their clinicopathological significance

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AIM: To explore the role of encoding protein of Kai1 and FasL genes in carcinogenesis and progression of NSCLC and its relevant molecular mechanisms.

METHODS: S-P immunohistochemistry was employed in specimens 79 cases of NSCLCS.

RESULTS: Kai1 was expressed in 55.7% (44/79) of NSCLCs, which was lower than that in the tissues adjacent to the tumor (82.6%, 38/46) ($P<0.05$). However, The positive rate (73.4%, 58/79) of FasL expression in primary NSCLC was significant, higher than that in its adjacent tissues (52.2%, 24/46) ($P<0.05$) .Consanguineous relationship between Kai1 and FasL expression in NSCLC

CONCLUSION: Down-regulation of Kai1 expression and up-regulation of FasL played an important role in carcinogenesis and progression of NSCLC. Reduced expression of Kai1 was involved in lymph node metastasis, progression and differentiation of NSCLC cells probably by increasing cell adhesion and decreasing cell mobility. FasL might contribute to lymph node metastasis, progression and differentiation of NSCLC by regulating tumor immune escape. They could be considered as molecular markers to reflect the progression of NSCLC. Additionally, positive correlation of FasL expression with Kai1 expression in NSCLC provided a novel insight into the regulatory effects of FasL expression on Kai1 expression, however, the regulatory action might not play an important role in the progress of the NSCLC.

Key word: Kai1 gene; FasL gene; Non-small cell lung cancer

The affective factors of the radiosensitivity in MGC803, ASPC-1 cell lines

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ABSTRACT

Objective: In solid tumors, hypoxia can promote malignant progression and confer resistance to irradiation and chemotherapy by altering gene expression. The aim of this study was to investigate the effect of hypoxia on cell cycles, expression of mitochondrion ATP6 (mt ATP6), mitochondrion Cyt-b (mtCyt-b), and PTEN mRNA, expression of vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) proteins, and radiosensitivity in a human gastric cancer cell line (MGC803). In addition, we studied the effect of heteroenous PTEN, LY294002 and PD98059, inhibitors of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), on human pancreas cancer cell line (ASPC-1) under normal oxygen and hypoxia conditions, probed into the mechanism that PTEN, LY294002 and PD98059 affect the proliferation and radiosensitivity of ASPC-1 cell under normal oxygen and hypoxia conditions. **Methods:** We measured the MGC803 cell cycles by flow cytometry, expression of mtATP6, mtCyt-b and PTEN mRNA by RT-PCR, and expression of VEGF and EGFR proteins by western blot assay in different time after hypoxia exposure. The survival rates of the cells irradiated(4Gy) or not were assessed by cologenic survival assay after 14days culture. And ASPC-1 cell was transfected with a eukaryotic expression plasmid (Peak8) containing PTEN or not in vitro by lipofectin, and then positive cell clones were selected and amplified. We measured hypoxic ASPC-1 cells irradiated (4Gy) or not by flow cytometry, western blot and cologenic survival assay, which were treated with heteroenous PTEN, LY294002 and PD98059. **Results:** The expression of PTEN was evaluated in MGC803 cell after hypoxia exposure in vitro showing maximal expression at 24hr hypoxia. Semiquantitative RT-PCR analysis showed that the relative expression of mtATP6 in MGC803 cell line after 24hr hypoxia was significantly reduced comparing with the expression under normal oxygen condition, and mtCyt-b was transiently reduced after 8hr hypoxia. The expression of VEGF and EGFR proteins was significantly increased after 24hr hypoxia. The MGC803 G_1 phase cell and apoptosis cell transiently increased after hypoxia comparing with normal oxygen condition. After 24hr hypoxic exposure, cell cycles distribution was similar to that of 0hr hypoxia. The surviving fraction of MGC803 cell without radiation under hypoxia condition gradually decreased, whereas, that with radiation gradually increased. A good correlation was observed between hypoxia time and surviving fraction in MGC803 cell irradiated or not. But there were no association between hypoxia time and the changes of cell cycles. After exposure in either LY294002 (20 μM) or

