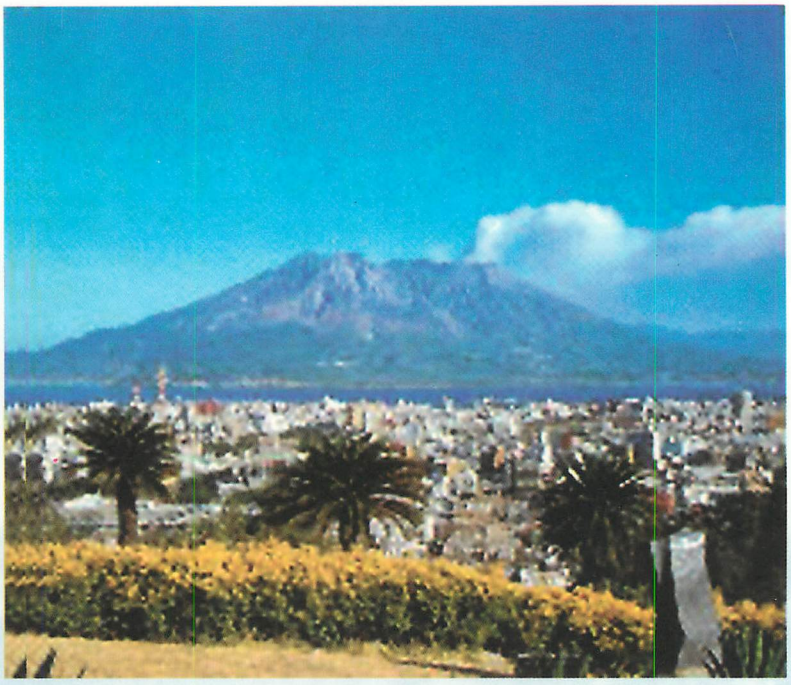
The Fifth International Symposium of Molecular Pathology

2007.7.31-8.2, Kagoshima, Japan



Supported by

Japanese Society of Pathology

Kagoshima University

China Medical University

第三届国际分子病理学研讨会

第四届国际分子病理学研讨会

CONTENTS

The Fifth International Symposium of Molecular Pathology

2007.7.31－8.2, Kagoshima, Japan

Organizing and Scientific Committee

President	Kazuhisa Hasui (莲井和久)	Kagoshima Univ. Japan
	Xinshan Jia (贾心善)	China Med. Univ. China

Japanese scientific committee

1)シンポジウム実施部門：蓮井和久、松山隆美、栄鶴義人、出雲周二、
西村俊秀、永井拓、田中将志、音田道治、奥村晃久

2)文化交流部門：佐藤栄一、野添良隆、早田隆

3)社会連携部門：野添良隆、日高旺、早田隆、平山申清

Chinese scientific committee

1)シンポジウム実施部門：賈心善、王恩華、関一夫、周宝森、趙雨杰

2)文化交流部門：王曼林

Secretary	Japanese	Kazuhisa Hasui(莲井和久)	Kagoshima Univ.
	Chinese	Shanliang Sun(孙善亮)	China Med. Univ.
		Nan Sun(宋楠)	China Med. Univ.
		Xiaobo Ma(马晓波)	China Med. Univ.
		Zhi Qu(曲智)	China Med. Univ.

General Information of the
Fifth International Symposium of Molecular Pathology

Date: 2007.7.31－8.2

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

Language: The official language of the symposium is English.

Wear: Informal.

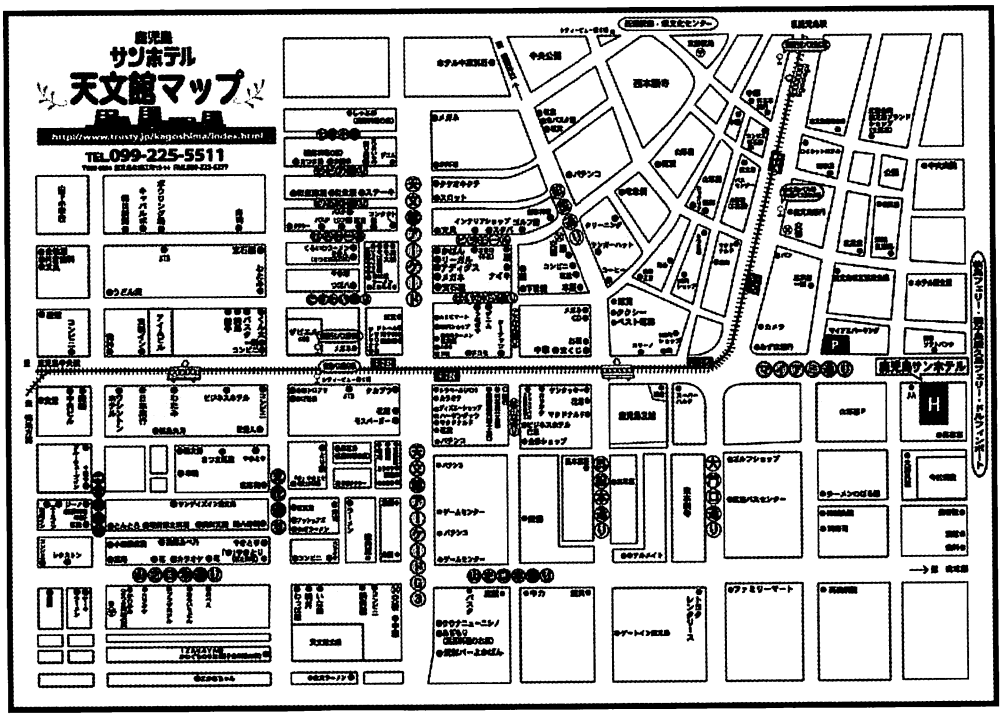
Registration: Most of the participants are pre－registered. People who want to attend the symposium must contact the Japanese side President of the symposium.

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 12min and left at least 3 min for discussion.

Map:



Greetings:

The Fifth International Symposium of Molecular Pathology
2007.7.31－8.2, Kagoshima, Japan

Greeting from Kagoshima:

Ladies and Gentlemen;

I am sincerely grateful that the Fifth International Symposium of Molecular Pathology will be held in Kagoshima through the long efforts of Dr. Sato, Eiichi, Professor Emeritus of Kagoshima University, Dr. Hasui, Kazuhisa, Senior Assistant Professor of our graduate school of Medical and Dental Sciences and Dr Jia Xinshan, Professor of China Medical University.

I have heard that the International Symposium of Molecular Pathology has been held continuously since 1998 especially on inland China with the goal of promoting young Chinese researchers' participation as a kind of grass－root academic exchange activity between China and Japan. Based on the committee members' efforts both in China and Japan including other regular participants from the Don Huang Association in Kagoshima, it is very significant that we are able to hold this memorable symposium in Kagoshima.

Since China Medical University and Kagoshima University concluded a cooperative relationship in 1993, we have pursued various exchange activities. I am convinced that this symposium is one of the most outstanding activities that we have organized so far between both our universities.

It is my sincere hope that this symposium will lead to the establishment of a viable network of researchers in Mainland China and Kagoshima not only in the field of Molecular Pathology but also other various important fields. This will enable the development of new joint research and projects between Chinese institutions and Kagoshima University.

Finally, I am certain that the results of this memorable symposium from the up－to－date research presentations and active discussion that will take place will be fruitful and above all beneficial to the medical world.

I look forward to seeing you in Kagoshima, soon.

Yoshida, Hiroki, M.D., Ph.D.

President of Kagoshima University

2007.07.31

Ladies and Gentlemen:

First of all, on behalf of China Medical University and in my own name, I wish to extend my warm congratulations to you for the opening of the Fifth International Symposium of Molecular Pathology held in the beautiful Kagoshima University. The First International Symposium of Molecular Pathology was held in 1998. During the past nine years, ISMP played a great role in communication between Japan and China, not only in the academic field but also in cultural areas. It has also strengthened friendship and cooperation between Japan and China. I think all these achievements could not have happened without the hard work of the sponsor and great support from the participants. Today, Kagoshima University is opening arms to welcome us with her beauty and hospitality. I believe that with their kind suport this symposium will be susscessful.

Kagoshima University is a sister univeristy of China Medical University. Over the years, the two universities have had constant cooperation and exchange in scientific research and teaching. I want to take this opportunity to express my sincere hope that the friendly relationship between the two universities will become even stronger.

In September, there will be a pathology seminar between Japan and China held in China Medical University. I am looking forward to seeing you in Shenyang.

Finally, let me wish the Fifth International Symposium of Molecular Pathology in Kagoshima a complete success.

Thank you.

Honorary Chairman of the 5th International Symposium of Molecular Pathology

President of China Medical University

Wanjin Dai

2007.07.31

Ladies and Gentelmen:

It is pregnant with meaning to held the fifth International Symposium of Molecular Pathology in Kagoshima, Japan.

According to the will of the late of Prof. Keiichi Watanabe to communicate in the field of Pathology between Japan and China and under the understanding of emeritus Prof. Eiichi Sato, Prof. Hiroshi Nagura and Prof. Kouki Inai (the Japan – side Presidents) and the members of Japanese and Chinese Scientific Committees about the communication, Prof. Xinshan Jia (China – side President) and I (Dr. Kazuhisa Hasui, Japan – side Secretary) have been promoting to held the previous symposiums in the landlocked area of China (Dunghuag, Chengdu, Kunming and Urmuqi in order to activate the communication between Japan and China in the field of pathology and in the arts of the history between Japan and China. The first symposium was the first scientific meeting hold in Dunghuang. Besides at the oral presentations, around the poster presentation set up on the floor of the hotel restaurant active participants hear the explanation attentively and discussed with presenters with eagerness. In the third symposium in Kunming, graduate school students of Prof. Xinshan Jia made the poster presentations, the Japanese active participants were eager to find out the talent in Pathology in them, and the symposium awarded the prize of the young Chinese researcher to them. In the fourth symposium we received a financial support in a part from Kagoshima University (President Yukihiro Nagata), awarded the prize from Kagoshima University President to the significantly active participants and held the post – congress meeting of Japanese participants to determine to hold the fifth symposium in Kagoshima, Japan.

On the other hand, sympathetic friends in Kagoshima associated these symposiums, organized a group "TonKouKai" in Kagoshima (President: emeritus Prof. Eiichi Sato, Adviser: Mr. Umashi Hidaka, Secretary General: Director Dr. Yoshitaka Nozoe), and promoted the friendship between Japan and China to awarded the prize from the TonKouKai" to the significantly active Chinese participants.

As for this fifth symposium in Kagoshima, the symposium is backed up by the Japanese Society of Pathology (President Yoshiyuki Osamura) and Kagoshima University (President Hiroki Yoshida). And this International Symposium of Molecular Pathology itself is qualified as the meeting of the lifelong education for the specialists for the pathology. Above all, the attendance of so many Chinese pathologists and friends to this symposium hold in Kagoshima, Japan, is the historical event in the communication between Japan and China. I believe that it is sure that this symposium will open the new friendly relationship between Japan and China in the fields of pathology, medicine and the arts.

Japan – side President of the fifth International Symposium of Molecular Pathology
Dr. Kazuhisa Hasui
Assistant Professor.
Div. of Persistent & Oncogenic Viruses
Kagoshima University Graduate School of Medical and Dental Sciences
2007.07.31

List of Chinese participants

Academic Part.

Prof.	Wanjin Dai	China Medical University
Prof.	Xinshan Jia	China Medical University
Prof.	Xueshan Qiu	China Medical University
Prof.	Daorong Zhang	China Medical University
Prof.	Guanping Wu	China Medical University
Prof.	Baosen Zhou	China Medical University
Prof.	Xiaoyi Mi	China Medical University
Prof.	Yifu Guan	China Medical University
Prof.	Huailiang Wang	China Medical University
Prof.	Yujie Zhao	China Medical University
Prof.	Xiubin fang	China Medical University
Prof.	Shuli Liu	China Medical University
Prof.	Guangyu Yu	Liaoning Panjin No.2 Hospital
Asso.Prof.	Jie He	China Medical University
TA.	Zhihua Yin	China Medical University
TA.	Miao He	China Medical University
TA.	Mingchuan Li	China Medical University

Social part

Chief.	Manlin Wang	China Medical University
Staff.	Dongfang Li	Shengjing Bank

Associating persons

Mrs.	Jiafeng Yang
Miss.	Yikang Zhang
Miss.	Zhaoqing Li
Mr.	Hao Zhou
Miss.	Xizi Luo

List of Japanese participants

President	吉田浩己	Haruki Yoshida	Kagoshima University
emeritus Prof.	佐藤栄一	Eiichi Sato	Kagoshima University
emeritus Prof.	名倉　宏	Nagura H	Sendai Shakaihoken Hospital
emeritus Prof.	高橋　潔	Kiyoshi Takahashi	Kumamoto University
Prof.	井内康輝	Kouki Inai	Hiroshima Univeresity
Prof.	堤　寛	Yutaka Tsutsumi	Fujita Health University
Prof.	大井章史	Akishi Ooi	Kanazawa University
Prof.	竹屋元裕	Motohiro Takeya	Kumamoto University
Prof.	渡邊昌俊	Masatoshi Watanabe	Yokohama National niversity
Prof.	朔　敬	Takashi Saku	Niigata University
Prof.	松山隆美	Takami Matsuyama	Kagoshima UNiversity
Prof.	栄鶴義人	Yoshito Eizuru	Kagoshima University
Prof.	出雲周二	Shuji Izumo	Kagoshima University
Dr.	蓮井和久	Kazuhisa Hasui	Kagoshima University
Dr.	田中将志	Masashi Tanaka	Kagoshima University
Dr.	王　嘉	Jia Wang	Kagoshima University
Dr.	浅川明弘	Akihiro Asakawa	Kagoshima University
Dr.	兒島真哉	Shinya Kojima	Kagoshima University
Dr.	西村俊秀	Hidetoshi Nishimura	Kagoshima University
Dr.	永井　拓	Taku Nagai	Kagoshima University
Dr.	音田道治	Michiharu Onnda	Kagoshima University
Director Dr.	野添良隆	Yoshitaka Nozoe	Chuuou Building Nozoe Dental Clinic
Dr.	奥村晃久	Teruhisa Okumura	Kagohsima Seikyo Hospital
Prof.	早田　隆	Takashi Hayata	Kagoshima Women's College
Director Dr.	平山申清	Koushin Hirayama	Hirayama Dental Clinic
Mr.	日高　旺	Umashi Hidaka	Previous President of KTSScientific

Scientific Program

2007.7.31

10:00 Opening

Chairman: Xinshan Jia(贾心善) China Medical University

Kazuhisa Hasui(莲井和久) Kagoshima University

10:00 Greeting

Haruki Yoshida (吉田浩己) President of Kagoshima University

Wanjin Dai (戴万津) President of China Medical University

10:10 CULTURAL LECTURE Chairman: Eiichi Sato

Yoshitaka Nozoe (野添良隆)

Chuu－Ou Building Nozoe Dental Clinic, TonKouKai

A Legend of the Coming of Jofuku in Japan.(徐福降临日本)

10:40 Photography of the symposium

11:00 SPECIAL LECTURE 1 Chairman: Kouki Inai

Yifu Guan (关一夫)

China Medical University

Ultrasensitive Nucleic Acid Detection with Nano－Structured Biosensor and Possible Applications to SNP Detection.

11:30 SPECAIL LECTURE 2 Chairman: Yifu Guan

Kouki Inai (井内康辉)

Hiroshima Univeresity

Seminar on Pathological Diagnosis of Mesothelioma.

12:00 Luncheon

13:30 SPECIAL LECTURE 3 Chairman: Xiaoyi Mi

Yutaka Tsutsumi (堤宽)

Fujita Health University School of Medicine

Application of Histo－ and Cytochemical Techniques to Pathological Diagnosis of Infectious Diseases.

14:00 LECTURE 1 Chairman: Nagura H

Daorong Zhang (张道荣)

China Medical University

Expression of TSG101 And its Correlation with MDM2 In Squamous Cell Carcinoma And Deocarcinoma Of Lung

14:15 LECTURE 2 Chairman: Daorng Zhang

Nagura H (名仓宏)

Sendai Shakaihoken Hospital

The Evaluation of a Diagnosis of Prostatic Adenocarcinoma in Needle Biopsy Specimens by Immunohistochemistry

14:30 LECTURE 3 Chairman: Akishi Ooi

Xiaoyi Mi (米小轶)

China Medical University

Expression of TRAF1 and TRAF2 and Their Interaction in the Different Metastasis Breast Cancer Cell Lines.

