International Symposium of Molecular Pathology

1998.8.26 - 29, Dunhuang, the People's Republic of China

Supported by

Japanese Chinese Medical Association

China Medical University

Organizing Committee

Second Department of Pathology, Kagoshima University

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International Symposium of Molecular Pathology

1998.8.26 - 29, Dunhuang, the People's Republic of China

Organizing and Scientific Committee

Japanese committee

President	Sato Eiichi	(Kagoshima Univ.)
Scientific committee	Watanabe Keiichi	(Tokai Univ.)
	Hata Jun-ichi	(Keio Univ.)
	Nagura Hiroshi	(Tohoku Univ.)
	Takahashi Kiyoshi	(Kumamoto Univ.)
	Kikuchi Masahiro	(Fukuoka Univ.)
	Sato Eiichi	(Kagoshima Univ.)

Chinese committee

Honoral president	Teng Wei Ping	(China Med. Univ.)
President	Jia Xin Shan	(China Med. Univ.)
Scientific committee	Liu Yan Fang	(The 4th Army Med. Univ.)
	Si Lue Sheng	(Xian Med. Univ.)
	Zhang Yue E	(Xiang hai Med. Univ.)
	Jia Xin Shan	(China Med. Univ.)

Secretary

Japanese	Hasui Kazuhisa	(Kagoshima Univ.)
Chinese	Han Yu Chen	(China Med. Univ.)

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Japanese Chinese Medical Association

China Medical University

General information of International Symposium of Molecular Pathology

Date: August 26 - 29, 1998

Venue: The symposium will be held in the meeting room of Shazhou Hotel Dunhuang.

The Japanese participants must arrive Xi'an Garden Hotel in Xi'an by August 26, 1998 from Japan or the other areas. The organizing committee will make the Japnese group to fly Dunhuang in the next day eary morning. In Dunhung the Japanese group stay in Shazhou Hotel Dunhuang. Promoting the symposium in success and in ecomonic aspect for attending the symposium, the oragnizing committee plan a group travel for the Japanese participants from Fukuoka (August 26-31, 1998).

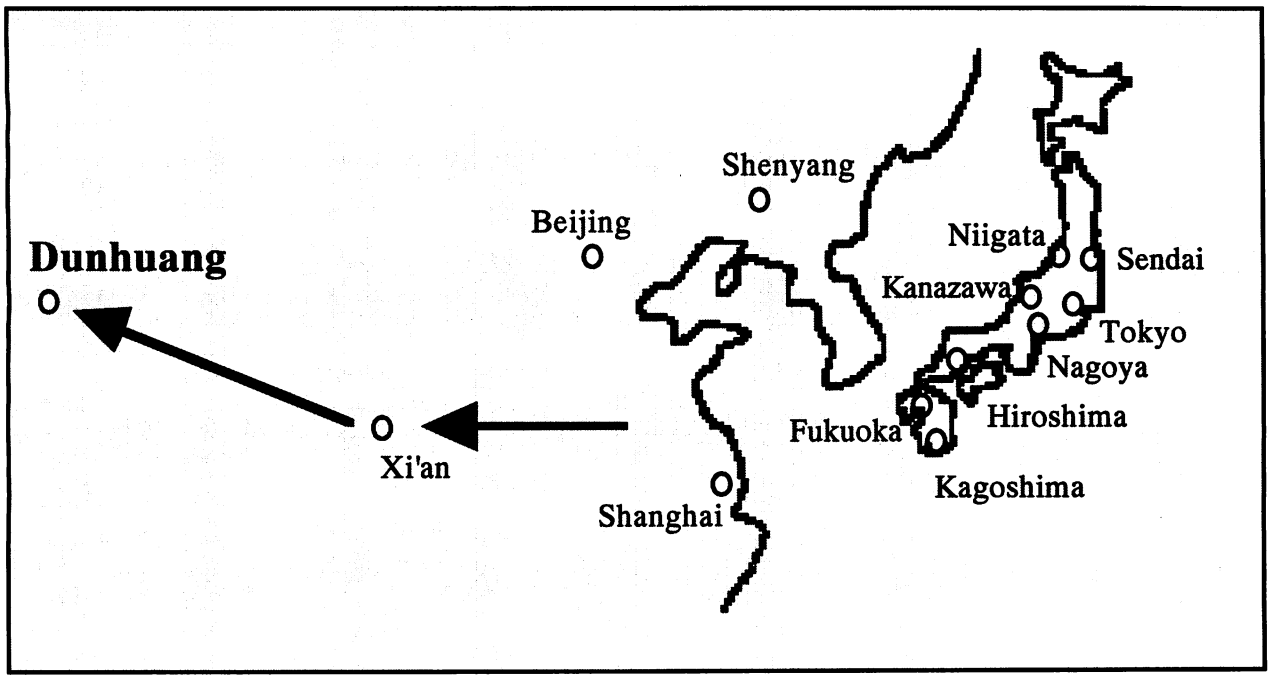
Language:
The official language of the symposium is English.

Wear: Informal. Considering the climate in Dunhuang that it is hot in the day, cool in the night and not hot in the morning and in the evening, all the participants prepare adequate wears.

Registration:
Most of the participants are pre-registered. People who want to attend the symposium, must contact the Chinese side Presisdent of the symposium, Prof. Jia Xin Shan at the meeting room in Shazhou Hotel Dunhuang.

Presentation in the scientific program:
Special Lecture: The presenter must finish the oral presentation within 25 min and left at least 5 min for discussion. **Lecture:** The presenter must finish the oral presentation within 25 min and left at least 5 min for discussion. **Oral presentation:** The presenter must finish the oral presentation within 12 min and left at least 3 min for discussion. **Poster presentation:** The presenter must prepare the poster in 90 (W) x 150 (H) cm on the plate where will be indicated before the opening of the symposium (in the morning August 27, 1998). Because the time of the symposium is limitted, there is no presentation time for the poster presentation. The presenter must stand by the presentation and answer the questions in the discussion time according to the program. When the presenter needs more time to answer, the presentere can use the coffee break time. The presentation must be removed after the closing of the symposium.

Social and associating person's program
The organizing committee plans programs for participants in the social part and associating persons to visit Dunhuang city and museums durining the scientific meeting. In the welcome party history of Dunhuang city and its periphery will be explained for 20 min. intermission.



Group travel for Japanese participants to attending International Symposium of Molecular Pathology from Fukuoka, planed by the Organizing Committee

(The travel will be organized by CITS JAPAN, Fukuoka TEL 092-441-8180 FAX 092-441-8160).

August 26, 1998	Fly from Fukuoka to Xi'an and stop over in Xi'an
	**********
	The meeting of the Japanese participants in Xi'an Garden Hotel
August 27, 1998	Fly from Xi'an to Dunhuang .
	International Symposium of Molecular Pathology in Shazhou Hotel Dunhuang
	Scientific meeting and Social and associating person's program
	Welcome party
August 28, 1998	Scientific meeting and Social and associating person's program
August 29, 1998	Fly from Dunhuang to Xi'an
	**********
	The meeting of the Japanese and Chinese participants in Xi'an Medical University
August 30, 1998	Visit Xi'an Medical University and the Fourth Military Medical University
August 31, 1998	Fly from Xi'an to Fukuoka

OPENING ADDRESS

Dear Participants,

Recent development in the field of life science has provided a tremendous possibility to detect extremely minute substances *in situ* by applying its technology to the morphology that is still a key field of the medical science. Although the technology itself is developing and technological application to many kinds of research is reported everyday in medical journals, it is furthermore important to get the deep insight into biological or pathological phenomena instead of compiling merely the data by the use of target substances in the mode of research. To realize this rather philosophical point of view, the international meeting to be held in the ancient cultural city Dunhuang has been planned by Chinese and Japanese members in Kagoshima, according to the suggestion of Drs. K.Watanabe, H.Nagura from Japan and Jia Xin Shan from China who had an opportunity to discuss about the Japan-China conference at the time of International Meeting of Histochemistry held in Chongqui in 1996.

It is of paramount importance to exchange the way of thinking and information about medical science for Japanese and Chinese researchers, in order to obtain a treasure ideas to explore future research. Thus, this International Meeting of Molecular Pathology focuses its purpose on the mutual exchange of ideas not only in the technical application of molecular biology to morphology, but also in the understanding of the basis of diseases, including infectious, nutritional, neoplastic, metabolic disorders as well as of the physiological processes of life phenomena in humans.

Lastly, I hope that the Meeting be successful and every participants are able to deepen the friendship for collaborative study between Japan and China and to get fruitful ideas for the future study, stimulated by seeing the creatures of ancient skillful artists that give us very cultural and religious atmosphere of this rare old city Dunhuang.

President of the Meeting

Eiichi Sato, M.D.

(Professor, Kagoshima, Japan)

Distinguished participants

of the international symposium of molecular pathology!

At first, I want to express my thank for your cooperation to prepare this meeting in Dunhuang far from Japan and from our home towns in China. As for the scientific purpose of this meeting, Japanese side president of this meeting, Prof. E Sato has already stated. In 1996.9 Chongqing (China), Prof. K Watanabe, Prof. H Nagura and Prof. A Kawai and I attended the fourth Chinese Japan symposium on histochemistry, we had eager to promote Chinese Japanese intercourse on the field of pathology, and we decided to hold this symposium in the sacred place called "Ancient Silk Road" where eastern and western cultures melted. In 1997.4, I had chances to cooperate in Japan, especially in the department of Pathology, Kagoshima University. I explained Prof. E Sato the thought to open the new Chinese Japanese intercourse in the field of Pathology and we ask him as the president of Japan-side to promote this meeting. And this meeting was subject to the help of the president of China Medical University and the Japan- China Association of Medical Science. With the efforts of everybody, the meeting has been running successfully. It is the days when recent development in molecular pathology has been introducing to the field of histopathology by means of in-situ techniques to see an extremely small amount of physiological and pathological substances in the relation to oncogenesis, infection of viruses and microorganisms, and immunology. This meeting succeeded to cover these fields where the new molecular pathological techniques are applied. I believe that the Chinese Japanese intercourse in this meeting will create the new relationship in the academic fields and also in the social fields.

Chinese side President of the meeting

Jia Xin Shan. M.D.

(Professor, China Medical University)

List of Japanese Participants

Academic part

Prof.	EIZURU Yoshito	Kagoshima University
Dr.	HARA Takuo	Kanazawa University
Dr.	HASUI Kazuhisa	Kagoshima University
Prof.	HATA Jun-ichi	Keio University
Prof.	HAYATA Takashi	Kagoshima Women's Junior College
Prof.	HIRAI Kanji	Tokyo Medical and Dental University
Prof.	INAI Kouki	Hiroshima University
Dr.	ITOH Johbu	Tokai University
Prof.	KIKUCHI Masahiro	Fukuoka University
Dr.	Li Ai Hua	Kagoshima University
Prof.	NAGURA Hiroshi	Tohoku University
Dr.	OH-ISHI Tsutomu	Saitama Children's Medical Center
Dr.	OKUMURA Teruhisa	Kagoshima Seikyoh Hospital
Dr.	OOI Akishi	Kanazawa University
Prof.	SAKU Takashi	Niigata University
Prof.	SATO Eiichi	Kagoshima University
Prof.	SHAMOTO Mikihiro	Fujita Health University
Prof.	TAKAHASHI Kiyoshi	Kumamoto University
Dr.	TSUTSUMI Yutaka	Tokai University
Prof.	WATANABE Keiichi	Tokai University
Dr.	WATANABE Masatoshi	Mie University

Social part (Kagoshima Japanese Chinese Association)

Mr.	KOMAKI Yuzo	President, Komaki Bilding Co.
Mr.	HIDAKA Umashi	President, Kagoshima Television Station
Mr.	KODAMA Kazuo	President, Maruco Food Co.
Mr.	HOSOYA Akio	Prof., Kagoshima Prefectural College
Mr.	HIRAYAMA Kosei	President Dr., Hirayama Dental Clinic
Mr.	NOZOE Yoshitaka	President Dr., Nozoe Dental Clinic

Associating Persons

Mrs.	HAYATA Kumiko	Mrs.	HIDAKA Hisako
Mrs.	HIRAYAMA Hideko	Mrs.	HOSOYA Fujimi
Miss.	HOSOYA Wakaba	Mrs.	INAI Junko
Mrs.	KIKUCHI Makiko	Mr.	KODAMA Keizou
Mr.	KOMAKI Ryu	Mrs.	NOZOE Keiko
Mrs.	OOI Shizue	Mrs.	SAKU Machiko
Mrs.	SATO Yuriko	Mr.	SHIOKURA Yasunobu
Mrs.	SHAMOTO Mihoko	Mrs.	TAKAHASHI Kazuko
Mrs.	WATANABE Noriko		