PD98059 (25uM), VEGF and EGFR expression remarkably decreased under hypoxia conditions. Cell cycles did not change significantly after hypoxia comparing with that under normal oxygen condition except for G_1 phase. The surviving fractions in hypoxia radiation group were higher than that in normal oxygen radiation group ($P<0.01$). Hypoxia cells treated with LY294002, with irradiation or not, had lower surviving fractions than those with PD98059. The surviving fraction of ASPC-1 cell under the cooperation of LY294002 and PD98059 had a remarkably effect than that under either one alone ($p<0.05$). We transfected plasmid pEAK8 contained PTEN or not into ASPC-1 cell and found that the PTEN mRNA and protein could be efficiently expressed in transfected ASPC-1 cell contained plasmid Peak8-PTEN, which could greatly enhance radiation-induced G_2/M arrest, apoptosis, growth inhibition and radiosensitivity. ASPC-1 cell transfected with the plasmid pEAK8 contained PTEN was preferentially killed through apoptosis under hypoxia conditions. The apoptotic peak of ASPC-1 cell transfected with PTEN was found much higher than that without transfection by FCM after 8hr hypoxia. **Conclusion:** Our findings suggested that hypoxia played an important role not only in modulating cell cycles of MGC803 and ASPC-1 cells but also in regulating the radiosensitivity of them, mightly, through stimulating the autocrine of VEGF, EGFR and upregulation of PTEN gene expression. It is concluded that surviving fractions that accepted irradiation or not in MGC803 cells were related to hypoxia time. Treatment with either LY294002 or PD98059 might remarkedly increased the radiosensitivity of ASPC-1 cell by inhibiting VEGF and EGFR expression under hypoxia condition. It is the inhibition of PI3K, but not MAPK, that led to a significant decrease in VEGF protein levels and an increase on the radiosensitivity after hypoxia exposure in ASPC-1 cells. The results demonstrated that PTEN could significantly increased the radiosensitivity of ASPC-1 cell by regulating VEGF and EGFR expression under hypoxia conditions.

Clinicopathological Significance of PTEN and Caspase-3 Expression in Breast Cancer

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Abstract

AIM: To discuss the possible biological significance of the PTEN and Caspase-3 protein in the tumorigenesis and progress in breast cancer, and the possible molecular pathologhic mechanism.

METHODS: Mouse anti-human PTEN monoclonal antibody (1:50 dilution) and rabbit anti-human Caspase-3 polyclonal antibody(1:100 dilution) was applied to detect the expression level of PTEN and Caspase-3 respectively in 95 cases of paraffin-embedded specimens of breast cancer by immunohistochemical method.

RESULTS: The expression of PTEN in breast cancer was significantly lower than that in control group ($P<0.01$). PTEN expression level was significantly negative correlated with TNM stage, histological grade, axillary lymph node status, recurrence and metastasis ($P<0.05$). The expression of Caspase-3 in breast cancer was significantly lower than control group ($P<0.01$). Five years survival rate (86.1%) with positive expression group of PTEN protein was significantly better than negative group (70.5%), and the difference between the two curve's distribution was significant by Log Rank test ($X^2=4.94$, $P<0.05$).

CONCLUSION: Down-regulated expression of PTEN and Caspase-3 made an important role during tumorigenesis of breast cancer. PTEN was implicated in progression of breast cancer probably by decreasing cellular adhesion,increasing cellular mobility and angiodenesis, and could act as an objective and effective marker to reflect the pathobiological behaviors of breast cancer. Low expression of PTEN could decrease expression of Caspase-3 to make apoptotic dysfunction of tumor cells. The expression of PTEN protein could probably act as a significant prognosis factor in breast cancer.

Key words: breast cancer; tumor suppressor gene; PTEN; apoptosis: Caspase-3; prognosis

Clinicopathological significance of maspin and mutant p53 protein expression in gastric cancer

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AIM: To detect the expression of maspin and mutant p53 protein, to investigate its biological significance and related molecular pathological mechanism in the carcinogenesis and progress of gastric cancer.