14:45 LECTURE 4 Chairman: Yujie Zhao

Akishi Ooi (大井章史)

Kanazawa University

Non－incidental Co－amplification of Myc and ERBB2, and Myc and EGFR, in Gastric Adenocarcinomas.

15:00 LECTURE 5 Chairman: Kazuhisa Hasui

Guangping Wu (吴广平)

China Medical University

The Clinical Application of Liquid－based Cytological Test to the Screening of Sputum Cytology for the Diagnosis of Lung Cancers.

15:15 LECTURE 6 Chairman: Huailiang Wang

Motohiro Takeya (竹屋元裕)

Kumamoto University

Class A Macrophage Scavenger Receptor (CD204) Attenuates the Risk of Cardiac Rupture After Experimental Myocardial Infarction.

15:30 LECTURE 7 Chairman: Eiichi Sato

Mingchuan Li (李鸣川)

China Medical University

Impact of XRCC1 Polymorphisms Arg399Gln and Arg194Trp on Risk of Lung Cancer in Non－smoking Women.

15:45 LECTURE 8 Chairman: Baosen Zhou

Kazuhisa Hasui (莲井和久)

Kagoshima University

A Nasal NK/T－cell Lymphoma in the Northeast Region of China.

16:00 LECTURE 9 Chairman: Motohiro Takeya

Yujie Zhao(赵雨杰)

China Medical University

To Study Immunophenotyping of Acute Leukemia by a Cell Microarray.

16:15 LECTURE 10 Chairman: Yifu Guan

Masashi Tanaka (田中将志)

Kagoshima University

The Z39Ig: a Specific Marker of Synovial A Cells in RA Synovium.

16:30 LECTURE 11 Chairman: Izumo S

Jia Wang (王嘉)

Kagoshima University

An Immunohistochemical Analysis of Proliferation, Apoptosis and Stem Cell Phenotype Expression in Human Testicular Spermatogenesis.

16:45 LECTURE 12 Chairman: Yutaka Tsutsumi

Huailiang Wang (王怀良)

China Medical University

Pulmonary Hypertension Mechanism Relevance to 5－Hydroxytraptamine, Receptors and Transporters

17:00 LECTURE 13 Chairman: Yujie Zhao

Izumo S (出云周二)

Kagoshima University

HIV Encephalitis and Diffuse Microglial Activation Occur Independently in the Brain of HIV－1 Infected Patients.

17:15 LECTURE 14 Chairman: Huailiang Wang

Akihiro Asakawa (浅川明弘)

Kagoshima University

Ghrelin and Growth Hormone Secretagogue Receptor as Therapeutic Targets for Obesity and Type 2 Diabetes.

17:30 LECTURE 15 Chairman: Guangping Wu

Shinya Kojima (兒島真哉)

Kagoshima University

Altered Ghrelin and PYY Responses to Meals in Bulimia Nervosa.

17:45 LECTURE 16 Chairman: Xiaoyi Mi

Sukalyan Kundu

Niigata University Hospital

Differential Expressions of Retinoid Receptors in Oral Squamous Cell Carcinomas

18:00 Closing Chairman: Kazuhisa Hasui

CATALOG

A Legend of the Coming of Jofuku (徐福) in Japan

Yoshitaka Nozoe (野添良隆)
Director Doctor, Chuu－Ou Building Nozoe Dental Clinic
Secretary－general, TonKouKai (敦煌会)

It is elucidated by means of the analysis of mitochondrial DNA that Japanese are of mixed ancient Asian people (1). The family of the Japanese Emperor has being said by themselves to have ancestor who came to Japan through the Korean Peninsula, whereas the most ancient Japanese history books Kojiki (古事記) and Nihon Shoki (日本書紀) wrote a legend of the Emperor being descendant of the gods. In order to understand the contradiction between these two accounts, we should discuss the legends of Jofuku (徐福) and the dynasty of the gods in Japan.

In around 210BC, according to the order of the first emperor in China (秦始皇帝) to get the elixir of life, Jofuku having magic and medical knowledge came to Japan with about 3000 young Chinese. There were following events suggesting his arrival in Japan. As general aspects between China and Japan, 1) According to the Chinese oldest medical book (黄帝内径) recovered from the burning of the Chinese classics and the burning of Confucian scholars alive in China (213BC), the oriental medicine that was almost the same as the modern one had been already established. It was thought that this oriental medicine was introduced in ancient Japan. Jofuku with magic and medical knowledge probably looked to be as a god to Japanese at that time. 2) The shell－money (Crowrie (宝貝、子安貝)gotten only in the near sea of Japan, Okinawa) was used in the ancient China more than 3000 years ago, suggesting the trading between ancient China and Japan. On the above historical background, 3) the settlement of Jofuku in Japan was memorized in the legends in the all regions of Japan. 4) Chinese ancient coins (半両銭) from 350BC in the period of Shin were excavated in the all region of Japan, suggesting the largeness of the settlement of Jofuku. 5) The skeletal structure of Japanese changed suddenly between the Jomon (縄文) period and the Yayoi (弥生) period. The background of agricultural era, only the environmental change could not explain this skeletal structural change of Japanese at that time. The large scale of his settlement might explain it since the population of Japanese at that time was estimated as 50 thousands to 300 thousands.

There were mysteries in the description of the most ancient Japanese history books Kojiki and Nihon Shoki. a) Why did the descendant of the gods fall in the barbarous Hyuga (日向) region in the Kyushu island (九州) rather than the Yamato (大和) region near the first ancient Yamato imperial court (大和朝廷) in the Honshu island (本州)？It is curious for the first ancient Yamato imperial court not to have payed their respects to the Hyuga region. b) The descendant of the gods and an ancestor of Japanese emperors, Niniginomikoto (ニニギノミコト), married to Konohanasakuyahime (コノハナサクヤヒメ), a princess of ancient Kumaso people (熊襲族) in the Hyuga region. When ancestors of Japanese emperors have been defined as gods, their blood might be mixed. c) Since it is clear that a human can not fall from the heaven to a peak of mount Takachiho, why did they write the first step of the descendant of the gods in south Kyushu region？d) The places, where Niniginomikoto landed from the sea after his coming from the heaven, were recorded as Kasasa (笠沙), Osaki (大崎), Utinoura (内之浦), Saito (西都) and Miyazaki (宮崎). These places located in the south Kyushu region. The pronounciation "Ama" of the heaven (天) might be that of sea (海. e) The mistery about the long life of the Japanese ancient Emperors: The question when the Japanese first emperor Jinnmu (神武天皇) ascended the throne is a non－sense one in the period when a calendar could not be believed. On the other hand, it is unbelievable that 11 of 16 emperors after the Japanese first emperor Jinnmu spent a long life beyond 100 years.

Taken together the legends of Jofuku's coming and the mysteries in the description of the most ancient Japanese history books, Kojiki and Nihon Shoki, it seems possible to set up a bold hypothesis that the Japanese

first Emperor Jinnmu might be Jofuku. On this bold hypothesis, Japan would be founded about 210BC rather than 660BC and the mysteries in the description of the most ancient Japanese history books, Kojiki and Nihon Shoki, might be solved. However, there remains a problem that Jofuku in the Japanese legends means often people from the foreign countries over the sea rather than the Jofuku mentioned above

1) An origin of Japanese. in the DVD book, NHK Special "A human body, 3rd. A miracle space", NHK Enterprise. 2003/10/24, in Japanese

2) Yoshitaka Nozoe. Jofuku's coming, Kizuna "絆" Vol. 4 pp. 51 – 110, 2005

Ultrasensitive Nucleic Acid Detection with Nano – structured Biosensor and Possible Applications to SNP Detection

Ying Yuan, Liancheng Sun, Tianbiao Zhang, Xianyu Piao, Yifu Guan

Department of Biochemistry and Molecular Biology, China Medical University, Shenyang, 110001, P. R. China

The accomplishment of Human Genome Project has stimulated our interest in identifying diseases based on the genetic information even before the abnormal symptoms can be noticed. Consequently, detection of particular gene mutation, copy number variation, and single – nucleotide polymorphism becomes extremely important for diagnosis and treatment of the gene – related diseases. Therefore, there is a great desire for sensitive, accurate, rapid, cost – effective, and user – friendly approaches for gene analysis and detection.

Recently, we have developed an approach to fabricate nano – structured biosensor for nucleic acid detection. Polyaniline (PANI) nanotube array was synthesized on a graphite electrode using the template – assisted method. Single stranded oligonucleotides were then covalently immobilized on the PANI nanotube array. Hybridization was accomplished by immersing this electrode in solutions containing perfect – match targets and mismatch oligonucleotides. The performance of this biosensor was evaluated with differential pulse measurement and daunorubicin (DNR) as the indicator. Experimental results showed that the PANI nanotube electrode improves the detection sensitivity significantly to the level as low as 9.0 – 16 mol/L. This PANI biosensor also demonstrated a great ability to identify different nucleotide fragments with even single – mismatch. The achieved technical advantages were rationalized in terms of the fact that the nanotube array structure provides a large surface area for single stranded oligonucleotide immobilization and a well – aligned structure for fast hybridization kinetics.

For point – of – detection purpose, we continued a thorough investigation to determine the parameters which affect the detection accuracy. A complete set of oligonucleotides with single – mismatch, double – mismatch, insertion, deletion was designed to examine their effects on the thermal stability of these hybrids. Experimental results demonstrated the significant effects of hybridization variations on the detection specificity, and the effects were interpreted on the basis of hybridization structures. Current investigations provide valuable information on the oligonucleotide probe design and hybridization condition selection as well as the guidelines on the quality control of microarray.

Seminar on Pathological Diagnosis of Mesothelioma

Kouki Inai

Hiroshima Univeresity

1. Purpose: The occurrence of mesothelioma has been increasing in Japan. In the former time, mesothelioma was

an occupational cancer related to asbestos exposure, however recently it occurs in the person with no occupational history. The huge use of asbestos in 1960's to 1990's in Japan is supposed to be cause of the increase of mesothelioma. In China, the amount of use of asbestos has kept to be high－level until now, therefore in the near future the incidence of mesothelioma will increase as well as in Japan.

The pathological diagnosis of mesothelioma is difficult because of its rarity and histological variation. However, recent progress of immunohistochemical stainings can lead to accurate diagnosis. In this seminar, the classical and rare cases of mesothelioma will be presented and the way of accurate pathological diagnosis will be introduced.

2. Contents: Photograph presentation and discussion

1) Epithelioid mesothelioma of pleura vs adenocarcinoma of lung

2) Epithelioid mesothelioma vs reactive mesothelial cell hyperplasia of pleura

3) Epithelioid mesothelioma of peritoneum vs serous papillary carcinoma of ovary

4) Sarcomatoid mesothelioma of pleura vs sarcomatoid carcinoma of lung

5) Desmoplastic mesothelioma of pleura vs fibrous pleuritis

6) Biphasic mesothelioma of pleura vs synovial sarcoma of pleura

Application of Histo－ and Cytochemical Techniques to Pathological Diagnosis of Infectious Diseases

Yutaka Tsutsumi

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

E－mail: tsutsumi@fujita－hu.ac.jp, HP: http://info.fujita－hu.ac.jp/pathology1/

It is of no doubt that the detection of infectious agents within the lesion is essentially important for the pathological diagnosis of infectious diseases. Immunohisto－ and cytochemical and non－isotopic in situ hybridization demonstration of the pathogens is useful enough for this purpose. When one knows the cross－reactivity and specificity of the antibodies and probes, as well as the sensitivity and specificity of the methods to be applied, the results will lead the patients directly to the appropriate diagnosis and treatment.

In the present review lecture, variegated applications of the histo－ and cytochemical techniques to the diagnosis of infectious diseases in surgical pathology and clinical cytology are presented. Five technical and practical aspects are described, including A) representative immunostaining with commercially available antibodies, B) use of diluted patients' sera as the primary antibody, C) comparison of immunostaining with non－isotopic in situ hybridization, D) use of cytology specimens for cytochemical demonstration of pathogens, and E) ultrastructural demonstration of pathogens in routine material. Representative infectious cases in which histo－ and cytochemical techniques considerably contributed to settling the final diagnosis are also presented.

A) Use of commercially available antibodies

1) Mycobacterial antigens are demonstrated in tuberculosis, non－tuberculous mycobacterial infection and leprosy by using a BCG antiserum, with much higher sensitivity than the Ziehl－Neelsen's acid－fast method. It is applicable to archival leprosy tissues fixed in formalin for more than 60 years.

2) Chlamydial and bacterial epididymitis are distinguishable by immunostaining for C. trachomatis and E. coli. The chlamydial antigens were shown in pap－stained cytology preparations after bleaching the dyes in acid alcohol. Malakoplakias or xanthogranulomas in the urinary bladder, kidney, colon, oviduct, gallbladder and bile duct were positive for the E. coli antigens.

3) Antiserum to Treponema pallidum is quite powerful to make a definite diagnosis of clinically unsuspected syphilitic lesions.

4) Distinction of hyphae – forming fungi such as Aspergillus, Mucor and Candida, as well as yeast – form fungi such as Cryptococcus and Histoplasma, is assisted by immunostaining when the specific antibodies are available.

5) Other examples include immunostaining for Helicobacter pylori, human herpes viruses, hepatitis viruses, parvovirus B19, adenovirus, polyoma viruses, influenza viruses, measles virus, Toxoplasma, Pneumocystis jiroveci and so on.

6) Some monoclonal antibodies require heating treatment (pressure pan heating) of paraffin sections prior to immunostaining. The targets include heat shock protein (HSP) – 60 of bacterial origin, penicillin – binding protein 2' (PBP2') expressed on MRSA, pneumolysin in Pneumococcus, hepatitis C viral antigen, rickettsial antigen, etc. It should be notified that protein A reactivity on Staphylococcus aureus and MRSA is retrieved by the heating pretreatment, so that nonspecific binding of human IgG to protein A can occur.

7) In general, the sensitivity of detection is more important than the specificity. The reasons for this are as follows. a) Basically, the histologic pattern of host reaction is the key aspect for suspecting the causative agent. b) Determination whether chlamydial, bacterial, mycobacterial, fungal or viral infections should be a tentative goal of the report of histologic examination.

B) Use of patients' sera

Patients' sera at a 1:500 to 1:1,000 dilution become convenient probes for indirect immunoperoxidase localization of pathogens in formalin – fixed, paraffin – embedded sections, particularly when cellular tissue reaction such as abscess and granuloma is evident histologically. Examples include staphylococcal pyoderma, cat scratch lymphadenitis, cryptococcosis, sporotrichosis, malasseziasis, alternariosis, amebic dysentery, acanthoamebiasis, cryptosporidiosis, isosporiasis, cutaneous and visceral leishmaniasis, schistosomiasis, gnathostomiasis, liver ascariasis, cysticercosis, etc. Endogenous human IgG in sections is scarcely detected by the peroxidase – labeled secondary antibody. In order to avoid the biohazard, sera from patients with hepatitis or AIDS must not be used.

C) Immunostaining vs. non – isotopic in situ hybridization

Both methods are comparable in detecting infection of cytomegalovirus (CMV) in paraffin sections. In contrast, human papillomavirus (HPV) and Epstein – Barr virus (EBV), the oncogenic viral antigens are less commonly detectable than the viral genomes in uterine cervical lesions such as condyloma and dysplasia, and in Hodgkin's and non – Hodgkin's lymphomas and gastric or nasopharyngeal carcinomas, respectively. In replicative carriers of hepatitis B virus in which HBV genome is demonstrable by in situ hybridization, HBs antigen is mainly expressed on the plasma membrane of the hepatocytes, HBc antigen in the cytoplasm and HBe antigen in the nucleus.