List of Chinese Participants

Academic part

Prof.	Deng Zhong Duan	Tongji Medical University
Dr.	Dong Wen Rui	China Medical University
Dr.	Fang Jun	China Medical University
Dr.	Fu qing Guo	China Medical University
Dr.	Gao Hua	China Medical University
Dr.	Han Qing Shong	Liaoning Public Security
Dr.	Han Yu Shen	China Medical University
Prof.	Jia Xin Shan	China Medical University
Dr.	Li Jian Hua	China Medical University
Prof.	Li Yu Lin	Norman Bethune University of Medical Science
Dr.	Li Xu	Xi'an Medical University
Dr.	Liu Gui Nan	China Medical University
Prof.	Liu Yan Fang	Fourth Military Medical University
Dr.	Ma Xiao Chun	China Medical University
Dr.	Qu Xue Shan	China Medical University
Dr.	Ruan Qin Rong	Tongji Medical University
Prof.	Shi Lu Sheng	Xi'an Medical University
Prof.	Shi Yu Xiu	China Medical University
Dr.	Wang Yi Li	Xi'an Medical University
Dr.	Yang Xiang Hong	China Medical University
Prof.	Yuan Yuan	China Medical University
Dr.	Zhai Wei Rong	Shanghai Medical University
Dr.	Zhang Lei	China Medical University
Prof.	Zhang Yue E	Shanghai Medical University
Dr.	Zhang Zhong	Shenyang Medical College

Scientific Program

1998.8.27

pm 00:30 **Opening**

Greeting Japanese side President : Sato E

Introduce the participants Chinese side President: Jia XS

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

pm 01:00 **SPECIAL LECTURE 1** Chairman: Liu YF

Watanabe Keiichi, Takekoshi Susumu, Nagata Hidetaka and Itoh Johbu.
Department of Pathology, Laboratories for Structure/Function Research, Faculty of Medicine, Tokai University, Isehara
δ isoform specific activation of protein kinase C by peroxidized diacylglycerol and its pathogenetic signiirfidance in Alzheimer disease like neuronal degeneration.

pm 01:30 **SPECIAL LECTURE 2** Chairman: Watanabe K

YF Liu, ZW Sun, CM Hu, LJ Chan, SJ Yang, SM Chen.
Department of Pathology, Fourth Military Medical University, Xi'an.
From monoclonal to engineered bifunctional single chain antibody against hepatocellular carcinoma

pm 02:00 **The First Session** Chairman: Hata J
Chairman: Zhang Yue-e

Oral 1
Tsutomu Oh-ishi, Takashi Arai, Minoru Okazaki, Seiichi Kagimoto, Kosuke Job, Ryoji Hanada, Kanji Hirai.
Saitama Children's Medical Center, Saitama, Tokyo Medical and
Dental University, Tokyo, Japan: Quantitative assessment of Epstein-Barr virus (EBV)-infected cells in the peripheral blood of the children with EBV-associated maligant lymphoma and LGL lymphocytosis

Oral 2
YF Liu, SJ Yang, Sf Huang, Ps Yan, CM Wang, J Liu
Forth Military Medical University, Xi'an, China
The effects of intracellular immunocomplexes on the pathologenesis of epidemic hemorrhagic fever

Oral 3
Tsutsumi Yutaka
Department of Pathology, Tokai University School of Medicine
Diagnostic histochemistry of infectious diseases

Oral 4
Han Yuchen, Jia Xin shan, Yonezawa Suguru, Sato Eiichi
China Medical University, Shenyang, China
The expression of MIB-1 and thrombomoduin in nasopharyngeal carcinoma

pm 03:00 **Coffee break**

pm 03:15 **Poster discussion**

Poster 1
Saku T, Cheng J, Tokunaga M, Liu A, Zhang W, Wu L, Lu Y, Zhou Z, Li Y, Li R, Rao H, Lin H, Ouyang J, Yang L, Yu S, Lou T, Wang S, Chen S, Tsay C
Niigata University, Niigata, Kagoshima University, Kagoshima, Japan, Shanghai Second Medical University, Shanghai, West China University of Medical Science, Chengdu, Norman Bethune University of Medical Science, Changchun, Fourth Military Medical University, Xi'an, Beijing Medical University, Captal University of Medical Sciences, Beijing, Hubei Medical University, Wuhan, China, National Taiwan University, Taipei, Chung Shan Medical and Dental College, Taichung, Taiwan
p53 mutation in salivary gland tumors; with a special attention to EBV-related-lymphoepithelioma-like carcinoma

Poster 2
Zhong Zhang, Xiao Wei Pan, Fan Wu, Yuan Yuan, Hua Gao, Ming Dong, Yie Qiu Wu, Lan Wang.
Shenyang Medical College and China Medical University, Shenyang, China
In situ observation of apoptosis and proliferation of gastric mucosa with Helicobacter pylori infection

Poster 3
Jia Xin Shan, Kazuhisa Hasui, Shuiji Izumo, MoeMoe Aye, Eiichi Sato
Department of Molecular Pathology and Genetic Epidemiology, Center for Chronic Viral Diseases, Second Department of Pathology, Kagoshima University, and Department of Molecular Pathology, China Medical University, Shenyang
Infection of human T-cell leukemia virus type 1 (HTLV-1) in malignant lymphomas in the northeast region of China: Modified ImmunoMax of HTLV-1 pX-related proteins and polymerase chain reaction (PCR) analysis of HTLV-1 proviral DNA

Poster 4
Hua Gao, Yie Oiu Wu, Lan Wang, Ming Dong, Mei Xian Wang, Yuan Yuan.
China Medical University, Shenyang, China
The study on the expression of PCNA and P16 protein in Helicobacter pylori associated gastric diseases

Poster 5
Eizuru Yoshito, Ueno Kazuyoshi, Mori Seiichiro, Tokunaga Masayoshi
Kagoshima University, Kagoshima, Japan
Nasal T/NK-cell lymphoma in southern Japan.

Poster 6
XiaoChun MA, RunJang YU
The institue of respiratory disease, China Medical University, Shen Yang, China 110001
The expression of HLA-DR antigen in pulmonary carcinomas.

pm 03:30 **LECTURE 1** Chairman: Lushemg

Kanji Hirai
Tokyo Medical and Dental University, Tokyo, Japan
Detection of Epstein-Barr virus genome-positive cells by in situ hybridization and in situ PCR

pm 04:00 **LECTURE 2** Chairman: Inai K

Yue-e Zhang, Hao Wu, Zude Xu, Yuanding Xu
Shanghai Medical University, Shanghai, China
Study of effects of platelet-derived growth factor and transforming growth factor-$\beta 1$ on expression of integrin $\alpha 5\beta 1$ of fibroblasts during rat pulmonary fibrosis.

pm 04:30 **LECTURE 3** Chairman: Zhang Yue-e

Inai Kouki, Takeshima Yukio, Nishisaka Takashi, Kitaguchi Souichi, Yamasaki Masahiro
Hiroshima University, Hiroshima, Japan
Characterization of preneoplastic lesions of lung cancer

pm 05:00 **The Second Session** Chairman: Kikuchi M
Chairman: Liu YF

Oral 5
Fang Jun, Zhao Huiru, Du Guangye, Cui Xiujuan, Fang Ce, Tian Xin
China Medical University, Shenyang, China
The expression of standard and variant CD44 in human lung cancer

Oral 6
Hara Takuo, Ooi Akishi, Kobayashi Masako, Nakanishi Isao
Kanazawa University, Kanazawa, Japan
Gene amplification of c-MYC, c-ERBB-2, c-MET, and K-SAM in gastric cancers: Detection by fluorescence in situ hybridization

Oral 7
Zhang Lei, Tang Tian-bao, He An-guang, Zhang Bao-geng
China Medical University, Shenyang, China
Cytosolic calcium and its relation with TNF-β inducing apoptosis in human pancreatic carcinoma cells

pm 05:45 **LECTURE 4** Chairman: Wei-rong Zhai

Takahasi Kiyoshi
Kumamoto University, Kumamoto, Japan
Significance of macrophage scavenger receptor in atherogenesis and granulomatous inflammation: An approach through experimental and molecular pathology

pm 06:15 **LECTURE 5** Chairman: Takahasi K

Wei-rong Zhai, Yui-jing Wu, Yue-e Zhang, Li Zhuang
Shanghai Medical University, Shanghai, China
Investigation of 72kD type IV collagenase and its inhibitor in liver fibrosis model induced by heteroserum.

pm 06:45 **Poster discussion**

Poster 7
Dong Wenri, Hasui K, Han Yuchen, Sato E
China Mecical University, Shenyang, China, Kagoshima University, Japan
Immunohistochemical study of ENV GP46 in lymphoma

Poster 8
Hasui Kazuhisa, Sato Eiichi
Kagoshima University, Kagoshima, Japan
HTLV-1-associated non-neoplastic lymphadenopathy

Poster 9
Hayata Takashi, Sakoda Akiko, Taki Chiaki
Department of Pathology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan
A case of endometrial cancer concomitant with uterine adenomyosis - A comparative immunohistochemical study with adenomyosis in elderly patients-

Poster 10
Ai Hua Li, Kazuhisa Hasui, Sadao Tanaka, Takasi Hayata, Eiichi Sato
Kagoshima University, Kagoshima, Japan
An immunohistochemical comparison between old and recent colorectal neoplasia using antibodies against Ki-67, P53, MUC2 mucin and α-smooth muscle actin

Poster 11
Qiu Xueshan, Gao Aifeng
Department of Pathology, China Medical University, Shenyang, 110001
The expression of Rb. p16 in large intestine carcinoma and its significance.

Poster 12
Qingguo Fu, Renxuan Guo, Tao Bai, Jun Liu, Zhihong Zhong
China Medical University, Shenyang, China
Detection and analysis of HSP70 protein expressed on the surface of mouse hepatocellular carcinoma cells.

pm 07:00 **Welcome party**

1998.8.28

pm 01:00 **LECTURE 6** Chairman: Li Yulin

Nagura Hiroshi, Sasano Hironobu, Hoshi Tatsuya, Yabuki Noritaka
Department of Pathology, Tohoku University Graduate School of Medical Science
Increased cell proliferation and DNA damage in Helicobacter pylori-infected gastric mucosa and the effect of its eradication

pm 01:30 **LECTURE 7** Chairman: Nagura H

Li Yulin, Han Yimgjie, Zhang Lihong, Li Yan Yun, Li Wei
Norman Bethune University of Medical Science, ChangChun, China
Expression and role of transforming growth factor $\beta 1$ in laryngocarcinoma

pm 02:00 **LECTURE 8** Chairman: Liu YF

Kikuchi Masahiro, Kanda Motonobu, Suzumiya Junji, Haraoka Seiji, Ohshima Kohichi
Fukuoka University, Fukuoka, Japan
Somatic hypermutations in the VH segment of immunoglobulin genes of intravascular large B-cell lymphoma. A direct sequence analysis

pm 02:30 **LECTURE 9** Chairman: Deng Zhong-Duan

Shamoto Mikihiro, Qian Bin, Osada Akiko, Shinzato Masanori.
Fujita Health University, Toyoake, Japan
Immune characteristics of interdigitating cells and Langerhans cells: Distribution, differentiation and migration

pm 03:00 **Coffee break**

pm 03:15 **Poster discussion**

Poster 13
Okumura Teruhisa
Department of Pathology, Kagoshima Seikyoh Hospital
Expression of p53 tumor suppressor gene and proliferating cell nuclear antigen (PCNA) in adrenal cortical tumors and its relationship to biological characters

Poster 14
Wang Yili, Si Huaxin, Geng Yiping, Shen Wenhong, Jiang Xiaoying, Si Lusheng
Xi'an Medical University, Xi'an, China
Construction of hIL2- perforin recombinant immunotoxin and its bioactivity

Poster 15
Hasui Kazuhisa, Sato Eiici, Jia Shin Shan, Sueyoshi Kazunobu, TANAKA Sadao, Nakagawa Masanori and Osame Mitsuhiro
Kagoshima Univ., Kagoshima, JAPAN
Polymerase chain reaction analysis and DNA sequencing of immunoglobulin heavy chain gene in gastric B-cell malignant lymphomas in China and Japan

Poster 16
Ruan Qiu-rong, Deng Zhong-duan
Tongji Medical University, Wuhan, China
Study of the mechanism of foam cell formation by northern blot analysis

Poster 17
Itoh Johbu, Hasegawa Hideaki, Umemura Sinobu, Yasuda Masanori, Takekoshi Susumu and Watanabe Keiichi
Lab for Struct/Funct Res, Dept of Pathol, Tokai Univ Sch of Med, Isehara, Kanagawa, Japan
The simultance observation of apoptotic nuclei by TUNEL method and methyl green nuclei countersting in the absorptive epithelial cells of rat small intestine with CLSM.