METHODS: Mouse anti-human maspin monoclonal antibody (1:50 dilution) and mouse anti-human p53 polyclonal antibody (1:100 dilution) were applied to detect the expression level of maspin and p53 respectively in 156 cases of paraffin-embedded specimens of gastric cancer by immunohistochemical method.

RESULTS: There were significant differences of maspin expression among the gastric cancer tissues (51.3%), intestinal metaplasia (95.3%) and normal gastric mucosa (0.0%) ($P < 0.001$). The positive rates of maspin protein expression in gastric carcinoma of early, middle and late periods were 56.3% (18/32), 39.3% (11/28) and 53.1% (51/96) respectively, there was no significant difference ($P > 0.05$). The mutant p53 protein was detected in 128 cases, and positive rate of expression was 46.1% (59/128). There was no correlation between expressions of maspin protein and p53 protein in gastric carcinoma tissues ($P > 0.05$).

CONCLUSIONS: The positive rate of maspin expression in gastric carcinoma was higher than that in the normal gastric mucosa, but lower than in the intestinal metaplasia, the cause of which might be due to the methylation status of maspin gene promoter. Inactivation of maspin expression may play an important role during the process of intestinal metaplasia transforming to the gastric carcinoma.

KEY WORDS: maspin, gastric cancer, p53

The study of HLA-DQA1 genotyping by oligonucleotide microarry

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Abstract

background and object

Human Leukocyte Antigen is the most complicated and polymorphism heredity system that has been discovered up to the present. The 13th International Histocompatibility Workshop and Conference (IHWC),which was held in February 2001, declared that the number of the discovered HLA alleles has reached 1340. The HLA system plays an very important role in antigen identification and presentation, immune response and regulation, and is also one of the key factors that affect the result of organic transplantation and hematopoietic stem cell transplantation. Most of all the clinical types HLA by serology at the present. But the general cross- reactivity and the lacks of the ideal antisera make it difficult in technique and the false result. Furthermore, the lack of the standard antisera also cause the "blank" result in a few newly discovered alleles. Labeled by the 12th IHWC held in 1996, HLA typing techniques enter DNA stage in general. For DNA typing in the single locus, PCR-SSP and PCR- SSOP were used more often now in the world. Although their result is more accurate than serology, the operation is too elaborate to use in large sample test. Gene-chip is a newly developed method which characterized as high-throughput and intensive, whose superiority fits the tedious research of HLA system. Though there are a large number of reports in domenstic and abroad about this, no mature HLA genotyping chip was produced until now .I'd like to fabricate the HLA genotyping chip by the method developed in our lab, and typing HLA allele in DQA1. I am in an attempt to establish a mature technique process to typing the complicated HLA system.

Materials and methods

1.prepare samples

extract genome DNA from 100 human anticoagulated whole blood by phenol/chloroform method, exam the concentration and purity respectively.

2. PCR amplification of the target sequence

design a pair of primers for HLA-DQA1 exon 2,and lable the sence strand by FITC. Make the fluorescenced strand the advantage strand in product by assymmetric PCR.

3. prepare the oligonucleotide probes

design 16 probes as four groups according to HLA-DQA1 sequence in GenBank and modify each probes by ammonia at 5'end. Then resuspended the Lyophilized probes in PH=9,0.1 carbonate buffer to 100 μM as explored in

advance.

4. prepare the spotting slides

rinse the glass slides in chromic acid solution for more than 12hrs, wash to clean, rinse in NaOH, in acetone, in arm molecule solution for connecting, roaste to fix, ready for use.

5. prepare the oligonucleotide microarray

add 16 prepared probes into 384-pole plate, meantime, add P2 ,low homologic probes and probe buffer in the same concentration as positive control , negative control and blank control respectively. Spot the microarray as designed by microarrayer. Then hydrate and roast to fix the probes, ready for use

6. block and hybridizate

block and wash the prepared slides by the method developed in our lab. Mix the PCR products and the hybridization buffer, cover each microarray, preserve in the capsule, 55 ℃,30 min to hybridize.