D) Use of cytology specimens for cytochemical demonstration of pathogens

In order to use cytology specimens for histochemical study, the "cell transfer" technique can be applied. After removing the cover slip of a pap – stained cytology preparation in warm xylene, mounting resin such as Mount – Quick or Malinol is covered. The solidified resin membrane is removed in warm water, when all the cells are transferred to the resin side. The resin membrane is tightly placed on another silane – coated glass slide. After enough drying, the resin is removed in xylene. If the resin membrane is cut into several pieces, multiple cytology specimens are ready for cytochemistry and partial preservation of the pap preparation. HPV genome can be demonstrated on the pap – stained smear of cervical dysplasia in such a way. This technique can be applied also to paraffin sections and adhesive cells cultured on plastic plates. Of note is that Giemsa – stained preparations are not suitable for this purpose.

E) Ultrastructural visualization of pathogens in routine material

The antigens of C. trachomatis, E. coli, CMV and HPV can be seen directly in paraffin sections or pap – stained cytology preparations by applying the technique of pre – embedding immunoelectron microscopy. This ap-

proach is useful to confirm the presence of pathogens within the lesions and the specificity of the antibodies. Ultrastructural identification of the HPV genome is described: In a biopsy specimen of severe dysplasia of the uterine cervix, the HPV16 genome is localized on a part of the chromatin network, while the viral antigen can be seen on the viral particles in the nucleoplasm in biopsied verruca vulgaris.

Case presentations

Case 1. 45 year－old male (syphilis)

The patient treated for malignant lymphoma four years complained of skin rash (papules) on the neck and face measuring 8－10 mm. Skin biopsy was performed under the clinical diagnosis of skin recurrence. Histologically, marked lymphocytic and plasmacytic infiltration was shown in the dermis. Immunostaining for T. pallidum revealed numerous spiral pathogens in the epidermis, confirming the diagnosis of secondary syphilis. Serological confirmation followed in this case. Reaching the exact histopathological diagnosis is solely dependent upon whether or not the pathologist can suspect the possibility of syphilis in H&E preparations.

Case 2. 45 year－old male (CMV esophagitis in AIDS)

His chief complaint was heartburn. Endoscopy pointed out erosive esophagitis, and the biopsy specimen revealed chronic active inflammation with suspected nuclear inclusions in endothelial cells. CMV antigen was detected. The pathologist's advice prompted the serological confirmation of the HIV carrier state.

Case 3. 32 year－old male (visceral leishmaniasis)

After working in India for four years and then in Australia for one year, this businessman complained of high fever, headache and malaise. His general condition was not good because of thrombocytopenia (DIC) and liver dysfunction. Liver biopsy revealed multifocal microgranulomas, histologically suggesting Q fever, brucellosis and mycobacterial infection. The 1:500 diluted patient's own serum showed protozoa－like agents in the cytoplasm of activated Kupffer cells and epithelioid cells, and visceral leishmaniasis (Kala Azar) was then suspected. Serological confirmation followed.

Case 4. 60 year－old male (acanthoamebic meningoencephalitis)

The patient suffering from alcoholic liver cirrhosis manifested progressive left hemiparesis. CT scan revealed multifocal low－density areas in the right cerebral hemisphere. Brain biopsy demonstrated perivascular gliosis and mononuclear cell accumulation without giant cell formation. Macrophage－like cells resembling amebic trophozoites and cysts were scattered around the vessel. The patient's own serum was strongly reactive with these infectious agents. The mouse antiserum panel against the Acanthoamoeba species immunohistochemically confirmed the opportunistic infection by A. culbertsoni: Antisera against A. polyphaga and A. castellani were non－reactive.

Case 5. 15 year－old boy (influenza encephalopathy)

This fat boy weighing 90 kg was mildly febrile for two days in May. He fell down when he ran around on the ground during the course of athletics hour in the junior high school. It was a warm day. After vomiting, he took a bed rest for a while, and went back to his house with his friends. About 10 minutes later, he was found dead in the washroom. The family suspected heat stroke, and called the sport teacher to account. The autopsy revealed bronchitis and brain edema without rhabdomyolysis and renal lesions. Immunostaining for influenza virus A antigen was positive in the bronchial ciliated cells, but not in the brain. The cause of death was regarded as influenza encephalopathy, since the history of Indomethacin (NSAIDs) intake was ascertained.

Case 6. 20 year－old male (chronic active EBV infection)

This cook manifested muscle weakness in association with elevated serum enzymes of muscle origin. High fever, skin rash, hepatosplenomegaly, eosinophilia and ascites soon followed. Autoimmune disorder was clinically suspected. Ascites cytology revealed atypical (large granular) lymphocytosis with eosinophilia and lym-

phophagocytosis. EBV genome (EBER1) was demonstrated in the nuclei of the atypical lymphoid cells in the cell block specimen. The diagnosis of chronic active EBV infection with hypercytokinemia was made. The patient died of duodenal ulcer perforation and multiorgan failure 7 months later. Autopsy revealed systemic infiltration of CD56 – positive atypical NK – type cells.

Case 7. 48 year – old female (actinomycosis co – infected with Entamoeba gingivalis)

A contraceptive device inserted for years was took out of the uterine cavity in the outpatient clinic. Yellowish white inflammatory exudates were attached to the device. The cytological study exhibited infection of actinomycotic grains, as well as amebic trophozoite – like cells. PCR analysis using primers specific for Entamoeba gingivalis was performed using DNA of part of the specimen obtained by the "cell transfer" technique described above, and the result was positive. Hence, opportunistic co – infection of these two pathogens, residents in the oral cavity, transmitted by oral sex was confirmed.

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Expression of TSG101And Its Correlation with MDM2 In Squamous Cell Carcinoma And Denocarcinoma Of Lung

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Object we investigate the expression of TSG101 and MDM2 by IHC and Westen Blot method and the relationship of their expression in squamous cell carcinoma and adenocarcinoma of lung in order to evaluate their possible roles in the oncogenesis and progression of lung cancer.

Materials and Methods Immunohistochemistry S – P method and Western Blot.

79 lung cancer tissue samples(48 lung squamous carcinoma and 31 lung adenocarcinomas). were obtained from the First Affiliated Hospital of China Medical University

Result 1. Expression of TSG101 and MDM2 in squamous cell carcinoma of lung, adenocarcinoma of lung and normal tissues. TSG101 is expressed in lung cancer tissues and localized in cytoplasm and/or nucleus, it is expressed strongly in normal tissues . TSG101 is not expressed in poorly differentiated lung cancer or is expressed weakly in cancer cell's cytoplasm. MDM2 is located in nucleus and /or cytoplasm, MDM2 is not expressed in normal lung tissue, MDM2 is expressed strongly in poorly differentiated lung cancer.

2. Clinical significance of TSG101 and MDM2 There was no relationship between expression of TSG101 and age, sex, tumor size, histological type and P – TNM stages($P>0.05$); otherwise, there was closely relationship between TSG101 expression and cellular differentiation, lymph node metastases($P<0.05$). The expression rate of TSG101 in well and moderately differentiated cells was significantly higher than that in poorly differentiated cells($P<0.05$, $P<0.01$)The positive rate of MDM2 protein was correlated with the cellular differentiation, lymph node metastases and P – TNM stages($P<0.05$). We find that MDM2 is expressed in 32 of 47 with expressed TSG101, the expression level of TSG101 is negatively correlated with MDM2($P<0.05$).

Conclusion

1. TSG101 is expressed strongly in normal lung cancer tissue, it alters coincidentally accompanied with tumor grade.

2. MDM2 is not expressed in normal lung tissue, it is expressed opposite with differeation of tumor cell.

3. In lung cancer, expression of TSG101 and MDM2 shows a significantly negative correlation.

The Evaluation of a Diagnosis of Prostatic Adenocarcinoma in Needle Biopsy Specimens by Immunohistochemistry

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The number of cases of prostate cancer has continuously inceased over the past decades. The serum prostate specific antigen (PSA) test came into common usage over decades ago, and cancer mortality could be reduced by the PSA screening. In addition, histological diagnosis and grading play a major role in the management of prostate cancer. Benign prostate glands contain a basal layer beneath the secretory cells. A feature common to prostatic adenocarcinoma glands, in contrast, is the presence of only single cell lining without a basal cell layer. Confirmation of the presence of basal cells is helpful in the differential diagnosis of prostatic adenocarcinoma. The recognition of basal cell, however, on hematoxylin and eosin stained sections is not straightforward. Useful immunohistochemical markers for prostatic secretory and basal cells in the formalin－fixed paraffin embedded tissues are available.

In the present study, needle biopsy specimens from patients suspicious for prostate cancer in the Sendai Shakaihoken Hospital were immunohistochmically analyzed, and discussed the role of immunihistochemistry in aiding the evaluation of difficult cases, such as atypical small glandular foci suspicious for adenocarcinoma and small glands of Gleason grade 3B adenocarcinoma resembling atrophic benign glands.

PSA is a marker of prostatic differentiation, localized to cytoplasm both non－neoplastic and neoplastic prostatic glandular cells, but not to basal cells and urothelial cells. PSA is diagnostically helpful in distinguishing prostatic adenocarcinoma from other neoplasms and metastatic carcinoma of unknown origin.

As basal cell markers, high molecular cytokeratin detected by 34βE12 (cytokeratin903) and P63, as a nuclear protein encoded by a gene on chromosome 3p27－29 with homology P53. An important criterion of carcinoma is identifying a complete absence of basal cell layer. The diagnosis carcinoma can often be accomplished with help of immunohistochemistry. Thus PSA in conjunction with the basal cell markers 34βE12 and P63, and also general epithelial marker AE1/AE3 is useful in the distinguishing prostatic adenocarcinoma from hyperplastic secretory and basal cells. The immunohistochemical analysis of high grade intraepithelial neoplasm (PIN) associated with atypical small glandular foci or acini provides supportive evidence to establish a diagnosis of cancer.
Although the gold standard for a diagnosis of prostatic adenocarcinoma still remains with the diagnosis usually being based on a constellation of histologic features on the hemotoxylin and eosin－stained slides, prostaic adenocarcinoma may be diagnosable even on two or three atypical glands fulfilling the immunohistochemical staining pattern. In our experience approximately 75% of cases with serum PSA higher than 4.0ng/ml diagnosed as "atypical small acinar proliferation suspicious for adenocarcinoma" or "adenocarcinoma composed of two or three atypical small acini without basal cells" by a needle biopsy are from patients subsequently proven to have prostatic adenocarcioma.

Three－dimensional Tissue Culture Models in Prostate Cancer Biology

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Prostate cancer is one of the most common cancers in men of the Western countries, especially the United States of America. Recently, the number of clinical cases has been increasing yearly in Japan, but it is well known that the incidence of prostate cancer is still higher in American Caucasians than in Asians. Androgens play a major role in promoting the development and progression of prostate cancer. As a result, androgen ablation or blockade of androgen action has been the cornerstone of treatment of advanced prostate cancer. Although this hormonal therapy produces a significant clinical response in most of the patients, most responders eventually lose dependency, resulting in mortality. Thus, a pre－clinical experimental model simulating the clinical profile of prostate cancer is necessary to explore the progression of prostate cancer.

Multicellular tumor spheroids, one type of three－dimensional culture, are a well－studied in vitro tumor tissue model and can mimic some of the in vivo microenvironmental characteristics of solid tumors because preservation of the three－dimensional structure is important for cell－to－cell and cell－to－matrix interactions. The tumor microenvironment may play a key role in responses to environmental stress, including responses to drugs. Since multicellular tumor spheroids reproduce the tumoral microenvironment more accurately than conventional monolayer culture systems, they might act as better models for the biological and biochemical characteristics of prostate cancer. In addition, these morphological changes can cause intrinsic resistance to radiation and chemotherapeutic agents.

This study will present biological alterations in prostate tumor cells under different growing conditions.

Expression of TRAF2 and its Relation with Invasion in Breast Cancer

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Objective: Tumor necrosis factor receptor－associated factors (TRAFs) were initially discovered as adaptor proteins that couple the tumor necosis factor receptor family to signaling pathways. They have emerged as the major signal transducers for the TNF receptor superfamily and the interlukin－1 receptor/Toll－like receptor (IL－1R/TLR) superfamily. They can mediate the activation of NFκB and JNK signaling pathway and play diverse and widespread physiological functions. This study investigated TRAF2 expression in breast cancer and the correlation between its expression and invasion of the breast cancer.

Methods: (1)We examined TRAF2 expression in normal breast and breast cancer with immunohistochemistry. (2)We also examined TRAF2 expression with immunohistochemistry and Western blot in cultured human breast cancer cells, respectively MDA－MB－435s, MDA－MB－231 and MCF－7 and a non－tumorigenetic epithelial cell line, MCF－10A. (3)Statistics with SPSS12.0 software and appling χ^2 test and mean square analysis. $P<0.05$ means significance.

Results: 1. TRAF2 does not express in normal breast. Positive expression rate of TRAF2 in breast cancer increases to 37.14%. Compared with that in intraductal carcinoma, TRAF2 positive rate in invasive carcinoma increase slightly($P>0.05$). 2. Compared with that in MCF－7, TRAF2 express in MDA－MB－435s、MDA－MB－231 enhances notably. The expression of TRAF2 in MCF10A is slightly and evidently lower than that in MCF－7. TRAF2 protein expression quantity in the four cell lines is coincidence with the result of the immunohistochemistry

Conclusions: 1. TRAF2 protein expression in breast cancer increase more than in normal breast tissue. 2. The expression of TRAF2 in cultured breast cell lines is evidently higher than in non－tumorigenic cell line, and high

metasis cell lines enhances notably when compared with low metastasis cell line.

Non－incidental Co－amplification of Myc and ERBB2, and Myc and EGFR, in Gastric Adenocarcinomas.

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Abstract This study was conducted to assess the frequencies of protein overexpression and gene amplification of Myc and to identify the mechanisms of Myc gene amplification, especially with regards to its possible co－amplification with ERBB2 or EGFR in gastric adenocarcionomas. By immunohistochemical analysis of a total of 300 formalin－fixed and paraffin－embedded gastric adenocarcinomas, the nuclear overexpression of MYC was found in 47 tumors (16%). A fluorescence in situ hybridization analysis revealed that 9 (19%) of the 47 tumors with protein overexpression had cancer cells with high levels of Myc amplification, while only 7 (6%) of the 122 tumors without protein overexpression showed high－level Myc gene amplification. Such Myc amplification was significantly correlated with positive nuclear protein overexpression. The co－amplification of ERBB2 or EGFR with Myc that was found in six and four cases, respectively, is believed to be non－incidental because those frequencies were significantly higher than the individual frequencies observed for the total examined cases (ERBB2: 7%; EGFR: 4%). The high levels of gene amplification of these three genes, as visualized by fluorescence in situ hybridization, could be broadly classified into two typical types, namely, "multiple scattered signals" and "large clustered signals". Using two－color fluorescence in situ hybridization, the coexistence of co－amplified Myc and ERBB2, or Myc and EGFR, within single nuclei in various combinations of amplification types and copy numbers, could be ascertained in all nine cases, including one in which the synchronous "multiple scattered type" co－amplification of Myc and ERBB2 was observed. In three tumors, co－amplification of ERBB2 and EGFR was found; however, ERBB2－ and EGFR－amplified cell populations were separate and mutually exclusive. We propose that the non－incidental co－amplification of Myc and either ERBB2 or EGFR occurred through translocation and subsequent rearrangement.

The Clinical Application of Liquid－based Cytological Test to the Screening of Sputum Cytology for the Diagnosis of Lung Cancers

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Abstract

Liquid－based Cytological Test (LCT) has been successfully and widely applied to cervical cytology diagnosis. The purpose of this study was to compare the cytologic finding and diagnostic sensitivity of LCT method with those of pick－and－smear (PS) method in the diagnosis of lung cancer. From January to October 2004, sputum samples of 101 patients diagnosed as lung cancer were studied. LCT slides had less area of smear membrane, clearer background, distinct and stereoscopic cytological pictures. LCT method had significantly higher diagnostic sensitivity for lung cancer (80.2%) than PS method (63.4%, $P<0.05$), particularly for small cell lung carcinoma ($P<0.05$). The combined LCT and conventional PS methods had significantly higher diagnostic sensitivity

for the detection of ADC (80.6%) than PS method alone (55.6%, $P<0.05$). It was operated easily for LCT to be a novel technique of worth being widely used, and was suitable for manual screening for the early diagnosis of lung cancer.