Poster 18
Li Jianhua, Li Zongxuan, Liu Guinan, et al.
Department of Pathology, School of Basic Medical Sciences, China Medical University, Shenyang, 110001
Immunohistochemical reaction of anti-fibroblast monoclonal antibody and anti-mononuclear monoclonal antibody in experimental thickening carotic artery intima.

Poster 19
Liu Guinan, Wang Enhua, Qi Guoxian, Zheng Dingyo
Internal Medicine of Circulation, the First Clinical College, China Medical University, Shenyang
The study on c-myc antisense RNA inhibiting the proliferation of vascular smooth muscle cells.
Poster 20
Jingsong Han, Yuxiu Shi
China Medical University, Shenyang, China
Ultrastructure of myocyte in tetralogy of Fallot and the observation of its Ca-ATPase activity
Poster 21
Yu Xiu Shi, Li Li, Kazuo Ogawa
China Medical University, Shenyang, China
Enzyme cytochemical study on nematolysosome (NLY) in cell of Betz in cerebrum under high voltage electron microscope

pm 03:30 **The Third Session** Chairman: Sato E
Chairman: Zhang Yue-e

Oral 8
Watanabe Masatoshi, Fukutome Kazuo, Nakayama Tsuyoshi, Shiraishi Taizo.
Mie University, Tsu, Japan
Molecular pathology of human prostate cancers
Oral 9
Li Xu, Si Lu Sheng, Chen Wei, Yan Chun Fang, Fu Wei, Yang Yu Cong
Xi'an Medical University, Xi'an, China
Two newly established human uterine cervical carcinoma cell lines: Karyotype analysis and molecular genetic characterization
Oral 11
Deng Zhong-Duan, Xia Chun-Zhi, Yu Guang-Yao, Meng Feng, Qu Zhi-Ling, Zhang Xu-Ming
Tongji Medical University, Wuhan, China
Application of immunohistochemistry and in situ hybridization in the study on atherosclerosis
Oral 12
Yang Xiang-Hong, Wang Yue-Zhong, Chen Tie-Zhen
China Medical University, Shenyang, China
Effects of lipid peroxidation on mitogenic activities of endothelial cells.

pm 04:30 **LECTURE 10** Chairman: Liu YF
Hata Jun-ichi
Department of Pathology, Keio University School of Medicine
Wilms' tumors -Their pathology and molecular genetics-

pm 05:00 **LECTURE 11** Chairman: Watanabe K
Si Lusheng, Guo Jianfen, Liu Zi, Wang Yili, Xu Changfu
Xi'an Medical University, Xi'an, China
The expression of co-stimulatory molecules in human cervical canrcerous tissues

pm 05:30 **Closing** Chinese side President: Prof. Jia XS

Abstracts

2 Special lectures
11 Lectures
12 Oral presentations
21 Posters

δ ISOFORM SPRCIFIC ACTIVATION OF PROTEIN KINASE C BY PEROXIDIZED DIACYLGLYCEROL AND ITS PATHOGENETIC SIGNIIRFIDANCE IN ALZHEIMER DISEASE LIKE NEURONAL DEGENERATION

WATANABE Keiichi[1)], TAKEKOSHI Susumu[1)], NAGATA Hidetaka[1)] and ITOH Johbu[2)]
[1)]Department of Pathology, [2)]Laboratories for Structure/Function Research, Faculty of Medicine, Tokai University, Isehara

Phorbol ester (PMA), an potent activator of protein kinase C (PKC) is known to cause neurodegeneration related Alzheimer disease (AD). We reported that oxidized diacylglycerols (DAG-OOH) exhibited outstanding activation of PKC, which is equivalent to that of PMA (S. Takekoshi, et al, BBRC, 1995). PKC is known to have nearly 10 isoforms, and the DAG-OOH effect was tried on 7 isoforms which are the representative ones in rat brain. Among them, only α and δ isoforms responded to the DAH-OOH. In the present study, attempts were made to prove the neurodegenerative effects of DAG-OOH on cultured neurons. [Materials and Methods] As the cultured cells, ① neuronal cells established from 18-day rat fetus cerebral cortex (PN cells) and ② NGF stimulated PC12 cells were employed for the experiments, since they showed clear contrast in expressing PKC δ, i.e. PC12 cells were strongly positive for PKC δ but PN cells were negative for it in both immunohistochemical and Western blot tests. These cultured neurons treated by streptolysin O to let the penetration of natural DAG and DAG-OOH (4 μ M) into cells ease. Those treated cells were observed by 1) phase contrast microscopy, 2) electron microscopy, 3) immunohistochemistry to localize the PKC δ isoforms and cytosketal proteins (tubulin, tau, phosphorylated tau, MAP-2 etc.) at both LM and EM levels. [Results and Discussions] Within 12h of exposure to DAG-OOH, PC12 cells (PKC δ +), exhibited neuritic thinning and characteristic beading, while PN cells did not shows such changes. The PKC inhibitor staurosporine prevented its neurodegeneration caused by DAG-OOH. Those lesions were observed both routine EM and tubulin immunohistochemistry which revealed the microtubule (MT) disassembly in the lesions. This MT damage may have caused in the stagnation of the neurosecretory vesicles to accumulate in the beaded lesions. Interestingly, phosphorylated tau was specifically accumulated in the beading lesions in the DAG-OOH stimulated cells, while it was evenly localized throughout the processes of the unstimulated control cells. Those findings indicate that the pronounced activation of PKC δ by DAG-OOH may specifically be responsible to elicit the neurodegeneration related to AD.

FROM MONOCLONAL TO ENGINEERED BIFUNCTIONAL SINGLE CHAIN ANTIBODY AGAINST HEPATOCELLULAR CARCINOMA

YF Liu, ZW Sun, CM Hu, LJ Chan, SJ Yang, SM Chen
Dept. Pathology, Fourth Military Medical University, Xi'an 710032, P.R. China

In order to prepare antibodies for targeting diagnosis or therapy of human hepatocellular carcinoma (HCC), many monoclonal antibodies (MAbs) have been prepared against this malignancy. Some of them were conjugated with ^{131}I and used for radioimmunoimaging. As for engineered antibody few other than our work were found in the literature.

We devoted ourselves to the preparation of MAbs against HCC since 1982. At first we tried it use soluble antigen and HCC cell lines to immunize BABL/c mice, but failed to get rather specific MAbs. Then we changed to use cell suspensions from fresh liver cancer specimens as antigen with various modifications. Toyally we got 10 strains of rather specific MABs to HCC. We took HAb25 as an example to show their specificity.

1, Immunohistochemically HAb25 reacts with paraffin sections of 83.15% of HCC without reaction to normal liver cells.

2, Internalization of golg labeled HAb25 into cultured HCC cells under electron microscope.

3, Concentration of ^{131}I into the HCC born in nude mice with T/L ratio 6.84.

4, Four cases among 5 patients of HCC showed positive images under ECT.

In order to overcome the limitation of mouse MAb (induce human anti-mouse antibody) in clinical use to human patients. Antibody enginering has been done on HAb25.

1, Cloning of the genes of the various region of light and heavy chain of HAb25 by RT-PCR. The sequences showed variation of the sequences of light and heavy chains and hemogenisity of the frame work compring with the standard Ig.

2, Formation of single chain antibody (HAb25scFv). The genes of light chain variable region and that of the heavy chain were connected to form a single chain antibody (HAb25scFv) by a linker.

3, A bifunctional single chain antibody was formed: MAb25scFv was fused with the gene of TNF-α to form a recombined gene HAb25scFv-TNF-α.
Coli. The product was purified by chromatography.

4, The affinity of HAb25scFv-TNF-α was showed 1) by immunohistology ABC method when it was used as first antibody to stain positively the HCC cell line. 2) by inhibitory test, to block the antigen cells first, and then to stain the cells with parent antobody, the engineered antibody could inhibit about 50% of the intensity of the parent antibody showing by the flow cytometer.

5, killing effect of HAb25scFv-TNF-α: MTT test showed the killing effect of the bifunctional single chain antibody of HAb25 is very strong. Its therapeutic effect on HCC xenograft in nude mice was very promising. It induced complete remission of a tumor 2 mm in diameter.

Detection of Epstein-Barr virus genome-positive cells by in situ hybridization and in situ PCR

Kanji Hirai
Department of Tumor Virology, Division of Virology and Immunology, Medical Research Institute, Tokyo Medical and Dental Univerisrty, Tokyo

In situ hybridization (ISH) with EBER1 (Epstein-Barr virus (EBV)-encoded small RNA1) probes is widely used for in situ detection of EBV-infected cells. To examine whether EBV-negative cells determined by in situ hybridization (ISH) with EBER1 probes are truly-negative or - positive for EBV DNA, we established a method for detection of one copy of EBV DNA per cell by in situ PCR. ISH with an EBER1 probe showed that 10 of 40 nasopharyngeal carcinoma cases were negative for EBER1 expression. Of the 10 EBER1-negative cases, three cases including one each of well- and poorly differentiated carcinomas and undifferentiated carcinoma were EBV DNA-positive by in situ PCR . The remaining seven were truly negative for the presence of EBV DNA. These results indicate that there are EBV genome-positive NPC cases expressing no EBER1 and that in situ PCR can be suitable for in situ detection of EBV-infected cells, especially those expressing no EBER1 in paraffin sections. Next, non-neoplastic tonsils were analyzed for detection of EBV-positive cells by *in situ* hybridization and *in situ* PCR.Using *in situ* DNA-DNA hybridization, the EBV-positive signals were observed in the upper epithelial cell layers of the tonsils. In addition, *in situ* PCR detected EBV DNA-positive cells in the lower epithelial cell layers and lymphoid cells. Furthermore, we attempted to detect cells infected dually with EBV and human cytomegalovirus by in situ PCR. I will discuss the specificity and sensitivity of in situ PCR method for detection of virus-infected cells.

STUDY OF EFFECTS OF PLATELET-DERIVED GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR-β_1 ON EXPRESSION OF INTEGRIN $\alpha_5\beta_1$ OF FIBROBLASTS DURING RAT PULMONARY FIBROSIS

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Deposition of fibronectin(FN) played an important role and was the prelude of collagen deposition during pulmonary fibrogenesis. In order to study the role of FN receptor $\alpha_5\beta_1$ and the effects of platelet-derived growth factor (PDGF) and transforming growth factor-β_1(TGF-β_1) on the expression of FN and $\alpha_5\beta_1$ of pulmonary fibroblasts(PFb) both in vitro and in vivo, Northern blot analysis and Immunohistochemistry(IHC) techniques were performed to detect the expression of α_5, β_1 and FN mRNA and their relevant proteins in cultured PFb after PDGF and TGF-β_1 treatment respectively, and IHC method was used for detecting the dynamic changes of PDGF, PDGF receptor, TGF-β_1 and $\alpha_5\beta_1$ positive staining cells in different stages of bleomycin-induced rat pulmonary fibrosis. The results were as follows: (1) The enhancement of expression of α_5 , β_1 and FN mRNA of PFb reached their peaks at 2-6 hours after PDGF or TGF-β_1 treatment. The increase of positive staining of $\alpha_5\beta_1$ and FN of PFb by IHC was visualized after 24 hours of treatment. (2) The primary mesenchyma cells, PFb, smooth muscule cells, pneumocytes and endothelial cells expressed integrin $\alpha_5\beta_1$ in normal lung tissue. The activated and proliferated mesenchyma cells, PFb and myofibroblasts, which were regarded as the main collagen producing cells(CPCs) during pulmonary fibrosis, enhanced in expression $\alpha_5\beta_1$ remarkably. (3) In addition to macrophages and other inflammatory cells, the CPCs expressed PDGF, PDGF receptor and TGF-β_1. It was concluded that by both paracrine and autocrine mechanisms PDGF and TGF-β_1 up-regulated the expression of $\alpha_5\beta_1$ of CPCs. Integrin $\alpha_5\beta_1$ with its ligand FN played very important roles in pulmonary fibrogenesis.