7. wash and exam

flush the cover slide, wash the microarray by 1×SSC/0.1％SDS, 0.5×SSC/0.1％SDS.0.1×SSC and ddH_2O which was preheated to 60℃ respectively, blow to dry. Exam and analyze the hybridization signal by Laser Cofocal Scanner and CCD imaging software. Determine the allele type of the sample.

Result

1. PCR amplification of HLA-DQA1

use the prepared samples as templates, amplify the target sequence. Examine the products by PAGE. The band is clear and 538bp in length as it shows, which matches the design.

2. the accuracy of the genotyping chip

SBT results match the genotyping result

3. the stability of the genotyping chip

to test the stability and reappearance rate of our chip, we choose 10 samples from 100 randomly, and genotype 5 times. The reappearance rate is 91.2%.

Conclusion

Genechip, as a newly developed method, has the prominent advantage of not only the high-throughput and intensive process, but also the direct, portable and easy-conserved result, Whose superiority fits the tedious typing research of HLA system. Relevant papers suggest, the oligonucleotide typing chip in our lab has a relatively simple technic route, and easy to operate. The specficity, sensitivity and stability can meet the genotyping of HLA system, which is a qualified method and can be used widely.

Key Words: HLA-DQA1;genotyping; oligonucleotide chip

Pathological features of endometrial stromal sarcomas

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Objective: To study the pathological features and immunophenotypes of endometrial stromal sarcomas.

Methods: 31 cases endometrial stromal sarcomas from The Affiliated Hospital of China Medical University were studied. The samples were stained with haematoxylin and eosin for histological examination. Immunohistochemistry studies were performed using monoclonal antibodies, against Syn, AAT, Lysozyme, Vimentin, CD34, myoglobin, SMA, EMA, CK.

Results: The patients' ages were from 32~76 years old, the mean age is 53 years old. 24 cases were low grade endometrial stromal sarcomas and 7 cases were high grade endometrial sarcomas. Endometrial stromal sarcomas were positive for Syn, AAT and Lysozyme and negative for CD34, myoglobin, SMA. Vimentin was focal positive. In stroma, SMA was strong positive.

Conclusions: It's important to distinguish low grade endometrial stromal sarcomas from high grade one, according to cellular atypia, invasive degree and immunotypes. It may deliver a significant guide to clinical therapy.

1 case uterine adenomoid tumor

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1. Case information:

The patient, female, 42 years old, was found uterine tumor and hospitalized.

Body examination: uterus was bigger, was touched 5cm×6cm tumor with smooth surface, mobile, no tenderness on the right.

Color ultrasonography: uterus was bigger, 5.5cm×6cm strong echo light mass was found on the right subserous, edge was not clear with uterine wall. It showed subserous myoma with vesicular and capsular surface and no adherence with surrounding tissues, but edge was not clear with cervix. No abnormality was found in bilateral adnex.

Fast cryopathological examination in operation: uterine benign tumor, may be adenomoid tumor.

Pathological examination: a tumor, volume 5cm×4.5cm×3cm with part smooth surface, a detachment surface. area 5cm×4cm, grey, many different size sac, the maximum diameter was 3cm with yellow viscofluid in it. Knife edge was found in right uterine serosa, length 3cm, cut uterus along front wall, length of uterus was 6cm. thickness of wall was 1.2cm, thickness of endometrium was 0.6cm. No abnormality was found in bilateral adnex.

Microscope examination: most tumor cells arranged different size and shape adenoid compartment with flat, cubic. columnar epithelium lined. Few tumor cells arranged irregular streak or snip. Part of tumor cells were vacuole. part were similar to signet ring cell. round or oval nuclei. thin chromatin, no karyokinesis. Stroma was few loose connective tissues and smooth muscle tissues. Tumor tissues infiltrated to myometrium, but no cellular atypia. PAS (+). Immunohistochemistry: catreti-nin (+), CKAE1 (+), Vimentin (+), Ⅷ factor (-), Actin (-).