Key words: Cytopathology; Sputum; Lung neoplasms; LCT

Class A Macrophage Scavenger Receptor (CD204) Attenuates the Risk of Cardiac Rupture After Experimental Myocardial Infarction

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<Background>

Cardiac rupture remains a serious complication of acute myocardial infarction (MI). The process is caused by the disruption of extracellular matrix (ECM) structures triggered by early inflammatory response to ischemic damage. Recent studies demonstrated that class A macrophage scavenger Receptor (SR-A, CD204) that recognizes an unusually broad range of ligands, is actively involved in modulation of inflammatory responses. This study was conducted to evaluate the relationship between SR-A and healing process of left ventricle after MI by using mice deficient in SR-A gene (SR-A-/-).

<Methods and Results>

Experimental MI was produced by ligating the left coronary artery. We examined cardiac rupture rate, survival rate, echocardiography, ECM degradation, and expression of inflammatory cytokines at the infarcted and noninfarcted regions after MI in SR-A-/- and wild type (WT) mice. Although there were no significant differences in cardiac function evaluated by echocardiography and in infarct size, WT mice had significantly better survival after MI compared with SR-A-/- mice (Log-rank $P=0.03$). Importantly, 31% (17/54) of SR-A-/- mice died of cardiac rupture, whereas 12% (6/51) of WT mice died of the same cause within 1 week ($P=0.01$). Immunohistochemical analysis revealed that infiltration of inflammatory cells into infarcted myocardium was identical between 2 groups. The real-time RT-PCR and in situ zymography showed augmented expression of matrix metalloproteinase (MMP)-9 mRNA and increased gelatinolytic activity in the infarcted myocardium in SR-A-/- mice compared to WT mice at 3 days after MI. Furthermore, it was suggested by in vivo and in vitro experiments that the presence of SR-A suppressed tumor necrosis factor-alpha (TNF-β) expression through the induction of interleukin-10 (IL-10).

<Conclusions>

These findings demonstrated that SR-A deficiency might impair wound healing of infarcted myocardium and cause cardiac rupture eventually. The augmentation of MMP activity and pro-inflammatory cytokine expression via insufficient production of anti-inflammatory cytokine might be a mechanism of these deleterious phenomenon during acute phase of MI.

Impact of XRCC1 Polymorphisms Arg399Gln and Arg194Trp on Risk of Lung Cancer in Non-smoking Women

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Abstract

The etiology of lung cancer has been shown to be associated with genetic and certain environmental factors that produce DNA damage. Single nucleotide polymorphisms in XRCC1 which is a major base excision repair gene may alter protein function and individual capacity to repair damaged DNA; deficits in repair capacity may lead to genetic instability and carcinogenesis. To establish our understanding of possible relationships between XRCC1 polymorphisms Arg399Gln or Arg194Trp and the susceptibility to lung cancer, we performed a hospital - based case - control study of 350 patients with newly diagnosed lung cancer and 350 cancer - free controls in non - smoking women using logistic regression models. Our results show that patients have a higher prevalence of cooking oil fume compare with controls (OR = 2.60, 95% CI: 1.87 - 3.61, P < 0.05). Subjects having the XRCC1399Arg/Gln or Gln/Gln genotype in adenocarcinoma group had an odds ratio (OR) of 1.64 (1.12 - 2.41 adjusted for age, cooking oil fume) or 2.48 (1.37 - 4.50 adjusted for age, cooking oil fume), respectively (P < 0.001), compared with those having the Arg/Arg genotype. Gene - environment interaction of XRCC1399Gln polymorphism and cooking oil fume increase risk of lung cancer in a supermultiplicative manner (OR for the presence of both XRCC1399Gln allele and cooking oil fume exposure was 3.30(2.04 - 5.32, P < 0.001), although the XRCC1 polymorphism itself was not associated with the risk. Furthermore, the OR increase to 4.65 (2.67 - 8.06, P < 0.001) in adenocarcinoma group. In conclusion, XRCC1Arg399Gln polymorphism may serve as a risk modifier.

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A Nasal NK/T - cell Lymphoma in the Northeast Region of China

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Nasal NK/T - cell lymphomas are peculiar Epstein - Barr virus - related ones, occurred often in Asian people and called previously as gangrenous rhinitis etc. When many Chinese cases have been reported from Sichuan Sheng and HongKong, we have counted near 100 of nasal cases in the registry of Pathology in China Medical University Hospital located in Shenyang with a population of about 7 million. The lymphoma cells show natures of NK/T - cells rather than B - cells, positive for CD3ε, cytotoxic granules, CD56 and EBER - 1 signals, and are associated with many CD204 - positive macrophages in the background. The lineage of these lymphomas cells has been believed as NK - cell or T - cell, however in a small scale of study employing anti - LAT - 1 (Linker for activation of T cell) antibody with CD5, CD3ε, CD56 and EBER - 1 these nasal NK/T - cell lymphomas could be categorized in T - cell, NK/T - cell and NK - cell types. On the other hand, These lymphomas revealed marked degenerative tendency but a series of studies about p53 protein expression and apoptosis could not explain their degenerative tendency. In a small scale of study employing a simple panel of CD204, cleaved caspase - 3 and beclin - 1 for programmed cell death, enhanced autophagy and autophagic cell death were found in these lymphomas. Taken together, these results of these studies suggested that the enhanced autophagy with cell death and EBV infection characterize nasal lymphomas of T - cell, NK/T - cell and NK - cell types as the entity of nasal

NK/T－cell lymphoma. The relationship among the enhanced autophagy with cell death, EBV infection and the occurrence in the nose/upper respiratory tract in the nasal NK/T－cell lymphomas should be understood from view points of the site concept and the unknown extrinsic factors.

To Study Immunophenotyping of Acute Leukemia by a Cell Microarray

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Objective: Leukemia is one of major malignant tumors resulted in the death. Acute leukemia accounts for the first cause of death in adult younger than 35 years. According to statistics, the mortality rate of leukemia is the highest in Western Europe and the North American for 3.27.4/100 thousand population; the mortality rate of leukemia is the lower in Asia and the South American for 2.8～4.5/100 thousand population. Chinese annual incidence and mortality rates are 2～4/100 thousand population with a significant difference from Europe and the United States. In the diagnosis and treatment of leukemia, the type of leukemia is the key. Different types of leukemia has different clinic program and prognosis. Before the 1980's leukemic diagnosis is by the FAB classification criteria. Now MICM Typing is benefiting to leukemia diagnosis, standard detection of residual disease and the prognosis. At present, the doctors usually choose immunohistochemical techniques or flow cytometry. But both of them need large specimen volume, high price, complic procedures, and other restrictions such as analysis of 1～4 surface antigens, missing a lot of indicators to body immune system. Reseachers have begun to use a biochip technological platform which is high－throughput, paralleled for the integration of leukemia immunophenotyping. On the basis of previous experiments, I conducted in－depth research in the application of the cell microarray in immunophenotyping of acute leukemia, in order to decide the best spotting conditions , the best incubation conditions, to comfirm the stability, I have used the microarrays to immunophenotyped 72 leukemias with peripheral blood samples. Get a quick, simple and complete immunization data for the clinical diagnosis and antibodies－targ treatment.

Methods

1. to determine the best spotting conditions;
2. to determine the best incubating conditions.
3. to confirm the stability.
4. Immunophenotyping clinical 72 samples.

Results

1. In 50μg/ml, signal intensity is the saturation, in 25μg/ml, 12.5μg/ml, 6.25μg/ml signal intensity gradually weakened.
2. When at room temperature (20℃), with the cell suspension (5 × 106/mL) , being incubated for 45 minutes, the chips capture lattice cell with the highest SNR.
3. Make certain to the stability

3.1 For the three samples, at (25.0±0.5)℃ and relative humidity 60%±5%, after 6 months, the Cell chips did not change the ability to capture cells.

3.2 Stored at －20 ℃for 12 months, chips did not change the ability to capture cells.

3.3 Stored at(4.0±0.5)℃、relative humidity 75%±5%, after 12 months, chips did not change the ability to capture cells.

4. Immunophenotyping results of 72 cases of acute leukemia: B－ALL: 14 cases express HLA－DR、CD19, 5 cases express CD20、CD22, 8 cases express CD79b, 9 cases expressCD10, 1 case expresses CD34. T－ALL: 8 cases

express CD2, 12 cases express CD7, 10 cases express TCRαβ or TCRγδ. AML: 46 cases express CD33, 30cases express CD13, 22 cases express CD15, 14 cases express CD11b and CD14, 1 case expresses CD235a, 1 case expresses CD4, 23 cases express HLA－DR.

Conclusions: In this study, using concentrations with 50μg/ml monoclonal antibodies, at room temperature conditions(20℃) and cell suspension (5×106/mL) incubated for 45 minutes, chips get to the highest SNR. This cell chips are of good stability. The experimental results meet with the immunophenotyping of leukemia reported. The cell microarray can be easy to use, good repeatability and stability. For clinical leukemia immunophenotyping, it provides a fast, high－throughput, parallel, integrated technology platform.

KeywordsCell chip; Immunophenotyping; SNR; CD antibody microarray

The Z39Ig: A Specific Marker Of Synovial A Cells In RA Synovium.

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Purpose: There is no synovial A cell marker in normal synovium and inflammatory arthritis synovium. Thus, it is difficult to determine the number and distribution of synovial A cells in inflammatory arthritis synovium. The Z39Ig is a membraneous complement receptor protein of the immunoglobulin super－family. It has been reported that the expression of the mRNA was limited to several organ tissues and correlated with the presence of activated macrophages. In this study, we developed monoclonal anti－Z39Ig antibodies and detected the expression of the Z39Ig protein in human organ tissues and inflammatory arthritis synovium.

Methods: Monoclonal anti－Z39Ig antibodies were produced by immunizing BALB/c mice with the Z39Ig gene－transfected murine B cell lines. Immunohistochemical analyses were performed on human organ tissues and inflammatory arthritis synovium by using the monoclonal antibody. Two color analyses were done using the anti－Z39Ig antibody for rhodamine and the anti－CD163, anti－cadherin11, anti－CD16, anti－27E10 antibodies for FITC.

Results: The expression of Z39Ig protein was observed in Kupffer cells, broncho－alveolar fluid macrophages, placenta macrophages including Hofbauer cells and synovial A cells from normal synovium. We also found the upregulation of the protein in monocytes differentiated with M－CSF, GM－CSF, vitamin D3 or dexamethasone but not in monocytes activated with TNF－α, IFN－γ or lipopolysaccharide. Also, a greater number of Z39Ig positive cells were found in RA synovial tissues compared to normal, pseudo－gout, or OA synovial tissues. Most Z39Ig positive cells were CD16 positive macrophages and contacted with cadherin11 positive cells in the lining layer. Furthermore, the protein was not expressed on 27E10 positive macrophages (known as inflammatory macrophages). On the other hand, the expression of Z39Ig protein was significantly low in the synovium from pseudo－gout.

Conclusions: These findings indicate that the Z39Ig protein is a specific marker of synovial A cells in RA synovium. The increase of Z39Ig positive cells in RA synovial macrophages might be related to the unique pathogenesis of RA.

An Immunohistochemical Analysis of Proliferation, Apoptosis and Stem Cell Phenotype Expression in Human Testicular Spermatogenesis

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In order to understand the relationship among the cell proliferation, cell death and stem cell phenotype expression in the human testicular spermatogenesis, 3 cases of formalin－fixed and paraffin－embedded specimens of the adult testis orchiectomized for hormone therapy against prostate cancer were investigated. The most target antigens were visualized by means of antigen－retrieval and polymer method. BECN1 (autophage marker) and Oct－3/4 (stem cell marker) were visualized by means of antigen－retrieval supersensitive method. Ki67－positive cells localized only in the basal and middle parts of seminiferous tubules were spermatogonia and primary spermatocytes before entering in meiosis. Cleaved caspase－3－ and BECN－1－positive cells were those from primary spermatocytes to spermatids. Many Sertoli cells were positive for BECN－1. Cells positive for ssDNA (single strand DNA marker) were observed at apical side of seminiferous tubules when it is unknown whether this phenomenon is physiological or not. The stem cells markers indicated a sequential expression in the order of Oct－3/4, Nanog, a group of CD34, CD117, and ABCG2, and Oct－3/4 once again according to the development from spermatogonia to primary spermatocytes. In conclusion, the expression of cell proliferation, cell death and stem cell phenotype markers in the human testicular spermatogenesis can be assessed by means of immunohistochemical analysis. Oct－3/4, Nanog and ABCG2 are useful marker for estimating the orientation in the germ stem cells development.

Pulmonary Hypertension Mechanism Relevance to 5－Hydroxytraptamine, Receptors and Transporters

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AIM: Pulmonary arterial hypertension (PAH) is characterized by elevated pulmonary vascular resistance which leads to right ventricular failure. Serotonin and the serotonin transporter play an important role in animal and human studies of PAH. We therefore hypothesized that monocrotaline－induced PAH rats treated with high－affinity selective serotonin reuptake inhibitors (SSRIs) would have a reduction of PAH. Methods: The chronic "inflammatory" pulmonary hypertension model of rat was established by i.p. administration of monocrotaline. Selective serotonin reuptake inhibiters (SSRIs) with high affinity (Kd＜1nmol) (sertraline or fluoxetine) were used in monocrotaline treated rats. Pulmonary homodynamic measurement and lung tissue morphological investigation were conducted. Serotonin transporter expression was assayed by RT－PCR or western blot. The effects of fluoxetine on concentration－response curves of 5－hydroxytramine (5－HT) in pulmonary arteries (PAs) were also studied. Results: Pulmonary artery pressure, right ventricular hypertrophy, the degree of muscularization of pulmonary arteries and the levels of serotonin transporter were significantly increased by monocrotaline ($P<0.05$ vs control) and these variables were significantly decreased either by sertraline or fluoxetine. Further investigation of transporter mechanism using cultured rat pulmonary artery smooth muscle cells (PASMC) and liposomal transfection to introduce ERK1/2 ODNs into cultured rat PASMCs shown that fluoxetine concentration－dependently inhibited proliferation of PASMCs induced by 5－HT. Meanwhile, antisense ODN to ERK1/2 also inhibited 5－HT－induced proliferation of PASMCs. Conclusion: SSRIs protect against monocrotaline－induced pulmonary hypertension, which was relevant to serotonin transporter reduction and this finding provides a novel therapeutic targets for PAH.

This study was supported by National Natural Science Foundation, China 30271510, and 30572194.

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HIV Encephalitis and Diffuse Microglial Activation Occur Independently in the Brain of HIV－1 Infected Patients: A Neuroprotective Role of Microglia in AIDS Encephalopathy.

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AIDS dementia is histopathologically characterized by microglial nodules with multinucleated giant cells, myelin pallor and axonal damage with abundant HIV－infected macrophages and microglia in the cerebral white mater. On the other hand, a variety of neuronal damage has been repeatedly reported in pathology of AIDS dementia. Our previous study using SIV－infected macaques suggested that there are two independent pathogenic processes in AIDS encephalopathy: 1) inflammatory process in the white mater occurring without immunodeficiency and 2) cortical degeneration caused without inflammatory changes.

To clarify whether these two pathologic events also occur independently in human HIV－1 infection, we examined 20 brains of patients with HIV－1 infection autopsied in Vienna University. Eleven cases showed typical pathology of HIV encephalitis in the white mater. Among theses 11 cases, following pathologic changes of the frontal cortex could be observed; diffuse microglial activation in 5 cases, astrocytic gliosis in 5 cases, and apoptosis of glia cells and/or neurons in 6 cases. However, presence of such cortical pathology was neither correlated with severity of encephalitis nor local presence of HIV－1－infected cells. The other 9 cases had neither HIV encephalitis nor any opportunistic lesions in the brain. In the frontal cortex, however, diffuse microglial activation was observed in 7 cases and astrocytic gliosis in 2 cases. In addition, apparent apoptosis of glia cells could be detected in 1 case. In order to investigate a role of microglial activation in the frontal cortex, we further examined expressions of harmful proinflammatory cytokines, IL－1β and TNF－γ, and neuroprotective EAAT－2, which is primarily expressed on astrocytes and keeps extracellular glutamate concentration low in the brain to prevent neurons from excitotoxic cell death. A quantitative analysis of EAAT－2 expression and microglial activation demonstrated that the number of Iba1－positive activated microglia was increased in 12 cases and the area of EAAT－2 expression was declining in 12 cases. There was a significant negative correlation between areas of EAAT－2 expression and numbers of Iba1－positive activated microglia ($P<0.01$) among the cases with decreased EAAT－2 expression. Expression of proinflammatory cytokines, IL－1β and TNF－γ were detected only in microglial nodules, but not in diffusely activated microglia.