CHARACTERIZATION OF PRENEOPLASTIC LESIONS OF LUNG CANCER

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Multistep carcinogenesis theory in human cancer was advocated by the study of morphological and genetic analysis and in lung cancer, the existence of preneoplastic lesions has also been recognized. That is, dysplasia in the tracheo-bronchial mucosa might be a preneoplastic lesion of squamous cell carcinoma. By contrast, the preneoplastic lesion of adenocarcinoma has not been fully understood, but recently atypical adenomatous hyperplasia (AAH) , which is a small lesion and consists of clara cells or type II alveolar cells is noticed. The aim of this study is to analyze preneoplastic characteristics of dysplasia and AAH by means of immunohistochemical staining and detection of genetic alterations.

Among 37 lesions of dysplasia in bronchial tree, p53 oncoprotein was expressed in 31% in mild dysplasia (16 lesions) , 50% in moderate dysplasia (12 lesions) and 67% in severe dysplasia (9 lesions) , however, mutation of p53 gene was detected only in 2 lesions of severe dysplasia. Loss of heterozygosity (LOH) of 3p, 9p of 17p was shown in 6% in mild dysplasia, 8% in moderate dysplasia and 22% in severe dysplasia by using microsatellite analysis.

On the other hand, 7 out of 20 lesions of AAH in peripheral lung immunohistochemically expressed p53 oncoprotein, but proportion of positive cells in most of those lesions was less than 10%. No mutation of p53 gene was detected. LOH of 3p, 9p or 17p by microsatellite analysis was shown in 18%, 13% or 6%, respectively.

From those results it is suggested that characteristics of dysplasia and AAH are heterogenous, but high-graded dysplastic or atypical lesions have a similar nature of carcinoma.

SIGNIFICANCE OF MACROPHAGE SCAVENGER RECEPTOR IN ATHEROGENESIS AND GRANULOMATOUS INFLAMMATION: AN APPROACH THROUGH EXPERIMENTAL AND MOLECULAR PATHOLOGY

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Scavenger receptors are important for macrophages in the uptake of not only oxidized low density lipoprotein (OxLDL) but also the other various substances *in vivo*. Until recently, several types of scavenger receptors have been identified and are classified into class A, B, and C. Among these receptors, type I and type II macrophage scavenger receptors are included in class A, together with MARCO receptor, and termed MSR-A. In the present report, the author shows the implication of MSR-A in atherosclerosis and granulomatous inflammation *in vivo*. MSR-A consists of six different domains: the collagen-like domain can bind to a variety of negatively charged macromolecular compounds, including OxLDL, negatively charged collagens, advanced glycation end products, and bacteria, and the a-helical coiled coil domain is involved in adhesion and dissociation of ligands. MSR-A is expressed on the cell membrane of macrophages, binds to ligands, and is internalized though coated vesicles and endosomes. In the endosomes, MSR-A is dissociated from the ligands and transported into a trans-Golgi system, while the ligands are directed into lysosomes and degraded. After packing into secretory vesicles in the trans-Golgi system, MSR-A is recycled to the cell membrane. To clarify the role of MSR-A during atherogenesis, we generated mice lacking both MSR-A and apoE or low density lipoprotein receptor (LDLR). In apoE-deficient mice, atherosclerosis spontaneously developed in the aorta and arteries. In the apoE/MSR-A double knockout mice, the size of atherosclerotic lesions was significantly reduced, compared with the apoE single knockout littermates. Similar evidence was obtained by comparing the lesion size of cholesterol diet-induced atherosclerosis in LDLR/MSR-A double knockout mice with that in LDLR single knockout mice. These results indicate that MSR-A is important for atherogenesis *in vivo*.

In our further studies, we produced hepatic granulomas by a single intravenous injection of heat-killed Corynebacterium parvum (C. parvum) in MSR-A deficient (MSR-$A^{-/-}$) mice and compared with wild type (MSR-$A^{+/+}$) mice. In the MSR-$A^{-/-}$ mice, the hepatic granuloma formation was delayed significantly by 10 days after injection, compared with the MSR-$A^{+/+}$ mice. In the early stage, the influx of monocytes into hepatic granulomas and the uptake and elimination of C. parvum by Kupffer cells and monocyte-derived macrophages were reduced in the MSR-$A^{-/-}$ mice, compared with the MSR-$A^{+/+}$ mice. Monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and interferon-γ (INF-γ) messenger RNAs (mRNAs) were not expressed in the liver of MSR-$A^{-/-}$ mice by 3 days after injection, while the MSR-$A^{+/+}$ mice expressed all these cytokine mRNAs in the liver at the first day after injection. These data suggest that MSR-A deficiency induces a marked delay in the uptake and elimination of C. parvum by macrophages, influx of blood monocytes into hepatic granulomas, their differentiation into macrophages, macrophage activation, and the expression of MCP-1, TNF-α, and INF-γ mRNAs in the early stage of hepatic granuloma formation. In conclusion, MSR-A is deeply implicated in atherogenesis and granuloma formation *in vivo*.

Lecture 5

INVESTIGEATION OF 72kD TYPE IV COLLAGENASE AND ITS INHIBITOR IN LIVER FIBROSIS MODEL INDUCED BY HETEROSERUM

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Objective To observe the role of 72kD IV collagenase (MMP-2) and its inhibitor played in heteroserum induced liver fibrosis model.

Method Sixty of Wistar rats were injected with 0.5ml porcine serum intraperitoneally twice a week for 8 weeks and sacrified at the end of 1, 2, 4, 8, 12, 16 week. Type IV collagen (Col IV), MMP-2, tissue inhibitor of metalloproteinase-2 (TIMP-2) and desmin(Dm) were detected in situ with immunohistochemistry (IHC). The activity of MMP-2 was measured by zymographic method.

Results Liver fibrosis developed in all of the rats at the 8^{th} week. The expression of MMP-2 was appeared markedly in the mesenchymal cells within the expended portal tracts and the cellular or cellular-fibrous septa at the 4^{th} and 8^{th} week, and reduced gradually afterwards. Most of the MMP-2 positive cells were also stained with Dm (a marker of activeted hepatic stellate cell and myofibroblasts). The distribution and degree of Col IV staining were very similar to that of MMP-2, but a narrow negative zone of Col IV was appeared in the perisinusoids right along the both sides of the cellular septa which contained numerous positive cells of MMP-2. TIMP-2 staining was detected only in a few cells in the septa from the 8^{th} week till the end of serum injection. Zymography of MMP-2 revealed high activity at 4-8 weeks of the experiment which was coincided with that in IHC.

Conclusion and Comments This experiment suggests that the increase of MMP-2 activity might contribute an initiating effect in liver fibrogenesis, by which the normal composition of matrices in the perisinusoids was denatured and the stellate cells were activated, the deposition of extracellular matrix was increased, and eventually liver fibrosis was formed.

INCREASED CELL PROLIFERATION AND DNA DAMAGE IN HELICOBACTER PYLORI-INFECTED GASTRIC MUCOSA AND THE EFFECT OF ITS ERADICATION

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Chronic and persistent infection with Helicobacter pylori (Hp) is considered as one of the earliest steps in gastric carcinogenesis. In the Corea's model of human gastric carcinogenesis, environmental factors are involved in the evolution of normal gastric mucosa to acute superficial gastritis, mulifocal atrophic gastritis, intestinal metaplasia, dysplasia, and finally to adenocarcinoma. Therefore, it is very important to examine the cell damage and proliferation of Hp-infected gastric mucosa and to analyse the mechanism to lead it into carcinogenic process.

We examined histopathologic features, cell proliferation, aberrant gene expression and both double and single strand DNA damage *in situ* in Hp-infected and its eradicated human gastric mucosa by immunolocalization of Ki67, DNA topoisomerase II, p53, TdT-mediated dUTP-biotin nick end labeling (TUNEL), and in situ nick translation.

Hp-infected gastric mucosa was histopathologically defined as "chronic active gastritis" with marked intraepithelial neutrophil infiltration and epithelial degeneration. The extent of mucosal damage and epithelial cell proliferation seen in Hp-infected chronic active gastritis was correlated with the degree of neutrophil infiltration. That is, intraepithelial neutrophil, Ki67, TUNEL, and *in situ* nick translation indices all increased with increasing grade of a gastritis, being highest in glands with incomplete intestinal metaplasia and lowest in those with complete intestinal metaplasia. All indices decreased following the eradication of Hp. p53 over expression by the immunohistochemical staining and *in situ* hybridization was observed in the Hp-infected mucosa with incomplete intestinal metaplasia.

The present result suggests that Hp-associated toxins and intraepithelial neutrophils are important in Hp-related epithelial injury, which induces cell proliferation and intestinal metaplasia. Such increased cell turnover, particularly in the Hp-infected chronic active gastritis with incomplete intestinal metaplasia may result in DNA instability and subsequest development of gastric carcinogenesis. The eradication of Hp infection can revert inflammation and DNA instability, and may prevent the process of gastric carcinogenesis progressing from chronic active gastritis through incomplete intestinal metaplasia.

Expression and role of transforming growth factor β1 in laryngocarcinoma

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ABSTRACT ***Objective*** To study the expression and role of TGF β1 in laryngocarcinoma. ***Methods*** Standard immunohistochemical method using polyclonal antibody of TGF β1 was applied to detect the expression of TGF β1 in 5 cases of normal larynx tissues, 36 cases of laryngocarcinoma tissues and paracarcinoma tissues. ***Result*** 31 laryngocarcinomas tissues expressed TGF β1, the intensities of which were from 1+ to 4+, while negative expressions were found in 2 laryngocarcinoma tissues. Weak or negative expressions were found in normal larynx tissues and paracarcinoma tissues, which was significantly different from the result found in laryngocarcinoma at statistical level ($p<0.01$). Positive expression of TGF β1 were no significant difference in largngocarcinoma tissues of different pathological and clinical T grading ($p>0.05$). But the intensities of expressions in laryngocarcinoma with positive lymphatic metastasis were significantly different from those in laryngocarcinoma with negative lymphatic metastasis ($p<0.01$). ***Conclusions*** These results suggest that TGF β1 may play a role in laryngocarcinoma genesis, malignant transformation and lymphatic metastasis, in addition, immunohistochemical staining of TGF β1 seems to be a morphological marker for determining the tendency of lymphatic metastasis of laryngocarcinoma.

SOMATIC HYPERMUTATIONS IN THE VH SEGMENT OF IMMUNOGLOBULIN GENES OF INTRAVASCULAR LARGE B CELL LYMPHOMA. A DIRECT SEQUENCE ANALYSIS

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Intravascular large B cell lymphoma is a very rare lymphoma and occasionally reacts with CD5. We investigated the sequence of the variable region of the immunoglobulin heavy chain (VH) using the direct sequence method in five cases to clarify whether or not lymphoma represents the pre-germinal center stage, as do other CD5 positive B-cell malignancies, or the post-germinal center stage, as is typical of diffuse large B cell lymphoma.

After an extraction of DNA from paraffin and/or fresh specimens, both CDR2, and the FW3 region were amplified by PCR and the direct sequence was examined from it products after purification. The sequence displayed somatic mutations with the percentage homology of VH from 86.4% to 99.3% (median 88.6%). These results indicate that intravascular large B cell lymphoma thus appears to usually originate in post germinal cell cells, such as CD5 negative diffuse large B cell lymphoma, however, a germinal center cell origin also remains a possibility such as diffuse follicular center cell lymphoma.

IMMUNE CHARACTERISTICS OF INTERDIGITATING CELLS AND LANGERHANS CELLS: DISTRIBUTION, DIFFERENTIATION AND MIGRATION

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Interdigitating cells (IDCs) and Langerhans cells (LCs) are very potent antigen presenting cells. They demonstrate similar morphological characteristics, and the presence of Birbeck granules (BGs) is the only characteristic difference in these two cell types at the ultrastructural level. We previously demonstrated that LCs with BGs were positive for both S-100 protein and CD1a antibodies, while IDCs without BGs were positive for S-100 protein but negative for CD1a based on sequential immunostaining, electron microscopy and immunoelectron microscopy. This difference makes it possible to differentiate between LCs and IDCs at the microscopic level. From these results, we clarified here, the distributions of LCs and IDCs in human organs and tissues. Immunostaining was performed using specimens embedded by the AMeX method. LCs were observed in the cervical, hilar, and inguinal lymph nodes, the skin, thymus, tonsils with squamous epithelium and in the trachea with metaplastic squamous epithelium. Flow cytometry was performed using axillary and abdominal lymph nodes. Positive cells for CD1a and S-100β protein antibodies with high FS and middle SS were enriched for electron microscopy. LCs with BGs were observed only in draining superficial lymph nodes but not in profound lymph nodes.