Pathological diagosis: uterine subserous adenomoid tumor

2. Discussion:

Adenomoid tumor was common in epididymis and rare in uterus. This case was similar to subserous smooth myoma in clinic. Edge of tumor was clear, but no theca. Accurate diagnosis was necessary before operation. About its tissue occurrence was argued for a long time. Have been raised had mesonephridium, mucerian epithelium and endothelium. Peritoneal adenomoid tumor was found occasionally, among cells with tubular structure had connection and accompanied with peritoneal typical papillary mesothelioma occasionally, which showed that adenomoid tumor origin from mesonephridium.

The immunohistochemistry results of this case also supported this idea. Adenomoid tumor may be distinguished from following disease. (1) Lymphangioma: Adenomoid tumor cells arranged irregular streak lined with flat, cubic, columnar epithelium. PAS (+). (2) Adenocarcinoma: Adenomoid tumor was no theca, tumor cells arranged adenoid, but no atypia and karyokinesis, slow growth and no necrosis. Uterine adenomoid tumor was benign. no metastasis and no recurrent after resection.

A case report of testicular malignant lymphoma

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Introduction. We report a case of testicular malignant lymphoma. A 67-year-old man presented with a rice-size mass in the scrotal contents in January 1996 .It was diagnosed as preputial calculus. It didn't ameliorate after anti-inflammation therapy. He underwent surgical operation in April 1996 when it grew to egg-size.

Methods. Mass was 4×3×2cm with smooth surface, the tumor tissue are gray-white in color and its consistency is soft crispy like fish-meat appearance on the section of the tumor. There was clear boundary with peripheral spermatic cord and lipid without obvious hemorrhage and necrosis.

Results. Microscopic observation: normal structure of testis didn't exist, structure of deferent duct remain partly. Tumor cells infiltrated the stroma and permeate the seminiferous tubules. Tumor cells were lymphocyte-like in same size, small and round, strong nuclear staining with nuclear notch, no cytoplasm. Atypia can be seen. It was diagnosed as non-Hodgkin's lymphoma of the testis firstly. Then it was diagnosed as testicular malignant lymphoma(B line) by professor SongJiye of China Medical University. Immunohistochemistry: CD3- (negative), CD20−+ (moderately positive).

Follow-up:3 months after operation, tumor transferred to vertebra and lung. After radiotherapy and chemical therapy, patients were dead in September 2002.

Conclusions. Lymphoma is rare in all testicular germinoma. Malignant lymphoma comprises only 2-4% of all germinomas, which is the most common testicular tumor in persons above 60 years old with poor prognosis. Five-years survival rate is 0-12%. Most of patients die in the first or second year after operation.6 years survival time as this case is very rare.

One case report of appendix duplication

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Patient: Mr. Sun, Male, 16 years old. Admission number: 0206010015. Pathological number: 618. Native place: Liaoning Province. Profession: student.

Clinical History: Paroxysmal pain in the right lower quadrant 14h, no vomiting and diarrhea. Examination: soft abdomen, liver and spleen were not touched, tenderness and rebound tenderness in the right lower quadrant were obvious. Local muscular tension, decreased bowel sounds.

Clinical diagnosis: Appendicitis

Pathology: Gross: length of appendix: 7.0cm. smooth surface, hyperemia in capillary, two luminal were found, the one diameter was 1.0cm. occlusive. The other diameter was 0.6cm. dark red, local occlusive. Microscope: two tubular appendixes were packed in one muscular layer. The one submucosa fibrous tissue proliferated. Serous membrane, mesentery edema. Neutrophilic granulocytes infiltrated from mucous layer to serous membrane and mesentery.

Pathological diagnosis: 1. Appendix duplication

2. Phlegmonous appendicitis

Discussion: Appendix duplication was the rare malformation and often with cecum duplication. Appendix duplication may be two tubular appendixes were packed in one muscular layer or one appendix with another aplasia appendix from cecum. This case was two tubular appendixes were packed in one muscular layer.

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