These data indicate that diffuse activation of microglia in the cerebral cortex is independent from HIV encephalitis, and may occur according with reduction of EAAT－2 expression on astrocytes in the brain of AIDS patients. Expression of EAAT－2 by activated microglia suggests its compensatory effect to prevent neurons from glutamate neurotoxicity.

Ghrelin and Growth Hormone Secretagogue Receptor as Therapeutic Targets for Obesity and Type 2 Diabetes

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Background and aims: Ghrelin, an endogenous ligand for growth hormone secretagogue receptor (GHS－R), is an appetite stimulatory signal from the stomach with structural resemblance to motilin. We examined the effects of the gastric peptide ghrelin and GHS－R antagonists on energy balance and glycaemic control in mice.

Materials and methods: Body weight, fat mass, glucose, insulin, and gene expression of leptin, adiponectin, and resistin in white adipose tissue (WAT) were measured after repeated administrations of ghrelin under a high fat diet. Gastric ghrelin gene expression was assessed by northern blot analysis. Energy intake and gastric emptying were measured after administration of GHS－R antagonists. Repeated administration of GHS－R antagonist was continued for six days in ob/ob obese mice.

Results: Ghrelin induced remarkable adiposity and worsened glycaemic control under a high fat diet. Pair feeding inhibited this effect. Ghrelin elevated leptin mRNA expression and reduced resistin mRNA expression. Gastric ghrelin mRNA expression during fasting was increased by a high fat diet. GHS－R antagonists decreased energy intake in lean mice, in mice with diet induced obesity, and in ob/ob obese mice; it also reduced the rate of gastric emptying. Repeated administration of GHS－R antagonist decreased body weight gain and improved glycaemic control in ob/ob obese mice.

Conclusions: Ghrelin appears to be closely related to excess weight gain, adiposity, and insulin resistance, particularly under a high fat diet and in the dynamic stage. Gastric peptide ghrelin and GHS－R may be promising therapeutic targets not only for anorexia－cachexia but also for obesity and type 2 diabetes, which are becoming increasingly prevalent worldwide.

Altered Ghrelin and PYY Responses to Meals in Bulimia Nervosa

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Abstract

Objective: In recent years great advances have been made in our understanding of the peripheral signals produced within the gastrointestinal tract that regulate appetite, such as ghrelin and peptide YY (PYY). While ghrelin elicites hunger signals, PYY elicites satiety. Therefore, alterations in hormone physiology may play a role in the pathogenesis of bulimia nervosa (BN). In this study, we investigated the postprandial profile of ghrelin and PYY levels in patients with BN.

Design and patients: Postprandial plasma ghrelin and PYY levels and insulin and glucose responses were measured in 10 patients with BN and 12 control patients in response to a standard 400 kcal meal.

Results: Basal ghrelin levels present in BN subjects (265.0±25.5pmol/L) were significantly higher than those in healthy controls (199.3±18.4pmol/L, $P<0.05$), while basal PYY levels were equivalent in BN (14.6±1.3pmol/L) and control (12.8±1.1pmol/L, $P=0.30$) subjects. Postprandial ghrelin suppression (decremental ghrelin area under the curve) was significantly attenuated in BN patients, compared to controls (－96.3 ±

26.8pmol/L×3hvs. −178.2±25.7pmol/L×3h, P<0.05). After a meal, the incremental PYY area under the curve in BN patients was significantly blunted from that observed in controls (9.2±2.6 pmol/L×3h vs.26.8±3.2pmol/L×3 h, P<0.01). Glucose and insulin responses to meals were similar between the two groups. **Conclusions:** BN patients exhibit elevated ghrelin levels before meals with reduced ghrelin suppression after eating. In bulimia nervosa subjects, the rise in PYY levels after meals is also blunted. A gut−hypothalamic pathway involving peripheral signals, such as ghrelin and PYY, may be involved in the pathophysiology of BN.

Differential Expressions of Retinoid Receptors in Oral Squamous Cell Carcinomas

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Background and purpose: Retinoids is known to take part in the maintenance of differentiation of normal squamous epithelial cells by acting through retinoic acid receptors (RARs) and retinoid X receptors (RXRs), members of the nuclear receptor family. We have recently demonstrated special types of keratin were differentially expressed in the developing process of oral mucosal malignancies. In order to elucidate the role of retinoids in the development of oral cancer, we studied the expressional modes of retinoid receptors in tissue sections of oral carcinoma in−situ (CIS) as well as oral squamous cell carcinoma cells in culture. Materials and methods: Sixty surgical specimens of oral superficial carcinoma (SCC) containing different histopathological grades of lesions in the same sections were studied by the immunohistochemistry for RAR and RXR subtypes α, β, and γ, in addition to for keratins 13 and 17. Oral squamous cell carcinoma cell systems, such as ZK−1, were determined for their expression levels of retinoid receptors by immunofluorescence and RT−PCR. Results: Retinoid receptors were characteristically immunolocalized in basaloid cells consisting of the lower half of epithelial dysplasia with two−phase appearances but not in its upper half, although they were not definitely localized in normal epithelium. In CIS, basaloid CIS cells showed strong nuclear staining for RXRγ, while there were almost no RXRγ positivities in acanthotic CIS cells. RXRα was strongly positive in most of the nuclei of verrucous or acanthotic CIS cells but not in basaloid CIS cells. It was not significantly expressed in epithelial dysplasia. In contrast, RARα was only positive in nuclei of the basal or parabasal cells of normal and dysplastic epithelia but not in CIS. In well−differentiated SCC, RXRγ was limited to be positive in the periphery, while RXRα was positive in the whole region of SCC cell nests, suggesting that the RXRγ expression was related to cell proliferation and RXRα to differentiation towards keratinization. There were no positive staining for RARβ and RXRβ in all of the specimens examined. Conclusion: The results suggest that retinoids play an important role in the carcinogenesis of oral epithelium through different subtypes of RARs or RXRs to induce receptor dependent proliferation as well as differentiation.

Salivary Gland Tumor Cells in Hypoxic Condition.

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Background: Salivary pleomorphic adenoma is histopathologically characaterized by its colorful stroma with myxoid, chondroid, and hyaline appearances, which are poorly vascularized. Hence, pleomorphic adenoma cells

embedded in such stromata are supposed to be able to survive in hypoxic conditions. However, their molecular mechanism for the proliferation of pleomorphic adenoma cells in such peculiar extracellular milieu is almost unknown. To understand the hypoxia－dependent manner of cellular proliferation, we determined both protein and gene expression levels of hypoxia－inducible factor 1α (HIF－1α), vascular endothelial growth factor (VEGF) which is one of the HIF－1α targets, and von Hippel－Lindau (vHL) and p53 genes, both of which induces HIF－1α degradation. Methods: Total cellular RNAs and proteins were extracted from SM－AP cells derived from a human palatal pleomorphic adenoma, ACC cells derived from human salivary gland adenoid cystic carcinomas cultivated under aerobic (5% CO_2/20% O_2) or hypoxia (5% CO_2/1% O_2 or 200－500 μM $CoCl_2$). These mRNA samples were further purified and subjected for RT－PCR, and the cell lysates were used for Western blotting. Cultured cells on chamber slides were fixed with 4% paraformaldehyde for immunofluorescence for HIF－1α and VEGF. Exons 5－7 of the p53 gene were PCR amplified for sequencing to examine mutational events. Results: Both SM－AP and ACC cells under hypoxia showed more enhanced gene expression levels for VEGF but did not those for HIF－1α. In contrast, however, HIF－1α protein levels were kept higher levels under hypoxia than aerobic condition in SM－AP cells, especially in SM－AP cells, which also showed lower expression levels for the VHL gene and shared a common deletion of the last base G of codon 249 (AGG to AG-) of the p53 gene. Conclusion: The results indicate that salivary gland tumor cells general can proliferate due to their highly maintained HIF－1α protein levels in the hypoxic condition, in which the degradation of HIF－1α is inhibited and which is more benefitable for SM－AP cells than aerobic conditions.

Whether Exists the Population of Cardiomyocytes Stem Cells in Newnatal Rat Heart

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Absract Objective: To study whether exists the population of cardiomyocytes stem cells in newnatal rat heart. **Methods**: cardiomyocytes (CMs) derived from neonatal SD rats were isolated and purificated in culture and the morphology and ultrastructure of CMs were observed by inversion microscope and transmission electron microscopy. Expression of cardiac－specific marker－troponin I (cTnI), identified by immunohistochemistry, was used as a marker of cardiac muscle. Besides, after 10 days when the CMs beating became slower and slower, cells were detached by addition of a solution containing 0.25% trypsin－EDTA and replated in order to observe whether the CMs has the ability of survival. The medium was refreshed at the following day, and the non－adherent cells were removed. **Results**: CMs were beating spontaneously and demonstrated positive staining for cTnI. Myofilament, Z band and the ubiquity of the cross－striation were seen in CMs. Between cells, there were intercalated disc. The surface of some CMs had a lots of micro－villi and dead cells were observed. There were still adhered several cells after refreshed the new medium and discarded most of the non－adhered cells, but to our surprised, 5－days later, several cells of those adhered cells became beating weekly. **Conclusion**: CMs can be successfully cultured in vitro and have the ability of survival. We hypothesized that these cells would be the population of cardiomyocytes stem cells. **Key words**: Cardiomyocytes (CMs), cardiomyocytes stem cells, beating spontane, in vitro

The Role of SH2－Bβin Asthma Mediated by NGF

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Asthma is characterized by airway obstruction, airway inflammation and increased airway responsiveness. Nerve growth factor (NGF) is increasingly recognized to play an important role in asthma. NGF regulates the airway inflammation, constractivity and hyperresponsiveness through direct and/or indirect effect on inflammation cells and the neurons in asthma . Our previous results confirmed that the expression of NGF increased remarkably in the lower respiratory tract and viscerosensory afferent sites of the asthmatic guinea pigs. NGF upregulates the expression of TrkA , the contents of calcitonin gene related peptide (CGRP), Tumor necrosis factor－α(TNF－α) and interleukin－1β(IL－1β). The intracellular signal transduction system mediated by TrkA might be one of the main routes for the involvement of NGF in the pathogenesis of asthma. SH2－B is a downstream protein of TrkA, has multiple protein－protein interaction motifs, including an Src homology 2 (SH2) domain, a pleckstrin homology (PH) domain, multiple proline－rich regions, and numerous potential phosphorylation sites. Four isoforms of SH2－B (α, β and δ) have been described to date. NGF stimulates the association of TrkA with SH2－B and phosphorylate it. SH2－B plays an important role in the neuron differen ciation and survival mediated by NGF through binding with TrkA. SH2－B is an important intracellular mediator of NGF/TrkA signaling in neurons and SH2－B also associate with the high－affinity receptor immunoglobulin E(IgE)FcεRIγsubunit. But the role of SH2－Bβ in asthma is stll unknown.

Asthma was induced in BALB/c mice by exposure to chicken egg ovalbumin(OVA). The changes of SH2－Bβimmunoreactivity and the regulatory effects of NGF and dexamethasone were investigated by means of immunohistochemistry and Western blot. The levels of IL－4 and IgE in the bronchoalveolar lavage fluid(BALF), serum and culture supernant of spleen cells were investegated after inhibition of SH2－Bβby means of ELISA. The changes of the airway resistance were investigated by AniRes 2003 animal lungs function analysis system.

The expression of SH2－Bβincreased significantly in lower respiratory tract and visceral sensory afferent sites(C7－T5 spinal ganglia and the corresponding posterior horn of the spinal cord) of the murine asthma model compared with the normal control group($P<0.01$), but decreased in the corresponding portion in anti－NGF group and DEXA group compared with the murine asthma model ($P<0.01$).

Increased airway resistance to methacholine(MCh) was observed in the asthmatic group compared with the normal control group, but decreased in anti－SH2－Bβgroup and DEXA group compared with the asthmatic group($P<0.01$).

Total cells and eosinophil counts in bronchoalveolar lavage fluid(BALF) of the asthmatic group were increased significantly compared with the normal control group($P<0.01$). Inhibition of SH2－Bβsignificantly reduced the total cells($P<0.01$) and eosinophil counts ($P<0.01$) in the BALF. Compared with asthmatic group, inhibition of SH2－Bβ significantly reduced IL－4 and IgE levels in the BALF and serum($P<0.01$). The results of cultured spleen cells showed that OVA enhanced the secretion of IL－4 and IgE from spleen cells, this effect was significantly inhibited by inhibition of SH2－Bβ($P<0.01$).

Advanced Glycation End Products Induce the Expression of Vascular Cell Adhesion Molecular－1 in Retinal Microvascular Endothelial Cells Through RAGE/NF－κB Pathway

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Key words: Advanced glycation end products; microvascular endothelial cells; receptor for AGEs; reactive oxygen

species; Nuclear factor - kappa B; vascular cell adhesion molecular - 1

Abstract: Aim To investigate the relationship of Advanced glycation end products and diabetic retinopathy. Methods Cultured Rat retinal microvascular endothelial cells(RMECs) in vitro and prepared advanced glycation end products - AGE - BSA. Cells were incubated with AGE - BSA in different concentration and the same concentration but different time, then the expression of Receptor for AGEs(RAGE) mRNA、Nicotinamide - adenine dinucleotide phosphate(NADPH)oxidase mRNA and Vascular cell adhesion molecular - 1(VCAM - 1) protein were analyzed by RT - PCR and Western blot method. Reactive oxygen species (ROS) were examined by flow cytometry and nuclear factor - kappa B(NF - κB) activation was detected by laser confocal microscope. Results AGE - BSA induced RAGE mRNA、NADPH oxidase mRNA and VCAM - 1 protein expression in the time/dose dependent pattern. After treatment with AGE - BSA in varying concentrations, intracellular ROS production fluorescence intensity units increased gradually. Fluorescence intensity units also increased for different time, which is the highest when RMECs were incubated by AGE - BSA for 2h, but apocynin can inhibit the production of ROS. NF - κB was mainly in cytoplasm without AGE - BSA . After RMECs were stimulated by AGE - BSA , NF - κB translocated into nucleus, and reached to the peak in 30 min. Conclusions We infered the signal transduction pathways of AGEs - RAGE→NADPH oxidase→ROS→NF - κB→VCAM - 1 may participate in the development of diabetic retinopathy.

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The Expression of RhoC and RhoGDIα in Lung Carcinoma Cells and Their Correlation with the Ablility of Invasion

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Rho protein family is one member of Small GTP - binding proteins of Ras superfamily. RhoC is one member of Rho subtribe, which take a pivotal role in the process of tumor invasion and metastasis. However, there is no studies about RhoC expression in lung carcinoma and its correlation with metastasis of lung cancer. RhoGDIs have been regarded as regulators to inactivate Rho proteins, however, evidence is accumulating that the classical function of rhoGDIs as universal chaperones for GDP - bound proteins may need to be revisited. Several studies have investigated the level of expression of rhoGDIs in various cancer cells compared with normal cells, revealing opposite patterns depending on the tumor cell considered. Likewise, rhoGDIβmRNA levels were increased in ovarian adenocarcinoma, and this upregulation correlated with the malignancy of tumors. In addition, the induction of motility of cancer cells by the autocrine motility factor cytokine appeared to induce an upregulation of rhoGDIβ mRNA and protein in vitro. However, less studies reported the correlation of Rho - GDIα expression and metastasis of cancer. In the present study, we investigate the expression of RhoC and Rho - GDIα mRNA and protein in four lung carcinoma cell (BE1, LH7, A2, A549) and Homo sapien Bronchus Epithelial cell(HBE) and their correlation with metastasis of lung cancer. The expressions of RhoC, Rho - GDIα protein were detected with western blot. The expressions of RhoC, Rho - GDIα RNA were detected with reverse - transcription polymerase chain reaction (RT - PCR). The expressions and locations of RhoC, Rho - GDIα were detected with immunofluorescence. The invasion ability of BE1 and LH7 in vitro was detected with BioCoat Matrigel Invasion Chambers with 8.0 micron pore transwells coated with extracellular matrix proteins. The SPSS 11.5 software was em-

ployed to ayalyze the data. $P<0.05$ was considered as statistical significance. The expression level of RhoC and Rho－GDIα protein in various lung carcinoma cell was significantly higher than that in HBE. The expression of RhoC and Rho－GDIα protein in BE1(the cell with high metastasis capability) was significantly higher than that in LH7(the cell with low metastasis capability). The expression of RhoC and Rho－GDIα mRNA in BE1(the cell with high metastasis capability) was significantly higher than that in LH7(the cell with low metastasis capability). The invasion ability of BE1 in vitro was significantly higher than LH7. The expression of RhoC and Rho－GDIα in BE1 and LH7 was all located in endochylema. Therefore, The expression level of RhoC and Rho－GDIα in lung carcinoma cell was higher than in HBE. The expression of RhoC and Rho－GDIα correlates with metastasis of lung cancer.