In animal experiments, LCs were observed in the squamous metaplastic epithelium of the rat urinary bladder induced by vitamin A deficiency, and in iliac lymph nodes which are regional lymph nodes of the urinary bladder. However, in normal rats, LCs were not observed in the iliac lymph nodes. These cells may directly migrate from the metaplastic squamous epithelium. These results suggest that the existence of squamous epithelium plays an important role in the maturation, migration and function of LCs.

WILMS' TUMORS
-Their pathology and molecular genetics-

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We have investigated mutation patterns of the WT1 gene in sporadic Wilms' tumors not involving other teratologies as well as in malformation syndromes and correlated these results with the pathological features of these tumors. As a result, we have been able to detect various mutations in the WT1 gene including previously unrecognized mutations in 11 of these 60 cases (18%). Furthermore, we analyzed WT1 in 8 patients who were diagnosed as "Drash syndrome". Surprisingly, five out of 8 cases were found to have intronic mutations in one of the splicing donor sites in exon 9. In order to know whether these intronic mutations affect alternative splicing, we constructed genes containing these mutational sequences and transfected them into COS cells. As a result, the KTS isoform retaining all three amino acids was not produced in transfected cells. All of these patients with intronic mutations had XY gonadal dysgenesis, late onset of nephropathy, and no development of Wilms' tumor. These clinical symptoms clearly correspond to those of Frasier syndrome rather than those of Drash syndrome. We will discuss major roles of WT1 in oncogenesis and organogenesis.

The expression of Co-stimulatory molecules in human cervical cancerous tissues

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To elucidate the mechanism underlying tumor escape from immunosurveillance, 64 samples of cervical cancer were analyzed for the expression of B7.1, B7.2, ICAM-1(CD54) and CD28, together with HLA-ABC and HLA-DR Ag on tumor cells and interstitial cells by using both immunohistochemistry and dig-probes, in situ hybridization techniques. The results showed as follows: (1) the tumor cells in most cases (28/34) expressed MHC Ⅰ Ag, and a couple of samples (6/34) also expressed MHC Ⅱ Ag. (2) The tumor cells in 25/42 cases expressed B7.1 Ag, in most cases analyzed for B7.1, all of the tumor cells had a very strong B7.1 Ag expression, but in several cases, only a portion of cancer parenchyma or a few of cancer cells in the nests were B7.1 positive. No tumor cells in all of samples inspected were B7.2 positive. (3) As for ICAM-1, the results were similar to that of B7.1 expression, in 26/42 cases the cancer parenchyma was ICAM-1 positive. (4) No cancer cells in whole group expressed CD28 Ag. (5) In the interstitials all nucleated cells were HLA-ABC-positive. and there were a lot of dendritic cells (DC) were HLA- DR positive, a few of TILs in most of samples expressed HLA-DR Ag. (6) As for B7.1 and B7. 2 expression, 6 samples had no B7.1 and B7.2 positive DC and lymphocytes, 3 samples had a great many of B7.1 and B7.2 dendritic cells and lymphocytes infiltrating in the interstitials and the nests, however, in most cases, only a few of B7. 1 and B7. 2 positive DC and lymphocytes was found in or around the nests. Compared with the results from HLA-DR and B7.1 or B7.2 staining, the postitive HLA-DR DC was much more than positive B7.1 or B7.2, implying that a considerable number of DC did not express B7.1 and /or B7.2 molecules. (7) In interstitials CD54 molecule was also expressed on endothelia of small vesseles except for DC and lymphocytes. (8) TILs were weakly stained with CD28 McAb, scattering or accumulating in the interstitials. The results from in situ hybridization with B7.2, B7.1 and CD54 probes was well correlated with that from histochemistry.

The present findings indicate that the dendritic cells infiltrating in the cervical cancers were deficient in B7.1 and B7.2 molecule expression, which might lead to their functional defect in immunostimulation; the tumor cells in most cases might be capable of Ag presentation since they expressed the corresponding accessory molecules. The escape mechanism of tumors might be heterogeneous, mainly attributed to the events after antigen presentation.

QUANTITATIVE ASSESSMENT OF EPSTEIN-BARR VIRUS (EBV)-INFECTED CELLS IN THE PERIPHERAL BLOOD OF THE CHILDREN WITH EBV-ASSOCIATED MALIGNANT LYMPHOMA AND LGL LYMPHOCYTOSIS

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Introduction. Using *in situ* DNA-RNA hybridization, we attempted to enumerate EBV-infected cells in the peripheral blood mononuclear cells (PBMCs) which were obtained from the pediatric patients with EBV-associated malignant lymphoma or LGL lymphocytosis.

Methods. PBMCs were obtained from 6 patients with EBV-associated malignant lymphomas of T-cell origin and 5 patients with EBV-associated LGL lymphocytosis in childhood. Twenty-five children without past EBV-infection, 10 children with infectious mononucleosis, and 45 healthy children with past EBV-infection were also investigated as controls. Serum samples were tested for anti-EBV antibodies by the conventional method (Henle et al, 1974). Ten μl of PBMC suspension (5×10^6 cells per milliliter) was spotted on a silane-coated slide glass. After the fixation of the cells, *in situ* DNA-RNA hybridization with alkaline phosphatase-conjugated oligonucleotide probe against EBV-encoded small nuclear RNA1 (EBER1) was performed on PBMCs. The fluorescence activated cell sorting and double-labeling immunohistochemical and *in situ* hybridization studies were done to identify the phenotypes of cells infected with EBV.

Results. EBV-infected cells, the number of which ranged from 8 to 25,000 per 50,000 PBMCs (mean ± SD; 4816 ± 9902 per 50,000 PBMCs), were observed in the PBMCs of all 6 patients with lymphoma, while more than 25,000 cells per 50,000 PBMCs were infected with EBV in each patient with LGL lymphocytosis. In all patients with lymphoma or LGL lymphocytosis, blast cells had not been found on the blood smears with the conventional morphological technique. Two to 121 EBV-infected cells (mean ± SD; 35 ± 36 per 50,000 PBMCs) were detected in the patients with infectious mononucleosis. Neither children without past EBV-infection nor healthy children with past EBV-infection showed any EBV-infected cells.

Conclusions. The quantitative assessment of EBV-infected cells in PBMCs indicates that a considerable number of EBV-infected cells exist in the peripheral blood of the patient with EBV-associated lymphoma or LGL lymphocytosis, even if the cytologic examination cannot clarify the blast cells on blood smear.

THE EFFECTS OF INTRACELLULAR IMMNUOCOMPLEXES ON THE PATHOLOGENESIS OF EPIDEMIC HEMORRHAGIC FEVER

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Introduction. Epidemic hemorrhagic fever is a world wide infectious disease. Immunocomplexes (IC) play an important role in its pathogenesis. This paper presents the results of studies on its site of deposition and its relation to primary cell death and the mechanism of IC on the pathogenesis of the disease. **Methods.** we have observed 18 autopsy cases, 52 biopsies of the patients. Experimental tracing of antigen in infected mice by colloid gold labeled antibodies and injection of IC into health mice were also done. Immunohistochemistry, double labeling, in-situ hybridization, confocal and immunoelectron microscopy were used. **Results.** The materials demonstrated that the IC and virus RNA were localized mainly in the cells especially in the epithelial cells including renal tubular epithelia, hepatocytes, ductal cells of glands and muscle cells .etc. Intracellular IC, beside IC in the extracellular fluid is a peculiar feature of epidemic hemorrhage fever. The gold labeled antibodies injected into the infected BALB/c could be internalized to combine with the antigen as phagolysosome. and caused shrinkage of the cell. The injection of the soluble IC prepared by purified excess G1,G2 and NP antigens with their antibodies induced also internalization, phagolysosomes and lysis of the cells. While the injected insoluble IC deposited mainly on the vascular and glomerular basement membranes, only a small amount was found in the cells. **Conclusions.** From the above material we could conclude that in the incubation period of the disease there is proliferation of the virus with increasing amount of antigen. Later production of antibodies form soluble IC due to antigen in excess and thus induces damages and causes the onset of the disease. So the soluble IC plays the key role in the course of the disease.

DIAGNOSTIC HISTOCHEMISTRY OF INFECTIOUS DISEASES

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Detection of infectious agents within the lesion is essentially important for the histopathologic diagnosis of infectious diseases. Demonstration of pathogenic antigens and genomes is one of the best choices in the diagnostic approach. The results will lead the patients to the appropriate diagnosis and treatment.

A) **Commercially available antibodies** have been utilized in routine diagnostic purposes to demonstrate bacterial antigens of *Mycobacteria*, *Chlamydia*, *E. coli*, and *H. pylori*. Detectable in paraffin sections are a variety of viral antigens, including HSV, VZV, CMV, EBV, HPV, parvovirus B19, JCV, adenovirus, measles virus, HBV and HCV. Protozoal antigens such as *P. carinii* and *E. histolytica* are also demonstrable.

B) **Sera of immunocompetent patients** become a powerful weapon to detect pathogens in paraffin sections. Diluted sera of the patients become convenient probes for the indirect immunoperoxidase diagnosis of chickenpox, staphylococcosis, cat scratch lymphadenitis, cryptococcosis, sporotrichosis, toxoplasmosis, amebic dysentery, cutaneous leishmaniasis, cryptosporidiosis, blastocystosis, liver ascariasis, angiostrongylosis cantonensis, and cutaneous gnathostomiasis. The specificity is sufficient in infectious diseases by protozoa and helminths. Sera of animals experimentally infected with *Treponema pallidum* are also useful.

C) **Non-isotopic *in situ* hybridization** (ISH) has been applied to detecting genomes of *H. pylori*, *P. aeruginosa*, *Aspergillus fumigatus*, *C. trachomatis*, CMV, EBV (EBER1), HPV (types 6, 11, 16, 18, 31/33/35 and wide spectrum), JCV, HBV, HCV in paraffin sections on silane-coated glass slides. Demonstration of ribosomal RNA is valuable to show the viability of the pathogens. In such oncogenic viruses as HPV and EBV, the viral antigens are less frequently detected than the viral genomes. Viral particles are hardly observed in neoplastic cells, so that ISH is primarily important for showing the pathogens in the neoplastic lesions. ISH is also applicable to detecting HPV genomes in cervical smear preparations. Direct comparison of ISH and pap morphology can be done by re-staining the bleached pap smear with biotinylated probes.

D) **Ultrastructural visualization of pathogens** has been performed in routinely prepared material. The antigens of *C. trachomatis*, *M. leprae*, MAI, *E. coli*, *H. pylori*, CMV and HPV are visualized in paraffin sections by applying pre-embedding immunoelectron microscopy. This approach is useful to confirm not only the presence of pathogens within the lesion but also the specificity of antibodies used. Viral genomes are also identifiable at the EM level. HPV genomes, types 16/18, are located in part of chromatin networks in severe dysplasia of the uterine cervix, a feature indicating the viral integration into host DNA. Chlamydial elementary bodies and HPV episomal particles can histochemically be shown in alcohol-fixed cervical smear preps.

THE EXPRESSION OF MIB-1 AND THROMBOMODUIN IN NASOPHARYNGEAL CARCINOMA

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Introduction: Nasopharyngeal carcinoma (NPC) has distinct epidemiological character, it usually seen in South China, but in recent years, the incidence of NPC in Shenyang has the trend of increasing. This paper presents the results of studies on the expression of MIB-1 and thrombomodulin (TM) in NPC. Methods: immunohistochemical ABC method was used. Results: MIB-1 positive rate was 95.7% (95/97) , it located in the nucleion of the proliferating NPC cells and some basal cells in normal mucosal cells. High MIB-1 PI related to poor prognosis, there was no relation of MIB-1 PI to subtype of non-keratinizing NPC. TM expressed in the surface of vessel endothelial cells and the bridge of normal squamous cells, but not in non-keratinizing NPC cells. Angiogeneic vessels in NPC losed polar, presented mainly two forms: cord and bud-like . Compared with the other two endothelial cell markers: F_8RA and CD_{34}, TM was well expressed and without collagen stroma expression, it was more sensitive than the others. In our experiment, most were nonkeratinizing NPC cells, we could not find the relation of TM expression and NPC cell differentiation, but the result suggest that in nonkeratinizing NPC, TM was not expressed. The number of angiogenic vessels in survival duration less than 3 years was higher than that of above 5 years. The more angiogenic vessels, the higher MIB-1 PI. Conclusion: MIB-1 and angiogenic vessels maybe helpful in NPC's prognosis , TM could be used in immunohistochemistry as an endothelial cells marker.

The expression of standard and variant CD44 in human lung cancer

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Abstract

CD44 transmembrane glycoprotein, a cell adhesion molecule, is expressed in most adult tissues and in the majority of neoplasias. Due to alternative splicing, this cell adhesion molecule exists in multiple isoforms, some of which have been associated with specific types of tumors as well as with tumor invasion and metastasis. In this study, we observed the expression of CD44 standard (CD44s) and variant forms (CD44v) in 96 primary lung carcinomas and 1 2 cases of metastatic lymph nodes by immunohistochemistry and associated the results with clinical outcome. **Objective:** to understand the correlation of CD44's expression with the histological types. differentiation, progression and metastasis of primary lung carcinomas. **Methods:** Formalin-fixed paraffin-embedded archival tissues from 56 squamous cell carcinomas (SCC), 34 adenocarcinomas (ADC), 6 small cell lung carcinomas (SCLC) and 12 metastatic lymph nodes were immunostained after microwave antigen repair with monoclonal antibodies against CD44s and CD44v6, and the results were associated with histological tumor type, tumor stage and metastasis. **Results:** (1) SCC showed much higher membranous expression of CD44s and CD44v6 than ADC ($P<0.01$), and there is no expression in all 6 SCLC cases. (II) In 12 metastatic lymph nodes, 10 metastatic lung carcinomas were found strong immunoreactivity for CD44v6 (83.3%), and in comparison with lung carcinomas without lymph node metastasis (50.98%), Primary lung carcinomas with lymph node metastasis showed strong expression of CD44v6 (91.2% $P<0.01$). (III) The expression of CD44s and v6 is independent from the tumor histopathological grading (well, moderate, poor differentiated). Among different tumor stage, however, there is a distinct statistic difference between stage I, II and stage III, IV ($P<0.01$) with the tendency of higher stage with higher expression of CD44v6. **Conclusion:** CD44 expresses mostly in NSCLC, moreover, there is an intense expression of CD44s and v6 in SCC over ADC. Immunopositivity of CD44v6 may suggest a potential of high risk for lymph node metastasis in NSCLC, CD44v6 reactivity may be useful to decide TNM stage of NSCLC, especially to discriminate stage I, II with higher stage.

Key word: CD44s, CD44v6, Iung carcinomas. Immunohistochemistry

GENE AMPLIFICATOIONS OF c-MYC, c-ERBB-2, c-MET AND K-SAM IN GASTRIC CANCERS: DETECTION BY FLUORESCENCE IN SITU HYBLIDIZATION

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Gene amplifications of the c-myc, c-erbB-2, c-met and K-sam were examined on cancer nuclei isolated from 154 primary gastric adenocarcinomas by fluorescence in situ hybridization (FISH) using cosmid probes for 8q24(c-myc locus), 17q11.2-12(c-erbB-2), 7q31(c-met) and the DNA probes for K-sam synthesized by polymerase chain reaction. The results were compared with Southern blot analysis. Dual-color FISH using the gene locus-specific and chromosome-specific probes was successful in 145, 134, 151 and 103 tumors respectively, and the gene amplification of c-myc was found in 23 tumors (15.8%), c-erbB-2 in 21 tumors (15.7%), c-met in 6 tumors (4.0%) and K-sam in 3 tumors (2.9%). Co-amplification of c-myc and c-erbB-2 was observed in 6 tumors, and c-myc and c-met in 6 tumors. This technique also could differentiate the amplicons located on homogeneous staining region(HSR), in non-HSR of the chromosomes, and double minute(DM) chromosomes in metaphase spreads and interphase nuclei of gastric cancer cell lines, KATOIII, SNU16, HSC39, MKN7 and MKN45. By referring to FISH images of these cell lines, high level amplification (10 and more copies) of c-myc was suggested to occur on DM in 4 tumors and on HSR in one, c-erbB-2 on DM in 3 tumors and on HSR in 15, and K-sam on DM in 2 tumors and on HSR in one. High level amplification of c-met was not found. The gene amplifications were also detected in most formalin-fixed and paraffin-enbedded primary gastric tumors, however low grade amplification was not detected by Southern blot analysis. It is concluded that FISH is an important tool providing the c-oncogene aberrations in intact cells in solid tumor.

CYTOSOLIC CALCIUM AND ITS RELATION WITH TNF-β INDUCING APOPTOSIS IN HUMAN PANCREATIC CARCINOMA CELLS

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Now new and effective methods are in urgent need for cancer therapy. Recently biological therapeutic method has been emphersized and would be put into widely use before long. Tumor necrosis factor-β (TNF-β) is one of the most promising one.

TNF-β is a 25KDa glycoprotein. Some studies abroad showed that it displayed 5 ~10 times more potent anti-proliferative effects than TNF-α.

In our laboratory, previous studies were undertaken to investigate the anti-cancer effects of TNF-β incombination with CDDP、DXR and MMC both in vitro and in vivo.

To uncover the underlying molecular pathologic mechanisms of antitumor potency of tumor necrosis factor-β, and to realize the path of tumor cell death and its interfering effects, terminal deoxynucleotidyl transferase (TDT) end labelling apoptosis in situ staining and optimal electron micorscopy were adopted to observe the effect of TNF-β inducing human pancreatic JF305 tumor cell apoptosis. In addition F-2000 fluoresence spectrophotometer (HITACHI) and Fura-2/Am was used to measure the intracellular free calcium. The results showed that when JF305 cells were incubated with TNF-β, apoptosis can be triggered, TdT in situ staining method revealed brown granules in nuclears. Transmission electron microscopy revealed classic morphologic alterations, i.e. compaction and margination of nuclear chromtin, condensation of cytoplasm, nuclear hole nearby disappears. After addition of tumor necrosis factor-β, cytosolic free calcium increased sharply; When incubated with TNF-β for 24 hours statistic calcium increased. So we can draw the conclution that apoptosis can be triggered by TNF-β. Meanwhile the cytosolic free calcium is obviously elevated. This is the pathway through which TNF-β destroy the tumor cells. Cytosolic calcium plays an important role in the cancer cell apoptosis.

Key words: Anti-cancer potency, apoptosis, TNF－β

MOLECULAR PATHOLOGY OF HUMAN PROSTATE CANCERS

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We have analysed genetic alterations of human prostate cancers since 1990. In addition, we have collaborated with some institutions and have collected frozen samples of human prosate as tissue bank. To date, 337 frozen samples containing 152 prostate cancers and 174 BPHs are stocked at -80C. At first, we will introduce our tissue bank system.

To clarify genetic alterations of prostate cancer, we have analysed mutations of the ras, p53, p73, and androgen receptor (AR) genes, microsatellite instability (MSI), and loss of heterozygisity (LOH) in Japanese human prostate cancers using PCR method. Mutations of the ras gene were detected in 20/81 cases (24%). Mutations of the p53 gene were detected in 11/90 cases (12%). However, there was no mutation of the p73 gene in 27 cases. Mutations of the AR gene were detected in 5/36 cases (14%). Using 12 microsatellite markers, the frequency of MSI in 25 cases was 28%. Analysis of LOH in 25 cases showed 48% of D8S201 and LPL loci, however very low frequency in BRCA1 and BRCA2 loci. These data shows that mutations of the ras gene and MSI are involved in early step of carcinogenesis of the prostate, and mutations of the p53 gene in late step. Now we tried to make cDNA library for further study.

TWO NEWLY ESTABLISHED HUMAN UTRINE CERVICAL CARCINOMA CELL LINES: KARYOTYPE ANALYSIS AND MOLECULAR GENETIC CHARACTERIZATION

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The derivation of permanent cell lines from 10 resected cervical carcinoma has been attempted . Two long-term lines were established both from squamous carcinomas and one grew as adherent cells and one as floating aggregates. Human Papillomavirus status was examined with polymerase chain reaction (PCR), employing HPV consensus primers from the L1 region. One cell line was HPV-positive and other HPV-negative. Cytogenetic analysis revealed karyotypes ranging from near triploid to near quadraploid with complex rearrangements of chromosomes 3, 9, 11, 13, 15, 21, 22, X and a del(3)(p14→pter) was involved. No normal chromosome 9 was found in the HPV negative cell line. Overexpression of p53 proteins were detected by Flow Cytomerter in both lines. Fragile Histidine Triad (FHIT) transcript of the expected size was identified by reverse transcription (RT)- PCR only in one cell line. Taken together, these cell lines would be very useful for studying the biology and genetics of the uterine cervical carcinoma.

APPLICATION OF IMMUNOHISTOCHEMISTRY AND IN SITU HYBRIDIZATION IN THE STUDY ON ATHEROSCLEROSIS

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The recruitment of monocytes/macrophages(MC/MØ) in the subendothelial space and the migration of smooth muscle cells(SMC) from media into intima and proliferation are the early events in atherogenesis. Migration of both MC/MØ and SMCs are influenced by a lot of factors including chemokines and other cytokines produced by arterial wall cells themselves. Our previous studies demonstrated that arterial wall cells including endothelial cells (ECs), SMCs and MC/MØ can express MCP-1 and MIP-1α and oxidized lipoproteins, lipid peroxidation and endotoxin induced a strong expression of both chemokines.

Immunohistochemistry Both antibodies rabbit anti-human MCP-1 polyclonal antibody and goat anti-human MIP-1a were for the assays as follows: (1) In vitro: a) Cultured arterial wall cells(ECs,SMCs and MC/Mα were grown on the glass cover-slips in the culture flasks. After exposure to the above-mentioned factors, the glass cover-slips were taken out and fixed , and then were immunostained. To evaluate the intensity of the MCP-1 expression, the mean absorbent value of the immunostained cells were determined using an image analysis system. Using this method,we obtained satisfactory results. b) To evaluate the MCP-1 protein content produced by the arterial wall cells, the media conditioned by the cultured cells were collected and determined by ELISA. ELISA can elucidate some problems from another angle. (2) In vivo: Dietary atherosclerosis in rabbits was developed by feeding the animals with standard pellets containing cholesterol. The sections of the plaques were immunostained using MCP-1 polyclonal antibody. The MCP-1 expression by the cells in the plaques was examined microscopically.By this method a quantitative analysis is difficult.

In situ hybridization (1) In vitro: a) The glass cover-slips grown with cells were collected according to the above-mentioned method. The probe for hybridization was MIP-1α cDNA labeled with digoxigenin-dUTP. An image analysis system was used for detection of the absorbance of the cells hybridized with the probe. The results we obtained were satisfactory. B) After exposure to the above-mentioned factors, the cultured cells were collected by trypsin digestion. The total cellular RNA was extracted by guanidinium isothiocyanate method, and each RNA sample from the cultured cells was spotted onto the nitrocellulose membrane. After hybridization with the probe, the absorbance of the spots on the membrane was detected using an image analysis system. The signal of the spots hybridized with digoxigenin-labeled probe was less strong than that of the spots hybridized with ^{32}P-labeled one. (2) In vivo: The paraffin sections of the atherosclerotic plaques from the above-mentioned animal models were used for in situ hybridization. A quantitative analysis is difficult, though this method can demonstrated which cells in the plaque express MIP-1α. The key to the achievement of in situ hybridization is how to prevent the degradation of the antigens in the samples. All the flasks, slides, tools, etc. must be thoroughly cleaned and baked at high temperature, and/or one can use the inhibitors of RNAase and DNAase.

Effect of Lipid Peroxidation on Mitogenic Activities of Endothelial Cells

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Abstract:

The purpose of this paper is to investigate whether the lipid peroxidarion of endothelial cells had any effect on the production of growth factors by them.

Methods: The bovine aortic endothelial cells were isolated and cultured using an enzyme-dispersal method, and the media conditioned by endothelial cells with lipid peroxidation induced by cumene hydroperoxide were collected. Then the endothelial cell-conditioned media (ECCM)were treated by heparin-binding sepharose, and the mitogenic activity of ECCM for swiss 3T3 cells were determined by incorporation of[^{3}H]-thymidine into DNA of the cells. The effect of lipid peroxidation of endothelial cells on their synthesis and secretion of PDGF was performed with neutralization assay using anti- PDGF antibodies and Northern blot analysis. The concentration of endothelin in the media of endothelial cells were analysed by radioimmunoassay.