The Studies on Isolation and Cultivation and the Mechanisms of Proliferation and Differentiation of Human Fetal Hepatic Stem Cells in Vitro.

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Objective: Our study is aim at separating and purifying hepatic stem cells from human fetus liver, and identify its biological functions. Meanwhile, we will detect their abilities of differentiation into mature hepatocytes and the mechanisms of proliferation and differentiation. Our work will supply theory basis for the future cells transplantation and human liver tissues reconstruction in mice and even large animals.
Methods and results: (1) By the combination of united enzymatic digestion, gravity sedimentation, monoclonal digestion, selective digestion, we isolated hepatic stem cells(hFHSCs) from human fetal liver in the early stage of gestation. The isolation method is simple and operated easily, and obtained cells have high vigor. (2) hFHSCs accord with foundamental characteristics of hepatic stem cells: they have strong proliferation ability in vitro, express both liver－connectors and biliary tree markers and have the normal karyotype. (3) HGF and EGF can stimulate hFHSCs to proliferate obviously. (4) HGF plays an important role on inducing hFHSCs to differeniate into mature hepatocytes, and EGF can be in coordination with it. (5) Inducing differentiation system was constructed in vitro, which will contribute to solving the problem of donator shortage on human mature hepatocytes treatment. (6) NF－κB activity was repuired for HGF－induced proliferation and differentiation in hFHSCs by phosphorylation and degradation of IκBα.
Conclusions: We obtained hFHSCs by a simple method and constructed an effective inducing －differeniating cultivation system, meanwhile, the investigations on the mechanisms of proliferation and differentiation in hFHSCs will provide the rationale for optimizing this system. The whole work will provide experiment basis and new clues for solving the problem of donator shortage of human mature hepatocytes and making hepatocytes and hepatic stem cells transplatation come into realities on the stage of clinical treatment.

Expression of Nanog of Rat Tracheal Epithelium During the Recovery of Injury Induced by 5－FU in Vitro

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Objective: To investigate the dynamic expression of Nanog, which is one of the correlative genome of embryonic stem cell genes, in rat tracheal epithelium during the recovery of injury induced by 5－FU in vitro, and discuss the molecular mechanism of the forming and retainning undefferentiated state of the tracheal stem cells.

Materials and Methods: 1. Preparation of tracheal epithelium regeneration model of rat. 2. Hematoxylin – eosin stain was used to observe the morphological changes during tracheal epithelium regeneration. 3. To observe the dynamic changes of Nanog during the process by indirect immunofluorescence. 4. Western blot analysis. **Results**: It was found that Nanog is negative in normal tracheal epithelium. The tracheal epithelium desquamated after 5 – FU treatment and the residual cells were trifle nude – nucleus distributed intervally on the basement membrane (G0 phase cells). Expression of Nanog was positive in G0 phase cells at 0h after 5 – FU treatment. The tracheal rings were covered with flattened epithelial cells at 3 – 6h after the removal of 5 – FU, and a lot of the epithelial cells were Nanog positive and to its top expression. At 24h, most of the epithelial cells were cuboidal cells, and Nanog positive cells decreased obviously. At 48h, pseudostratified mucociliary epithelium appeared in some region of tracheal epithelium, when Nanog was in minimal level. The result of western blot was consistent with it of indirect immunofluorescence.

Conclusions: Nanog expressed in G0 phase cells, and it is presumed that somatic cells had been reprogrammed after strong stimulus, and they obtained the function and feature of stem cells, and repaired the impaired tracheal epithelium. Nanog expressed strongly in the stem cells , then decreased gradually following the cell differentiation. It is suggested that Nanog plays a important role to retain undefferentiated ability of cells.

Key words: injury and recovery of tracheal epithelium; stem cell; reprogram

DNA Repair Capacity and the Risk of Non – small Cell Lung Cancer

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Worldwide, lung cancer is the most common malignancy and the leading cause of cancer mortality. The role of tobacco smoking in the etiology of lung cancer is well established, but only about 15% of smokers develop lung cancer. Why? The answer is the variation in susceptibility between individuals. Studies show that cells have evolved DNA repair capacity (DRC) to maintain the genome integrity. Because cells must face to the attacks from endogenous or exogenous genotoxic chemicals, the extent of damage repaired by DNA repair system is of great importance. Host cell reactivation assay (HCR) is a powerful tool, which allows researchers to measure the capacity of whole DNA repair system. We applied HCR to evaluate the effect of DRC on the risk of non – small cell lung cancer (NSCLC) and to evaluate the interactions of DRC and smoking on the risk of NSCLC.

There were 107 cases and 117 controls in this case – control study. They were frequently matched according to sex, age (± 5) and smoking status. Lymphocytes were isolated from peripheral blood, then stored in – 80℃. Plasmids (p – CAT – control) was treated by UVC (254nm) using the dose of 0J/m^2 or 800J/m^2 respectively. The lymphocytes were thawed and cultured in RPMI1640 medium (supplement of phytohemagglutinin) in batches. The cells were transfected with damaged or undamaged plasmids by DEAE – dextran methods after 72h. Cell lysis was extracted after 44h, stored in – 80℃. The cell lysis reacted with 3H – chloramphenicol after being thawed. With appropriate scintillation fluid, the radioactivities of the samples were measured by a liquid scintillation counter. The cpm values were recorded for the cells with undamaged plasmids (control reading) and UV – damaged (repair reading) plasmids. DRC (%) = (damaged plasmid value/undamaged plasmid value) × 100%.

The subjects were divided into two groups according to the DRC median of controls, named optimal DRC and suboptimal DRC. Suboptimal DRC was associated with the risk of NSCLC, OR value was 4.026 (2.211 – 7.329). OR for adenocarcinoma was 2.949 (1.397 – 6.225), OR for squamous cell carcinoma was 5.196 (2.144 – 12.596), and OR for other type was 6.390 (1.381 – 29.574). The subjects were divided into two groups

according to the smoking index median of controls, named heavy smoker and light smoker. Heavy smoker was associated with the risk of NSCLC, OR value was 2.227 (1.284 – 3.860) for NSCLC, 2.265(1.078 – 4.758) for adenocarcinoma, 3.195 (1.448 – 6.864) for squamous cell carcinoma and 3.800 (1.019 – 14.169) for other type. The subjects were divided into four types: optimal DRC + light smoker group (reference group), optimal DRC + heavy smoker group, suboptimal DRC + light smoker and suboptimal DRC + heavy smoker group. OR of suboptimal DRC + heavy smoker group was 7.154 (2.960 – 17.290) for NSCLC, 4.673 (1.659 – 13.164) for adenocarcinoma, 7.615 (2.032 – 28.537) for squamous cell carcinoma, and 11.423 (1.376 – 94.860) for other type. In suboptimal DRC + light smoker group, OR for squamous cell carcinoma was statistically significant. The value was 4.091 (1.071 – 15.621). The results indicated that poor DRC and heavy smoking associated with the risk of NSCLC. And the interactions between DRC and smoking increased the risk of NSCLC.

Suboptimal DRC is a very important susceptible factor for the risk of NSCLC. The interactions between DRC and cumulative smoking can increase the effect of either factor on the risk of NSCLC. The individuals with suboptimal DRC and heavy smoking are more susceptible to NSCLC.

The Localizational Study of β – Glucuronidase in Lysosome in Epithelial Cell of Breast Duct

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Abstract: Objective: To study the overtiny structure localization of β – Glucurinidase (β – G) in the epithelinal cell of the human breast duct. **Methods:** The technique of anti – β – Glucuronidase antibody and colloid gold bougue and immuno electro microscope were used. **Results:** Gold grains labeled β – G were localized in endoplasmic reticulum and lysosome. **Conclusions:** Successful localization of β – G in endoplasmic reticulum and lysosome in epithelial cell of breast duct could be used normal morphologic midel to study the breast and the tumor in breast.

Key Words: β – Glucuronidase; Colloid gold; immuno electro microscope; endoplasmic reticulum; lysosome.

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Research on Cytochip of Cell Membrane Carbohydrate Indetification

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Abstract: Carbohydrates cover the surface of most living cells and organisms in the form of diverse glycoconjugates, Glycans create a landscape of recognition sites, barriers, and carriers that help control the rhythms of metabolism from conception to catabolism. Generally, glycans represent cell's mediating recognition and communication processes and, they control immunological recognition, cell – cell adhesion, pathogen attack, and protein folding and placement. **Objective:** To present the use and applications of a lectin micro – array to observer the surface glycosylation pattern of cells. **Methods:** Lectin microarray: 20 kinds of lectins were printed on glass slides those have been conjugated with chemical groups; Cell: cell was collected form the normal serum of human and the different tissues of mouse; HE straining of cell. **Result:** the lectin microarray can capture cells through the cybonhydrate chain on the surface of cell, immobilized cells form different tissue were specifically and differently

detected on lectin micro－array. **Conclusion:** The lectin micro－array can be used to observed the the surface glycosylation pattern of cells.

Key word: lectin micro－array glyconjugate cell menbrane

A Case of Comamonas Testosteroni Isolation From Pleural Fluid of Lung Cancer

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Abstract: Comamonas testosteroni, a lesser－known member of the genus belonged to comamonas, is a kind of conditioned pathogen and metabolizes testosterone as the sole carbon source via a meta－cleavage reaction. According to the reports, it has been isolated from some clinical specimens such as blood, pus, urine and secretion of airway but not from pleural fluid of lung cancer. We here present a case of gram－negative bacteria isolation from pleural fluid of a male 51－year－old lung cancer patient with brain metastasis. By means of bacterial culture, biochemistry analysis and drug sensitivity test, we identified that the isolated bacteria was comamonas testosteroni. Lung hasn't been regarded as a target organ of sex hormone in the past, however, recent study indicated that the sex hormone may have a role in the carcinogenesis of bronchial lung cancer. Although further study need to be performed, our study suggests that testosterone probably exists in the pleural fluid of lung cancer and may thereby causes the growth of conditioned pathogen.

FISH of 20 Cases of Gastric Lymphoma

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Recent cell genetical studies have indicated that trisomy 3 is a feature of gastric lymphomas(GL). Twenty cases of these GL in Shenyang district were studied in total, including 10 mucosa－associated lymphoid tissue (MALT) lymphomas, 9 diffuse large B cell lymphomas (DLBL) and 1 mantle cell lymphomas(ML), stained by H&E and immunohistochemistry of CD3、CD5、CD20. To clarifing the pathogenesis and relationship between trisomy 3 and (H. pylori) HP、EBER－1 in these tumors, paraffin samples were stained by immunohistochemistry of antibody of HP (DAKO), fluorescence in situ hybridization (FISH) of α－satellite probes for chromosomes 3, and EBER－1 in situ hybridization. Trisomy 3 was found in 8 of the 20 cases (40%). The percent of HP and EBER－1 is respectively 85% and 20%. The coherence between HP and trisomy 3 is 100%. These finding suggest that, in Shenyang district, HP and trisomy 3 play important roles in pathogenesis of GL, and perhaps, there is some relationship between HP and trisomy 3. EBV concerns also with early oncogenesis of GL in Shenyang district.

Cytoplasmic Connexin32 Enhances Growth and Metastatic Ability of Human HuH7 Hepatoma Cells in Vitro and in Vivo

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A considerable number of studies have stated that gap junctional intercellular communication (GJIC) suppresses tumor development during carcinogenesis, i. e. GJIC is down－regulated in almost all tumors through whatever mechanisms, including no or reduced expression, aberrant localization, and aberrant phosphorylation or dephosphorylation of connexin (Cxs) and restoration of GJIC has been reported to reverse the malignant phenotype of some transformed cells. In the liver, normal hepatocyte express both connexin26 (Cx26) and connexin32 (Cx32) and they exhibit a high level of GJIC with neighboring counterparts. Conversely, GJIC is abolished in hepatocellualr carcinoma (HCC). While no expression of Cx26 mRNA or protein is detected in either human or rat HCCs, Cx32 protein very often continues to be expressed not in a cell－cell contact but in cytoplasm without mutation, at least in the coding redion, thus incapable of forming gap junction. Such an aberrant localization of connexin proteins is generally considered to be a "loss of function" as a component of gap junction. Recently, cytoplasmic expression of Cx26 was reported to induce invasion and metastasis in breast cancer, which raises a question that whether cytoplasmic Cx32 in HCCs exerts some other functions than gap junction. To elucidate the roles of Cx32 protein in HCC growth and progression, we introduced human Cx32 cDNA into HuH7 human HCC cell line by retroviral infection and established doxycycline(Dox)－ responsive Tet－off Cx32 clones where expression of Cx32 protein could be enhanced by Dox removal from the medium. Densitomeric analysis of the immunoblotting gels showed that the expression of Cx32 was approximately 4－fold up－regulated in the established HuH7 Tet－off Cx32 cells after Dox removal compared with in 8 μg/ml－Dox－supplemented medium. Furthermore, the expression of Cx32 protein increased gradually as the amount of Dox decreased. Indirect immunofluorescence revealed that overexpression of Cx32 induced by Dox withdrawal was not localized in cell－cell contact but cytoplasm in HuH7 Tet－off Cx32 cells. Further Golgi apparatus staining showed that the Cx32 protein was expressed in Golgi apparatus. Cell surface protein biotinylation indicated that no Cx32 protein was detected in the cell surface protein fraction in either the presence or the absence of Dox. In contrast, E－cadherin, an essential component of adherens junctions in hepatocytes, is successfully enriched in the cell surface protein fraction. No cells were dye－coupled in either the presence or the absence of Dox, as revealed by scrape－loading dye transfer assay to estimate the GJIC capacity, indicated that the overexpressed Cx32 protein was not functional as a component of gap junctions in HuH7 Tet－off Cx32 cells. The anchorage－dependent proliferation rate of the HuH7 Tet－off Cx32 cells was significantly higher in Dox－free medium than in the Dox－supplemented medium. Performed by precoating the 8－μm millipore filter of Boyden chamber with vitronectin or matrigel, Serum－stimulated transwell assay showed that Dox withdrawal enhanced both motility and invasiveness of the HuH7 Tet－off cells, while Dox did not affect motility and invasiveness of HuH7 Tet－off mock cells. To exclude involvement of undetectable GJIC in the upregulation of motility, cell motility was assayed in the presence of olealmide, a specific GJIC inhibitor. Oleamid did not have any effect on cell motility of either HuH7 Tet－off Cx32 cells or mock cells, confirming that GJIC is not implicated in the Cx32－mediated upregulation of motility in HuH7 Tet－off Cx32 cells. On the other hand, the Cx32－transfected Hela cell clone, which exhibited a high level of GJIC and a lower motility than mock－transfected Hela cells, regains its high motility after treatment with oleamide. Intrahepatic spreading is the major critical reason for liver failure caused by HCCs. We thus examined how HuH7 Tet－off Cx32 cells overexpressing cytoplasmic Cx32 protein behaved in vivo when thay were

grafted into the liver of SCID mice by subserosal injection of them along with the mock transductant. Dox was administrated in drinking water to repress the expression of Cx32 protein. Five out of six mice developed tumors at the implanted sites in each of the Dox(+) and the Dox(−) groups. Macroscopic metastatic lesions were found in all of the five tumor − bearing mice in the Dox(−) group but in none of the mice in the Dox(+) group, which clearly indicated that the overexpressed cytoplasmic Cx32 can give the metastatic ability to HuH7 Tet − off Cx32 cells. When the cells were subcutaneously xenografted into the backs of athymic nude mice, three out of six mice given HuH7 Tet − off Cx32 cell in the Dox(−) group but none in the Dox(+) group developed a tumor, which showed that the overexpression of cytoplasmic Cx32 upregulated tumorigenicity and growth of HuH7 Tet − off Cx32 cells in vivo. In a word, our results suggest that cytoplasmic Cx32 protein exerts favorable for HCC progression, such as invasion and metastasis, once the cells have acquired a malignant phenotype.

Heparanase Expression is Correlating with Metastasis and Poor Prognosis of Non − small Cell Lung Cancer

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Abstract: Heparanase (Hpa) is an endoglycosidase that cleaves HS side chains of HSPGs at a limited number of sites, thus playing a critical role in tumor metastasis and angiogenesis. In the present study, samples of 115 non − small cell lung cancer cases and 40 neighboring noncancerous tissue were studied with anti − heparanase, VEGF, VEGF − C, VEGF − R3 and CD34 antibodies. Expression of heparanase was positive in 91 lung cancer samples, but was negative in normal lung tissue. Heparanase expression was associating with clinical stages ($r=0.203$, $P=0.030$), vascular invasion ($r=0.344$, $P=0.000$), and lymphatic metastasis ($r=0.276$, $P=0.003$). Patients with positive heparanase expression had a worse prognosis than those with a negative haparanase expression ($P=0.0024$). Heparanase expression was associating with VEGF expression ($r=0.321$, $P=0.001$) and VEGF − C expression ($r=0.302$, $P=0.001$). Microvessel density (MVD) and lymphatic vessel density (LVD) in heparanase positive tumors were significantly higher than in heparanse negative tumors ($P<0.001$). VEGF expression was related to MVD ($P<0.001$), and VEGF − C expression was related to LVD ($P<0.001$). MVD and LVD were closely related to blood metastasis ($P<0.001$) and lymphatic metastasis ($P=0.010$) respectively. The proliferation, invasion capacity and locomotory ability of highly metastatic potential cell line of human lung giant cell cancer PG − BE1 were reduced by inhibition heparanase expression in it. At the same time, the expressions of VEGF, VEGF − C and pAkt in PG − BE1 were decreased. The results suggested heparanase in non − small cell lung cancer could increase tumor invasion and metastasis; heparanase maybe facilitate expression of VEGF and VEGF − C correlating with PI3K/Akt signal pathway.

Gene Therapy of Murine Orthotopic Liver Cancer with Inducible IL − 12 Gene Mediated by Hydrodynamic Injection

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Abstract Background: Most diseases are heterogenic and dynamic in nature. Therefore, the ability to control transgene expression in temporal and spatial in vitro and in vivo is very important, especially in tumor gene thera-

py. This study was aimed to evaluate the inducible ability of plasmids carrying an RU486 regulatory system and the antitumor effect of inducible mIL－12 gene. **Methods**: We used plasmid pRS22 containing RU486 regulatory system, liver－specific promotor TTR and mIL－12 gene or plasmid pRS－LacZ encoding LacZ gene. These plasmids were transfected into cells derived from different tissues or injected into mice by hydrodynamic injection. LacZ expression was examined in cultured cells and tissues by χ－gal staining. The mIL－12 level was tested in the supernatants of cultured cells and in serum by an ELISA kit. The safety of hydrodynamic injection was evaluated by histological analysis. The distribution and inducible expression of pRS22 in mice were assayed by measuring DNA, RNA and protein levels using PCR, RT－PCR and immunohistochemical staining. The antitumor effect of inducible mIL－12 gene was tested in mice bearing orthotopic liver cancer via hydrodynamic injection of pRS22. **Results**: The levels of ALT and AST in serum increased abruptly 10 h after hydrodynamic injection of saline or pCMVβ, then declined rapidly to normal level at 7 days. The necrotic areas in liver tissue recovered by proliferation of hepatocytes within one week. A kinetic study indicated that mIL－12 could be detected at 4 h after administration of RU486. At 10 h the serum concentration of mIL－12 reached a peak level that was 3578－fold higher than that of pre－induction with RU486. Then it decreased to a minimal level at 24 h and was undetectable by day 3. When induced every 7 days, the kinetic curve of mIL－12 expression was similar. Though peak values of mIL－12 decreased gradually after each induction, mIL－12 in serum could be detected until 4 months after plasmid administration. We only detedcted mIL－12 expression in liver tissue. The expression level of mIL－12 was related to the dose of RU486 and plasmid, increasing the dose of RU486 and plasmid caused higher expression of mIL－12 in serum. The sustained levels of mIL－12 could be achieved by administration RU486 every day within 6 days. The mIL－12 induced by RU486 could produced IFN－r and NO, inhibit tumor cells proliferation and angiogenesis of tumor, prolonged the survival of mice bearing orthotopic liver cancer. **Conclusion**: The expression of transgene mIL－12 can be switched on or off at desired time by addition or withdraw of RU486. The expression of mIL－12 is limited only in liver. We can not only give scope to the antitumor effect of mIL－12, but also decreased its toxicity to organism. Those apply new idea to gene thereapy of liver cancer.

Key words: IL－12; hydrodynmic injection; RU486; plasmid DNA; gene therapy; liver cancer

Electron Microscope Observation of the Relationship Between Mast Cells And Neovascularization in the Substantia Propria of the Cornea After the Wasp Stings the Cornea

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Pathological changes after corneal injury, infection and toxic material invasion will lead to the proliferation of mast cells and corneal neovascularization. Corneal neovascularization often cause tissue cicatrization and persistent inflammation, which is one of the most common causes that lead to corneal blindness. This article uses the technique of transmission electron microscope (TEM), we use some wasp－stinged corneal tissue, prepare the electron microscope sample conventionally, observe the relationship between mast cells and neovascularization in the substantia propria of the cornea, which aims at discussing the role they play in the blindness caused by corneal damage.

Under the electron microscope we see there are many mast cells distributed in the substantia propria of the cornea, they are large in volume, some thin processes extended from their surface, the karyotypes of the mast cells are usually irregular, they are usually eccentric and close to the cell membrane, there are few organelles and many secretory granules in the cytoplasm of the mast cells, most granules show high electron density, and they are dif-

ferent in size, some undergo fusion, some migrate to the margin of the cell membrane, many granules disperse among the connective tissue. We also see many corneal neovascularizations, there are mast cells and granules released from the mast cells distributed near the neovascularization. There is close contact between the mast cells and the vascular endothelial cells, the granules released from the mast cells move near or enter the vascular endothelial cells. There is dilation of the cavity of the newly formed blood vessels, many processes of the basal membrane of the endothelial cells extend to the peripheral connective tissue.

Neovascularization is the procedure that the vascular endothelial cells proliferate and migrate and form new blood vessels. In 1949, Campbell first put forward that neovascularization is because of a kind of electromigratory soluble factor that exist in the cornea. Now many polypeptide factors have been purified, such as leptin, shh, interleukin-1 (IL-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-4 (IL-4), and interleukin-13 (IL-13), nitric oxide synthase-II (NOS-II), matrix metalloproteinase-2 (MMP-2), cysteine protease, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), angiopoietin (Ang), they are all related to neovascularization. Mast cell can not only synthesize, reserve and release many active mediums, but also is the source of many cytokines. Some research use in vitro culture method, mix mast cells and vascular endothelial cells together, electron microscope observation show that the granules of the mast cells can migrate to the vascular endothelial cells near them, and the conclusion that mast cells can induce neovascularization has been confirmed by many researches. Human mast cells can produce cytokines such as SCF, IL-1, IL-4, IL-5, IL-6, bFGF, VEGF, platelet derived growth factor (PDGF), among them VEGF, bFGF, PDGF can induce more mast cells migrate to the neovascularization, and promote the neovascularization. The granules of the mast cells also contain biological active mediums such as heparin, histamine, tryplase, secretin, when the mast cells undergo degranulation, they are released to the peripheral environment, they have the function of modulating the proliferation of the vascular endothelial cells and promote neovascularization. The bee venom contains many biological amines (such as histamine) and non-enzymatic polypeptides (such as bee venom peptide) and phosphatase A, B and hyaluronidase, which can cause toxic reaction and allergic reaction. After the wasp stings the cornea, the direct action of the bee venom cause damage to the inhibitory factors of the neovascularization, and lead to the production of many mast cells, most mast cells show degranulation phenomenon, which means very active secretory electricity, thus the active mediums secreted by the mast cells and the overexpression of the cytokines and the hypoxia of the corneal microenvironment lead to the corneal neovascularization. The close contact between mast cells and newly formed blood vessels morphologically indicates the functional interaction between the two, they both take part in the modulation of the corneal microenvironment, and is the main cause of corneal blindness. Corneal neovascularization is a complicated pathological process, it results from the interaction of various factors.

Expression and Significance of Aquaporin-3 (AQP3) in Normal and Neoplastic Tissues of Multiple Human Organs

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Abstract Objective: To investigate the presence and expression pattern of AQP3 in normal and neoplastic tissues of multiple human organs and its differential diagnostic sense between different adenocarcinomas. **Methods:** Immunohistochemistry staining (EnVision Two-Step method) was used to detect the expression of AQP3 in 86 normal and adenocarcinoma cases including colon (15 specimens), stomach (5 specimens), prostate (5 specimens), breast (35 specimens), uterus body (11 specimens), ovary (10 specimens) and thyroid (5 specimens).?

2 test was used to analyze the results by the SPSS 12.0 software. **Results:** (1) In normal tissues, immunohistochemical expression of AQP3 was demonstrated in the membrane of gland secretory cells. The frequency of AQP3 immunoreactivity in normal tissues were: 100% (5/5) in prostate, 75.0% (6/8) in breast, 60% (3/5) in stomach, 14.3% (3/21)) in endometrium, and 37.5% (3/8) in colon, respectively. No specific immunoreactivity of AQP3 was observed in ovary and thyroid. (2) AQP3 was observed in 23.3% (20/86)of the adenocarcinoma tissues. The highest and strongest immunoreactivity of AQP3 was observed in prostate adenocarcinomas (100%, 5/5), while lower and fainter immunoreactivity was observed in breast (14.3%, 5/35)、stomach (80.0%, 4/5)、endometrium (27.2%, 3/11) and colon (20.0%, 3/15). No specific immunoreactivity of AQP3 was observed in ovary and thyroid adenocarcinoma. (3) There was a significant correlation between expression of AQP3 and tissue histological type in adenocarcinomas by X2 statistic analysis. **Conclusions:** AQP3 is expressed in normal and neoplastic tissues of stomach、colon、breast and endometrium in different extension, highly expressed in prostate. No specific immunoreactivity of AQP3 was observed in ovary and thyroid adenocarcinoma, suggesting that AQP3 may be of possibility to be a new candidate marker to exclude ovary and thyroid in identifying the origin of a metastasitic adenocarcinoma in the future, although the detailed mechanism of AQP3 expression in different adenocarcinomas remains to be clarified.

Key words: Aquaporin－3; adenocarcinoma; immunohistochemistry; differential diagnosis

Changes in Notch Signaling Pathway During Regulation of Rat Tracheal Epithelial Regeneration

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Abstract Objectives: To explore the role of Notch signaling during proliferation and differentiation of rat tracheal stem cells. **Materials and Methods:** We developed an ex vivo model of rat tracheal regeneration using fluorouracil (5－FU) to induce injury. Expression levels of members of the Notch signaling pathway were examined by RT－PCR in treated and untreated samples, and expression of Notch－3 and Jagged－1 proteins were examined by Western blot and immunofluorescence. In addition, expression levels of ABCG2, CK19, and PCNA were examined by Western blot in tracheal epithelia treated with and without a γ－secretase inhibitor. **Results:** Expression levels of Notch－3, Jagged－1 and Hey1 were increased in rat tracheal epithelial cells after treatment 5－FU, relative to untreated cells. Levels of Notch－3 and Jagged－1 reached peak expression within 6 h of treatment, while Hey1 expression peaked around 12 h. Levels of all three genes decreased gradually and returned to levels similar to those of untreated cells within 48 h. Disruption of Notch signaling by treatment with a γ－secretase inhibitor led to reduced recovery following treatment with 5－FU; expression levels of ABCG2 and PCNA were lower, while CK19 was higher than in cells not treated with γ－secretase inhibitor. **Conclusions:** In the normal rat trachea, Notch signaling maintains self－renewal of tracheal stem cells. During tracheal epithelial regeneration, Notch signaling via Hey1 maintains an undifferentiated state and promotes proliferation among a population of tracheal cells.

Key words: tracheal stem cells; Notch signaling; proliferation; regeneration

Expression of Integrin Beta3 and Tenascin－c and Their Significance in Breast Invasive Ductal Carcinoma

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Abstract Objective: To explore the expressions of integrinβ3 and Tenascin－c(TN－c) in breast invasive ductal carcinoma and their effect on the invasion and metastasis of breast invasive ductal carcinoma. **Methods:** The expressions of integrinβ3 and TN－c were detected in 80 cases of breast invasive ductal carcinoma by immunohistochemical(S－P) method. The relations of integrinβ3 and TN－c expression with thepathological features of breast invasive ductal carcinoma were evaluated. **Results:** The positive rate of integrinβ3 and TN－c expressions in breast invasive ductal carcinoma tissues was 56.25% and 86.25%, respectively. 41 cases showed TN－c positive in integrinβ3 positive and 29 cases showed TN－c negative in integrinβ3 negative. A significance positive correlation was found between integrinβ3 and TN－c expression ($P<0.05$). The expressions of integrinβ3 and TN－c was related to lymph node metastasis and TNM stage ($P<0.05$), and they were not related to age and tumor size ($P>0.05$). **Conclusion:** Integrinβ3 and TN－c may play a synergic role in the invasion and metastasis of breast invasive ductal carcinoma, and their expressions can be used as important indexes in detecting the metastasis and invasion of breast invasive ductal carcinoma.

Key words: Breast invasive ductal carcinoma; Integrinβ3; Tenascin－c; Metastasis.

Study on Effect of AGEs on Injuring of Rat Renal Microvascular Endothelial Cells and Protective Effect of Probucol in Vitro (Abstract)

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Objective: To explore the effect of AGEs on injuring of rat renal microvascular endothelial cells and protective effect of antioxidant probucol. **Methods:** Microvascular endothelial cells isolated and cultured from rat renal were divided into 3 groups: normal group, AGEs group and probucol group. The levels of MDA and NO were tested by the methods of TBA and nitrate reductase respectively. The expression of eNOS mRNA and NADPH oxidase protein was detected by RT－PCR and western－blot respectively. The intracellular disposition of NF－κB was observed by immunostaining and confocal microscopy. **Results:** In comparing to control groups, AGEs increased the level of MDA and upregulated the expression of eNOS mRNA and NADPH oxidase., both showing a dose and time dependment effect. Exposure of microvascular endothelial cells to AGEs for 30 minutes and 6 hours resultded in increasing the level of NO, but when the exposure time reached 12hours , the level of NO decresed. probucol decrease the level of MDA, inhibited the expression of eNOSmRNA and NADPH oxidase , Probucol also improved the level of NO. Exposure of microvascular endothelial cells to AGEs for 30 minutes resultded in NF－κB translocation to nucleus. **Conclusion:** AGEs can cause the injuring and dysfunction of renal microvascular endothelial cells via the following possible mechanisms: (1) activation of NADPH oxidase , increasing the production of ROS; (2) enhancing the mRNA expression of eNOS, influencing the production of NO; (3) activatin of NF－κB . Probucol can protect microvascular entothelial cells via inhibiting the activation of NADPH oxidase and NF－κB. decreasing the mRNA expression of eNOS.

Expression of RhoC and its Regulator RhoGDIα in Lung Squamous Cell Cancer and Adenocarcinoma

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Objective: In order to discuss the function and significance in lung cancer, we design to examine the expression of RhoC and RhoGDIα in lung Squamous Cell Cancer and Adenocarcinoma. Then analyze the relationships between

them and the clinicopathological characteristics and the survival time of patients.