Results: Cumene hydroperoxide induced storage of lipid peroxide in cultured endothelial cells, and there was an obvious increase in the incorporation of [^{3}H]-thymidine into DNA in Swiss 3T3 cells treated with ECCM compared with the control. However, the mitogenic activity of ECCM for Swiss 3T3 cells were in non heparin-binding parts., and were not inhibited by anti-PDGF-AA and anti-PDGF-BB antibodies. Conversely, a weaker PDGF-B chain mRNA expression in endothelial cells was observed after exposure to cumene hydroperoxide. Then the concentration of endothelin in the media of endothelial cells treated by cumene hydroperoxide were increased obviously compared with the control.

Conclusion: It seems reasonable to believe that lipid peroxidation of endothelial cells induced by cumene hydroperoxide leads to an increased synthesis and secretion of growth factors, and the mitogenic activities were due to non heparin-binding parts like as endothelin.

p53 MUTATION IN SALIVARY GLAND TUMORS -with a special attention to EBV-related lymphoepithelioma-like carcinoma

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In order to determine prevalence of Epstein-Barr virus (EBV) infection in salivary lymphoepithelioma-like carcinoma (LEC) in China, we have collected 159 cases of LEC from various areas of the main China and Taiwan (42, Shanghai; 40, Chengdu; 42, Guangzhou; 9, Beijing; 4, Wuhan; 4, Changchun; 3, Kunming; 3, Xi'an; 2, Urumqi; 0, Jinan; 6, Taipei; 3, Taichung) and examined the tissue samples by immunohistochemistry, in-situ hybridization (ISH) for EBER-1 and polymerase chain reaction (PCR) technique for EBV DNA BamHI W region for the presence of EBV. The LEC series consisted of 74 male and 78 female patients (9 unknown). Except for one Uygur and one Xibe, the patients were of Han race (3 unknown), ranging in age from 9 to 77 years (mean, 43.5 years; 35% of patients were younger than 40 years). EBV infection was demonstrated in all of the LEC specimens examined by ISH and PCR, but not in the other types of salivary gland tumors. Secondly, we wanted to study the mutational status of tumor-related genes in these EBV-related LEC cases. To this end, we extracted DNA from formalin-fixed paraffin sections of LECs and other salivary gland tumors and examined exons 5-7 of p53 by PCR-fluoro-SSCP analysis and compared their expression of p53 protein. PCR-amplified DNA fragments from cases with abnormal SSCP patterns were further examined with a fluoro-DNA sequencer. Various and extensive mutations were found in LECs which showed scarce p53 protein expression, whereas other salivary gland tumors had more enhanced expression of p53 protein but a small number of point mutations. The result indicates that such severe mutational events of p53 specifically found in LECs might be possibly evoked by the EBV infection.

IN SITU OBSERVATION OF APOPTOSIS AND PROLIFERATION OF GASTRIC MUCOSA WITH HELICOBACTER PYLORI INFECTION

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To understand the relationship between Helicobacter pylori (Hp) and gastric carcinoma, apoptosis and proliferation of Hp associated gastric diseases were quantitatively studied. Terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) technique for apoptosis and proliferative cell nuclear antigen (PCNA) immunohistochemical staining for proliferation were used in the research. 130 endoscopic gastric mucosal biopsy specimens were examined, including 30 chronic gastritis, 30 atrophic gastritis (intestinal metaplasia), 30 dysplasia, 20 gastric carcinomas and 20 normal gastric mucosa. Each kind of case, except normal gastric mucosa without Hp infection, was divided into two groups, and one group was Hp − positive and the other group was Hp − negative. The results showed that the apoptotic index(AI) was higher in chronic gastritis with Hp infection ($8.7 \pm 3.3\%$) than in Hp − negative group ($5.4 \pm 2.9\%$) and in the normal mucosa ($2.1 \pm 1.1\%$)($P<0.01$ and 0.001). In atrophic gastritis, AI of Hp − postiive group ($4.4 \pm 2.0\%$) was slight higher than that of Hp − negative group ($3.8 \pm 1.8\%$) ($P>0.05$). On the contrary, there was a higher AI in Hp − negative dysplasia ($3.1 \pm 1.3\%$) and carcinoma ($1.9 \pm 1.0\%$) than in Hp − positive group ($2.3 \pm 1.1\%$ and $1.3 \pm 0.7\%$), respectively ($P>0.05$). In each case, the PCNA index (PI) was higher in Hp − positive group than in Hp − negative group ($P<0.05$ or 0.01). From chronic gastritis to carcinoma, AI showed a gradual decrease and PI showed a gradual increase. Among atrophic gastritis, dysplasia and carcinoma, the apoptosis/proliferation ratios were higher in Hp − negative group than in Hp − positive group ($P<0.05$ or 0.01), and had a significant decrease along the progress of lesions. These suggest that Hp can strongly trigger cell proliferation, and induce apoptosis in early stage, but perhaps inhibit apoptosis in advanced stage via certain factors during the formation of gastric carcinoma. As a result of the two effects of Hp, there were a higher cell proliferation and a lower apoptosis on gastric mucosa. In other words, Hp caused the dissociation of gastric epithelial cell proliferation from apoptoisis, so Hp is an important carcinogenic factor of gastric carcinoma.

Poster 3

Infection of human T-cell leukemia virus type 1 (HTLV-1) in malignant lymphomas in the northeast region of China: Modified ImmunoMax of HTLV-1 pX-related proteins and polymerase chain reaction (PCR) analysis of HTLV-1 proviral DNA

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Recently we have established highly sensitive immunohistochemistry (the modified ImmunoMax) detecting HTLV-1 pX-related proteins (p40Tax and p27Rex). Employing the modified ImmunoMax and PCR analysis of HTLV-1 proviral DNA pX region on DNA extracted from paraffin sections, we analyzed malignant lymphomas (MLs) in China to see how many cases and what kinds of Chinese MLs have relation to HTLV-1 infection. Chinese 63 cases of MLs diagnosed in Department of Pathology, China Medical University were employed for this study. Based on paraffin-immunohistochemistry of monoclonal antibodies against CD3, CD4, CD8, MB-1, LN1, LN2, LN3, S100 protein and muramidase, these MLs were categorized according to the new WHO classification. These MLs comprised 1 CLL, 1 diffuse mantle cell lymphoma, 2 marginal zone B-cell lymphoma, 5 CB/CC, 1 follicular CB lymphoma, 7 diffuse large B-cell lymphoma, 14 immunocytoma (IC), 3 plasmacytoma, 1 Burkitt type lymphoma, 3 T-cell large anaplastic cell lymphoma (LAC), 5 AILD type T-cell lymphoma, 9 peripheral T-cell lymphoma NOS, 1 Angiocentric T cell lymphoma, 1 Castleman disease, 1 Mycosis fungoides and 8 Hodgkin disease (HD). The modified ImmunoMax employing WATM-1 against p40Tax and Rec-6 against p27Rex (Supplied from Dr. Y. Tanaka, Kitasato University) labeled lymphoma cells in 8 (12.7%) cases of the Chinese MLs, revealing various amount of positive product in nuclei or cytoplasm of lymphoma cells. The WATM-1 and Rec-6-positive eight cases of MLs were 3 cases of peripheral T-cell lymphoma, 1 case of AILD type, 2 cases of diffuse large B-cell lymphoma, 1 case of IC and 2 cases of HD. Fourteen cases including the HTLV-1 pX-related protein-positive cases were examined by means of PCR analysis employing a pair of primers SN443 and 444. The pX-related protein-positive cases except one HD were PCR-positive, four cases were pX-related protein-negative and PCR-negative and two cases were pX-related protein-negative and PCR-positive ($p=0.036$). Consequently, a few ML cases in the northeast region of China were associated with HTLV-1 infection.

THE STUDY ON THE EXPRESSION OF PCNA AND P16 PROTEIN IN HELICOBACTER PYLORI ASSOCIATED GASTRIC DISEASES

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The explore the relationship between Helicobacter pylori (Hp) infection and gastric carcinoma at molecular level, we investigated gastric epithelial cell proliferation and expression of p16 protein in Hp associated gastric diseases. 136 gastric mucosal biopsy specimens were examined with PCR techique and proliferative cell nuclear antigen (PCNA) immunohistochemical staining, including 24 normal gastric mucosa, 37 superficial gastritis (moderate－serious), 34 erosion and ulcer, 21 atrophic gastritis and 20 gastric carcinomas. Among the above cases, each kind of case, except the normal gastric mucosa without Hp infertion, was divided into two groups, namely Hp－positive group and Hp－negative group. The results showed that the PCNA index (PI) of normal gastric mucosa was very low and PI had a gradual increase along the progress of the lesions. PI of normal gastric mucosa, Hp－negative gastric benign lesions, Hp－positive gastric bengin lesions and gastric carcinoma was $9.8 \pm 3.7\%$, $13.94 \pm 7.34\%$, $20.71 \pm 11.26\%$ and $41.49 \pm 15.55\%$, respectively. PI of the normal mucosa was compared with that of gastric carcinoma, Hp－negative benign lesions and Hp－positive benign lesions, respectively, and the difference between these values was statistically significant ($P<0.001$, 0.05 and 0.01, respectively). The difference between PI of Hp－positive benign lesions and that of Hp－negative benign lesions was significant, too ($P<0.01$). The positive rate of P16 protein expression was 100% on normal gastric mucosa, and was 95.3% and 82.1% in Hp－negative and Hp－positive benign lesions . But the positive rate of gastric carcinoma was only 35%. There was a statistical difference between the above Hp－negative and Hp－positive group. These show that there are a higher PI and a lower expression of P16 protein in Hp－positive benign lesions similar to in gastric carcinoma, and this suggests that there can be a more significant canceration tendency in Hp－positive gastric benign lesions than in Hp－negative benign lesions. Perhaps Hp infection caused the overproliferation of gastric epithelial cell by inhibitting the activity of P16 suppressor gene and elevated the susceptibility of epithelial cell to canceration.

Poster 5

NASAL T/NK-CELL LYMPHOMA IN SOUTHERN JAPAN

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Nasal T/NK-cell lymphoma is presented as midline granuloma and is characterized by aggressive clinical course. We examined the clinico-pathological, cytological and virological aspects of 14 cases of nasal T/NK-cell lymphoma in Southern Japan. Clinically, these 14 cases were divided into two groups. The first group consisting of 8 patients showed good prognosis. All of them, except for one case, are still alive without relapse. One case died of another malignancy, gall bladder cancer. The other group consisting of 6 patients showed poor prognosis. Five of them had died within half or one year after diagnosis. One patient is still alive, but the tumor relapse.

In combination of EBER-1 in situ hybridization with immunohistochemistry, the characteristics of tumor cells were examined by double-labelling. CD4 and CD8 surface T-cell markers were negative in EBER-1-positive tumor cells in all cases . In 4 cases, the EBER-1-positive tumor cells were positive for CD3, however, the ratio of CD3-positive cells were less than 30% of EBER-1-positive cells. In contrast, the cytoplasm of all EBER-1-positive tumor cells in all cases was stained with anti-CD3e monoclonal antibody. Tumor cells were also positive for CD2 and NK-cell marker, CD56. In addition, there was no rearrangement of T-cell receptor (TCR) gene. Therefore, nasal T/NK-cell lymphoma in Southern Japan may be NK-cell origin.

The subtype of EBV was examined by amplification of EBNA-2 region and the hybridization with subtype-specific probe. EBNA-2 region was amplified in 10 out of 12 specimens. All of them were subtype A. Two cases were not amplified, suggesting the mutation and/or deletion exists in this region.

The Expression of HLA-DR Antigen in Pulmonary Carcinomas

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The purpose of this study was to investigate the expression of HLA-DR antigen in human lung cancers and to relate such expression to the presence of lymphocytic infiltration around the tumor cells. We have examined surgical specimens of lung cancers using immunohistochemistry and have carried out a subsequent in vitro experiment to induce HLA-DR antigen expression in human lung cancer cells. using recombinant IFN gamma. Twenty two cases (22/29) adenocarcinoma and two cases (2/21) squamous cell carcinoma were positive for the HLA-DR antigen, no positive expression showed in small cell carcinoma (0/3). The staining in adenocarcinoma generally was stronger and more extensive than that in squamous cell carcinoma, which showed only week and focal staining. Marked lymphocyte infiltration was seen in the HLA-DR antigen positive cancers. In vitro experiment HLA-DR antigen expression in two cancer cell lines depend on the concentration of IFN gamma. The data suggest that the expression of HLA-DR antigen in lung cancers may reflect the differented type of tumor, and influence the host immune response.