Methods: Immunohistochemical staining using 100 samples with their neighboring noncancerous tissue samples and using Western Blot to test protein expression of 60 fresh lung cancer samples.

Results: There are 68 cases of Lung Squamous Cell Carcinoma and Adennocarcinoma tissue that appeared brown particles in cytoplasm of tumor cell among 100 cases of RhoC, which are 73 cases of RhoGDIα. the level of RhoC and RhoGDIα expression were higher in cancer tissue than in paracarcinoma. And they had no obvious relationship($P=0.002$, $P=0.008$). The expression of RhoGDIα were both correlated with lymph node metastasis($P=0.014$) and clinic stage ($P=0.022$).

Conclusions: RhoC and RhoGDIα protein are overexpress in Lung Squamous Cell Carcinoma and Adennocarcinoma and they had no obvious relationship. The overexpression of RhoC and RhoGDIα are correlated with lymph node metastasis and clinic stage. And the upgrade of RhoC and RhoGDIα expression in lung cancer reduce the survival time of patients and imply the poor prognosis.

Key words: RhoC; RhoGDIα; lung cancer

Global Gene Expression Profile of Gastric Carcinoma by Complementary DNA Microarray

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Abstract: Gastric cancer is one of the most frequently diagnosed malignancies in the world. The expression profiling and molecular grouping of gastric cancer has been a challenging task because of their complexity and variation. To understand the molecular mechanism associated with gastric carcinogenesis, we analyzed gene expression profiles of 10 gastric cancer/nontumor mucosa couples using 14K cDNA microarray . Data for the different expressed genes were verified using reverse transcription－PCR, Western blotting and immunohistochemical staining in the gastric cell lines and tissues. Forty genes were identified as either up－regulated or down－regulated genes in human gastric cancer tissues. Among these, genes such as CDH17, ETV4, S100A6, S100A11 and Ephb4 were confirmed to be up－regulated genes in six gastric cell lines by semiquantitative reverse transcription－PCR. On the other hand, genes such as NK4 and PPP2R1B were identified as down－regulated genes. Western blotting and immunohistochemical analyses Ephb4 for showed overexpression and localization changes of the corresponding protein in human gastric cancer tissues. Evaluation of genome－wide gene expression profiles of gastric cancer tissue provides the significant genetic information to understand the cancer biology and characteristics of tumor. Those newly identified genes should provide a valuable resource for understanding the molecular mechanism associated with tumorigenesis of gastric carcinogenesis and for the discovery of potential diagnostic markers of gastric cancer.

The Relationship Between ERCC2 Polymorphism and Risk of Lung Cancer in Nonsmoking Females

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Excision repair cross – complimentary group 2 (ERCC2) is one of the important DNA repair genes. ERCC2 codon 751 polymorphism has been shown to modulate cancer risk. We therefore assess the relationship between the ERCC2 polymorphism and susceptibility to lung cancer in nonsmoking females via a hospital – based case – control study. We conducted a case – control study, which had 150 lung cancer cases and matched healthy controls. Information concerning demographic and risk factors was obtained for each case and control by a trained interviewer. After informed consent was obtained, each person donated 2ml blood for biomarker testing. ERCC2 genotypes were determined by PCR – RFLP method. All of the statistical analyses were performed with SPSS (v 12.0). All of the subjects in this study were nonsmoking females in Shenyang. There was a significantly difference between the frequencies of ERCC2 polymorphism in cases and controls ($P<0.05$). The individuals with Lys/Gln + Gln/Gln combined genotype were at an increased risk for lung cacner as compared with those carrying the Lys/Lys genotype (adjusted OR = 2.87, 95% CI = 1.49–5.82). We analyzed the environmental risk factors for lung cancer in nonsmoking females. Cases showed a higher prevalence of cooking fumes compared with controls (OR = 2.45, $P<0.05$). Furthermore, an interaction between cooking fume exposure and the ERCC2 751Gln allele on the risk of lung cancer was observed. Individuals with both risk genotype and cooking fume exposure have a higher elevated risk of cancer than those with only one of them. The above findings indicate the Lys751Gln polymorphism in the XPD gene is associated with the risk of lung cancer in nonsmoking females. Individuals with both XPD751Gln allele genotype and cooking fume exposure have a higher elevated risk of cancer than those with only one of them in nonsmoking female population.

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Our Experience on Application of Internet Telepathology Consultation

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Telemedicine is a new interdiscplinary field. Connecting medicine and communication via telemedia, telemedicine will stongly improve development of medicine. Telepathology consultation is an important part of telemedicine.

Some suspicious pathological case need consultations, especially for those small hospital. In present, sending slides to famous pathologist is main method of pathology consultation, which cost much and delay treatment in time.

Telepathology, pioneered by Weinstein and associates in the mid 1980s, described as the practice of pathology through communication via telemedia, has get more and more agreement from international pathologists. Telepathology consultation offers the advantage of sending microscopic images to pathologist via internet, allowing diagnosis of suspicious cases and treating patients in time. With rapid development of communication via computer multimedia, feasibility and availability of telepathology get much improvement. Now, telepathology can be applied on clinical pathology and allow effective transmitting digital images and management, multi – point transmission among international groups, and collecting clinical and educational information.

Established in 1999, our group researched and practiced 'internet telepathology consultation system'. After 8 years hard working, we successfully invented internet termination of 'Telepathology Center' based on internet platform, and took some research on management of medical information, transmission and compression of digital images at a distance, clinical application and management of telepathology. All of these provides firm basement for

further development of telepathology in China.

We have finished 200 cases consultations with ¥100/30min on each case, including diseases in every systems, such as gastrointestine, breast, thyroid gland, soft tissues and so on. Each consultation was finished in half hour, getting consultation from consultants without going out. These economical consultations were welcome by doctors and patients and get wide confirmation from pathologist in China.

On the other side, at the same time of bringing social interest, telepathology has some disadvantages, for example, expensive instruments costing several hundred thousand, setting up ' national system of internet telepathology consultaion', technical training members about pathological images and diagnosis in front of screen and computer practice, balancing rights and responsibility between hospitals which send or accept images, widely application and management of telepathology.

Expression of RET, HBME－1 and Ki67 in Benign and Malignant Thyroid Tumors

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Abstract

Objective: To explore the expression of RET, HBME－1 and Ki67 proteins in thyroid tumors.

Methods: The expression of RET, HBME－1 and Ki67 were detect by means of tissue microarray technique and immunohistochemistry method in the following thyroid lesions: 22cases of nodular goiters, 7follicular adenomas, 47 pappilary carcinomas, 7 undifferentiated carcinomas, 5 medullary carcinomas, 4 follicular carcinomas, 1 poorly differentiated insular carcinoma and 1 squamous carcinoma.

Results: Significant difference of all the three protein was found between benign and malignant thyroid tumors ($P<0.01$). But there was no difference among benign group ($P>0.05$). The positive rate of HBME－1 in papillary carcinomas were higher than that in follicular, medullary and undifferentiated carcinomas ($P<0.01$). Ki67 expression in undifferentiated carcinoma was higher than papillary carcinomas ($P<0.05$).

Conclusion: Allied test of RET, HBME－1 and Ki67 expression may be useful to determine malignancy of thyroid tumors. HBME－1 is the most sensitive among the three and RET is the next , upregulated expression of Ki67 may suggest de－differentiation, infiltration and metastasis of tumor. Simultaneous increase of all the three markers in benign thyroid tumor most probably implies strong tendency of malignancy.

Key Words: thyroid neoplasm, thyroid carcinoma, RET, HBME－1, Ki67

Infectious Mononucleosis

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Infectious mononucleosis is an is an infection caused by the Epstein－Barr virus(EBV). It is a disease seen most commonly in adolescents and young adults, characterized by fever, sore throat, muscle soreness, and fatigue. And some patients also display splenomegaly and/or hepatomegaly, skin rash and night sweats. The laboratory hallmark of the disease is the presence of atypical lymphocytes on the peripheral blood smear. In addition, the overall white blood cell count is almost invariably increased, particularly the number of lymphocytes. However, the signs and symptoms are variegated, and the atypical presentations of mononucleosis is difficult to make the diagnosis.

EBV is a lymphotropic DNA virus, belonged to the Herpesvirus. Mainly infect B cells, and it has five differ-

ent antigens: 1. viral capsid antigen; 2. early antigen; 3. nuclear antigen; 4. lymphocyte determinant membrane antigen; 5. membrane antigen.

After an initial prodrome of 1 – 2 weeks, the fatigue of infectious mononucleosisoften lasts from 1 – 2 months. The virus can remain dormant in the B cells indefinitely after symptoms have disappeared, and resurface at a later date. Many people exposed to the Epstein – Barr virus do not show symptoms of the disease, but carry the virus and can transmit it to others. This is especially true in children, in whom infection seldom causes more than a very mild illness which often goes undiagnosed. This feature, along with mono's long (4 to 6 week) incubation period, makes epidemiological control of the disease impractical. About 6% of people who have had infectious mononucleosis will relapse.

Date	Hospital	Resercher	Cases
2004.1 – 2007.4	China Medical University No.1 Hospital	Zhi Qu	40
2000.1 – 2003.12	Chengdu Children's Hospital	Ming Xu	341
2003.4 – 2004.1	Zhengzhou Children's Hospital	Ming Zhang	40
2001.1 – 2003.12	Jilin University No.1 Hospital	Jinhua Piao	142
2004	Chongqing No.1 People's Hospital	Surong He	12
2000.1 – 2004.9	Henan Ruzhou No.1 People's Hospital	Sumei Han	56
2002 – 2005.3	Sichuan Mianyang Children's Hospital	Wenying Wang	35
2000.7 – 2005.6	Chuan Bei Medical College Hospital	Zengrong Liu	57
2000.1 – 2005.6	Zhejiang Wenzhou Leqing People's Hospital	Wei Zhang	64
2003.4 – 2005.4	Heilongjiang Power Hospital	Yanhong Du	191
2004.1 – 2005.3	Beijing Friendship Hospital	Yingchao Zhang	30
2000.6 – 2006.6	Liuzhou People's Hospital	Linlin Sha	30

Relationship Between MTA1 Expression and Metastasis and Prognosis in Non – small Cell Lung Cancer

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Objective: Metastasis – associated protein 1 (MTA1) is one number of metastasis associated protein family. It's overexpression is correlated with esophageal carcinoma and breast cancer metastasis. But the generality of it's expression in cancer and the significance for judging biological behaviors of tumor and evaluating prognosis of patients is to be investigated. The aim of the study is to research the relationship between MTA1 expression and clinicopathological factors regarding metastasis and prognosis of human non – small cell lung cancer (NSCLC). **Methods:** The expression MTA1 was detected in 101 paraffin – embedded specimens by immunohistochemistry method, as well as in 35 freshly – taken NSCLC tissues by Western – blot. **Results:** There are 56 cases of NSCLC tissue that appeared yellow or even brown particles in nucleus of tumor cell among 101 cases, and in epithelia of bronchi or alveoli in neighboring noncancerous tissue, MTA1 protein showed negative expression. Western blot analysis showed the level of MTA1 was remarkably higher in NSCLC than that in normal tissues ($t = 3.953$, $P = 0.000$). Expression of MTA1 was higher in tumor with metastasis than that in tumor without metastasis ($t = 4.057$, $P = 0.000$). Expression of MTA1 significantly correlated with differentiation ($\chi^2 = 10.131$, $P = 0.006$), lymphatic metastasis ($\chi^2 = 8.535$, $P = 0.003$) and TNM stage($\chi^2 = 17.419$, $P = 0.000$). The survival time of patients with negative MTA1 expression was (44.866 ± 12.946) months, and that of patients with positive MTA1 expression was (23.714 ± 7.498) months($P = 0.002$). In multivariate analysis, only lymphatic metastasis and TNM stage could be considered as prognostic factors. **Conclusion:** MTA1 might play an important role in the development and metastasis of NSCLC. It is indicated that patients with MTA1 expression would have a greater

chance of metastasis and a poorer prognosis. However, MTA1 expression is not an independent prognosis factor.

The Localizational Study of β－Glucuronidase in Epithelial Cell of Breast Duct

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Abstract Objective: To study the overtiny structure localization of β－Glucuronidase(β－G)in the epithelinal cell of the human breast duct. Abstract: **Methods**The technique of anti－β－glucuronidase antibody and colloid gold bougie and immuno electro microscope were used. **Results**Gold grains labeled β－G were localized in endoplasmic reticulum and lysosome. **Conclusions**Successful localization of β－G in endoplasmic reticilum and lysosome in epithelial cell of breast duct colud be used as normal morphologic midel to study the disease and the tumors in dreast.

Expression of TRAF1 and Its Relationship with TRAF2 in the Different Metastasis Breast Cancer Cell Lines

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Objective: To study the expression of TRAF1 and the relationship between TRAF1 and TRAF2 in the different metastasis breast cancer cell lines.
Methods: Immunohistochemical S－P and Western Blot methods were used to detect the expression of TRAF1 in the normal human breast cell line MCF－10A, the low metastasis human breast cancer cell line MCF－7, the moderate metastasis human breast cancer cell line MDA－MB－231 and the high metastasis human breast cancer cell line MDA－MB－435s. Co－immunoprecipitation was used to study the relationship between TRAF1 and TRAF2 in the above－mentioned cultured cell lines except MDA－MB－231.
Results: 1. The results of Immunohistochemical S－P show that the expression of TRAF1 in MCF－10A is lower than that in MCF－7 ($P<0.05$), MDA－MB－231 ($P<0.01$), and MDA－MB－435s ($P<0.01$). It's expression in MCF－7 is lower than that in MDA－MB－231 ($P<0.01$) and MDA－MB－435s ($P<0.01$). It's expression in MDA－MB－231 is lower than that in MDA－MB－435s ($P<0.05$). 2. The results of Western Blot show that the expression of TRAF1 in MCF－10A is lower than that in MCF－7 ($P<0.05$), MDA－MB－231 ($P<0.01$), and MDA－MB－435s($P<0.01$). It's expression in MCF－7 is lower than that in MDA－MB－231 ($P<0.05$) and MDA－MB－435s($P<0.01$). It's expression in MDA－MB－231 is lower than that in MDA－MB－435s ($P<0.05$). 3. The results of co－immunoprecipitation show that the quantity of TRAF1 in combination with TRAF2 in MCF－10A is larger than that in MCF－7($P<0.05$) and MDA－MB－435s($P<0.01$). Its quantity in MCF－7 is larger than that in MDA－MB－435s ($P<0.05$).
Conclusion: 1. The expressions of TRAF1 in breast cancer cell lines are stronger than that in normal human breast cell line. As the metastasis of breast cancer cell lines increases, the expression of TRAF1 would be stronger. 2. TRAF1 would combine with TRAF2 in both the normal human breast cell line and different metastasis of human breast cancer cell lines. 3. The quantity of TRAF1 in combination with TRAF2 in normal breast cell line is larger than those in breast cancer cell lines. As the metastasis of breast cancer cell lines increases, the quantity would be smaller. It suggests that TRAF1 is a negative regulator of TRAF2 signaling pathways through the combination with TRAF2.

The Influence of GM－CSF on Proliferation and Functional Activity of Rat EPCs

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Key words: Granulocyte – macrophage Colony Stimulating Factor; Endothelial Progeni tor Cells; Atherosclerosis; Reendothelialization

Abstract: Aim To investigate the influence of GM – CSF on proliferation and functional activity of rat EPCs(endothelial progenitor cells) in vitro. Further provide experimental basement for the mechanism of reendothelialization and prevention and treatment of coronary artery disease. Methods EPCs were separated from bone marrow and spleen of rat with density gradient centrifugation method. Then observed growth and differentiation procession of EPCs with inverted phase contrast microscope. EPCs were identified with laser scanning confocal microscope (LSCM). After cultered seven days, EPCs were added stimulating factors. Then detect the influence of RatGM – CSF on proliferation、transfer and blood vessels formation of EPCs with MTT colorimetry、Transwell chamber、Matrigel. Finally analysis experiment results. Results After treated with RatGM – CSF, OD490、the number of transferred cells and tubes increased in a dose – dependent and time – dependent manner. Conclusions GM – CSF can promote proliferation、transfer and blood vessels formation of EPCs in vitro and in certain rages showing a dose – dependent and time – dependent manner.

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