Immunohistochemical study of ENV GP46 in lymphoma

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INTRODUCTION

human T cell leukemia virus type 1(HTLV-1) is a kind of C type reverse RNA virus which can cause adult Tcell leukemia. Iみ the past the detect methods of HTLV-1 had indirect fluorescent test. Elisa test. Western blot and PCR etc. rescently monoclonal antibody 6 C2 which can recongize HTLV-1 env gp46 and its precursor in paraffin section has been made. we studied the expression of 6C2 in Chinese lymphoma 70 cases, in tended to explore the related frequency of HTLV-1 with kinds of lymphoma in China.

MATERIALS and METHODS

70 cases of paraffin embodied lymphoma tissues sections were collected from Dept. of Pathology, China Medical Univ. 4 μ serial sections.

Monoclonal antibodies: MT-1. UCHL-1 .L26. BenH2

Method : immuno-peroxidase ABC staining on paraffin section. HE staining classify Tand B cells, then 6 C2 immunohistochemical staining. HE staining according to Keil's classfication (1992)

Second antibody: anti-mouse IgG.

Positive control: ATL cell line

Negative control:PBS instead of 6C2

RESULTS and DISCUSSION:

6C2 positive expression located in cell membrane granularly or membranoid, in some cases in cytoplasma, the former recognize gp46, the latter recognize its precursor. it expressed in 6/70(8.6%)cases. Among these, 3 cases Hodgkin's disease, 1 case ATL/lymphoma, 1 case NK/T cell lymphoma, 1 case Ki-1 undifferentiated lymphoma.

6C2 is a kind of antibody which can recognize HTLV-1 Env GP46 and its precursor. co-worker Hasui studied its expression in Kagoshima(ATLL high frequency) of 6C2 cases polymorphic T lymphoma, results suggestthat its expression has high specificity. in our study, result shows that a few cases of Chinese lymphoma have the infection of HTLV-1. The immuno-histochemistrical staning of 6C2 provided a valuable method for the study of HTLV-1's infection retrospectively, and may provide methods for studying the pathogenesis of leukemia and lymphoma, especially the relation with HTLV-1.

HTLV-1-ASSOCIATED NON-NEOPLASTIC LYMPHADENOPATHY

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HTLV-1-associated non-neoplastic lymphadenopathy (HANNLA) was reported to be not-neoplastic lymph node lesions in HTLV-1-carriers. HANNLA shows follicular hyperplasia with follicle-lysis and developing paracortex. In the developing paracortex there are many Ia-like antigen (LN-3)-positive dendritic cells suggesting an immunological histogensis with close relationship to HTLV-1 infection. We tried to analyze the histogenesis of the developing paracortex in HANNLA. Improving highly sensitive paraffin-immunohistochemistry (modified ImmunoMax) specificity of the highly sensitive immunohistochemistry using anti-p40Tax monoclonal antibodies (Lt-4 and WATM-1) was examined in one HTLV-1-not-related and 14 HTLV-1-related T-cell malignant lymphomas (T-MLs). Introducing the modified ImmunoMax to the capillary method of immunohistochemistry (MicroProbe) and evaluating quantitatively the imunohistochemical reaction, the paracortex in two lymph nodes with HANNLA and one lymph node of ATLL were examined by the immunohistochemistry of the monoclonal antibodies against p40Tax (WATM-1 and Lt-4) and against p27Rex (Rec-6) supplied by Tanaka Y (Kitasato University). Proviral dose of HTLV-1 in the two lesions of HANNLA was estimated by analyzing polymerase chain reaction (PCR) of proviral DNA pX region and by processing the elctrophoresis image of the PCR product with image analysis. In 13 cases of HTLV-1-related T-MLs Lt-4 and WATM-1 labeled lymphoma cells, revealing nuclear and dominantly cytoplasmal granular stain. In one case of HTLV-1-related and one case of HTLV-1-not-related T-MLs, Lt-4 showed strong nuclear stain but WATM-1 did not labele lymphoma cells. Lt-4 and WATM-1 revealed faint nuclear and dominantly cytoplasmal stain in lymphocytes and dominantly cytoplasmal stain in dendritic cells in the paracotex of HANNLA. The stain of Lt-4 and WATM-1 became stromger in order of HANNLA with small areas of paracortex, HANNLA with developed paracortex and ATLL, altough Lt-4 showed stronger reaction than WATM-1. Rec-6 showed the strongest reaction in HANNLA with developed paracotex. Quamtitiative analysis indicated that gradual increase of p40Tax from HANNLA with small areas of paracortex to ATLL and prominent increase of p27Rex in HANNLA with developed paracortex. Proviral dose of HTLV-1 was estimated by image analysis of the electrophoresis of the PCR product as 5.5 copies in HANNLA with developed paracortex and 1.6 copies in HANNLA with small areas of paracortex. Consequently, WATM-1 is more specific for p40Tax than Lt-4. The gradual increae of p40Tax according to the paracortex development in HANNLA and the prominet increase of p27Rex in HANNLA with developed paracotex suggested that the paracortical deveopment in HANNLA reflects activation of proviral DNA, reproduction of HTLV-1, re-infection of HTLV-1 and host immunity against HTLV-1-related proteins. On the other hand, HANNLA was not followed by the occurrence of adult T-cell leukemia/lymphoma (ATLL) and most patients with HANNLA were over 60 years-old. Then, HANNLA is thought to be an unique HTLV-1-related lymphadenopathy suggesting a mode of HTLV-1 infection enlargement in HTLV-1 carriers rather than a preceeding lymph node lesion of ATLL.

A CASE OF ENDOMETRIAL CANCER CONCOMITANT WITH UTERINE ADENOMYOSIS -A COMPARATIVE IMMUNOHISTOCHEMICAL STUDY WITH ADENOMYOSIS IN ELDERLY PATIENTS-

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Objective) To evaluate the potential character of adenomyosis proposing base of cancerization, a case of endometrial carcinoma with adenomyosis was investigated immunohistochemically.
Case and Method) Sixty three year old female was operated for the diagnosis of endometrial cancer. Sections having both of endometrial carcinoma and adenomyosis were immunohistologically stained with such antibodies as estrogen receptor (ER) , progestrone receptor (PR) , α smooth muscle actin (SMA) , Ki-67 (MIB-1) , Vimentin (Vim) and p53. For the comparison, 7 cases of adenomyosis without carcinoma in elderly were also examined with same antibodies.
Results) 1) HE revealed endometrial carcinoma in some adenomyotic island, accompanied by a few adenomyotic glands, some of which showing changes to cancer. 2) ER was positive in cancer cells, particularly in invading portions as well as adenomyotic nucleus. 3) PR was shown in adenomyotic gland as in carcinoma, mostly in basal portion of surface carcinoma. 4) SMA was detected in 5 cases of 7 adenomyosis and also in periglandular portion of some cancer nests in the myometrium, but not in the endometrium. 5) MIB-1 was positive in some cancer cells. 6) Vim was distinct in cytoplasm of adenomyotic gland, but not in cancer. 7) P53 was not shown in carcinoma, except for in one adenomyotic gland.
Discussion) Although features of adenomyotic glandular change to cancer were not so frequently pointed out in this case, SMA positive pericancerous tissue suggested some relations to periadenomyotic gland stain for SMA in elderly. Adenomyotic gland in these elderly might be a favorite site for cancer formation or route for its growth.
Conclusion) Endometrial cancer with adenomyosis would be a good model to survey carcinogenesis by immunohistochemistry.

AN IMMUNOHISTOCHEMICAL COMPARISON BETWEEN OLD AND RECENT COLORECTAL NEOPLASIA USING ANTIBODIES AGAINST Ki-67, P53, MUC2 MUCIN, AND α-SMOOTH MUSCLE ACTIN

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In order to elucidate the recent trend in biological behavior of colorectal carcinomas in a southern district (Kagoshima) in Japan, we investigated immunohistochemically polypectomized materials of adenomas and incipient carcinomas with adenoma components (CaA) of the large bowel, comparing the recent (after 1995) and old (before 1985) cases, by using such antibodies as Ki-67 (MIB-1), P53 (NCL-P53-1801), MRP against MUC2 mucin as a marker of cytoplasmic differentiation, and α-smooth muscle actin (SMA) for pericryptal fibroblast sheath (PCFS). The materials are composed of group A (88 cases polypectomized between 1969 and 1984), group B (34 cases between 1988 and 1994) and group C (85 cases polypectomized after 1995). The histological atypia in the adenoma and adenoma component in CaA were graded in mild, moderate and severe. The MIB-1 positive cells were counted in three portions of a neoplasia, and the labeling index of a neoplasia was decided by the average positive percent in the three portions. We counted the population of α-SMA positive glands in every neoplastic lesions. We defined such gland as α-SMA positive gland in which over half of the periglandular area was encircled by the α-SMA positive PCFS, irrespective of continuously or discontinuously. In adenomas of the group C (recent cases), the degree of histological atypia was more advanced than those in group A ($P<0.001$). The population of MIB-1 positive cells in adenoma were A < B < C (A & B vs C: $P<0.001$). The expression rate of P53 protein in adenoma cells and in carcinoma cells of CaA was A < B & C ($P<0.05$). The expression rate of MUC2 mucin in the neoplastic cells was A > B > C (A vs B: $P<0.01$, A & B vs C: $P<0.001$). The prevalence of the α-SMA positive glands in the adenoma area of CaA was A > C ($P < 0.001$).

These results indicated that the recent adenomas and CaAs show more rapid growth and more aggressive biological behavior than the previous colorectal neoplasia, which may be reflected not only to the nature of recent non-hereditary advanced colorectal carcinomas but also to the strongly increasing incidence of colorectal carcinomas in Japan.

The Expression of Rb. p16 in Large Intestine Carcinoma and its significance

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For studying the expression of Rb. p16 in large intestinal carcinoma and its significance, we examined their proteins by immunohistochemistry in 40 primary large intestinal carcinoma. The results showed that the expression of Rb was revealed in 29/40 samples (72.5%) and of 16 was in 15/40 samples (37.5%). The expression of p16 in large intestinal carcinoma with lymph node metastasis was very low, only 2/15. Interestingly, 23/24 Rb positive carcinomas had no or low p16, while 9 cases of Rb negative or light positive carcinoma showed high levels of p16. These results suggest that the abnormal expression of Rb. P16 was followed during happening of large intestinal carcinoma, undetect of p16 expression was related to metastasis of lymph node in large intestinal carcinoma, while there was a reciprocity between Rb inactivation and p16 expression.

Key Word: large intestinal carcinoma, Rb, p16

DETECTION AND ANALYSIS OF HSP70 PROTEIN EXPRESSED ON THE SURFACE OF MOUSE HEPATOCELLULAR CARCINOMA CELLS

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Heat shock proteins(HSPs), expressed by cells undergoing stress that activates the heat shock genes, are commonly exist in organic cells. Recent research has found the followings: 1. HSPs expression is much higher in tumor cells than in normal cells and inducible; 2. HSPs expression may be a protective response of tumor cells against chemotherapy, hyperthermia and radiotherapy, it has something to do with tumor host's prognosis. 3. Tumor HSPs chaperoning antigenic peptides, called HSP－Peptide Complexes, can elicit a strong antitumor immunity within tumor host's body. Hence, it is practically significant to research and detect the HSPs of tumor cells.

We carried out our research with hepatocellular carcinoma cell line Hcaf, chemically induced in 615 strain mice. The cells were cultured under 37℃、5% CO_2 in RPMI－1640 containing 10% FCS, part of them were stressed under 43℃ in water bath for 2 hours, after 8－hours recovery under 37℃, both the stressed and unstressed cells were detected by Flow Cytometric Analysis using FAC－SCAN(Bection Dickinson USA). The tumor cells were stained with anti－HSP70 mAb(Sigma Co. USA), labled with FITC－conjugated fragment of goat antimouse IgG. 62.2% of the unstressed cells expressed HSP70 on their surface and 75.5% of the stressed cells, significant difference was found($p<0.01$). The cells were homogenized and centrifuged at 100,000－g, the supernatant was applied to a Con A－Sepharose column, the unbound substance was collected and resolved on a FPLC system(Mono Q, Pharmacia, Sweden). Proteins of each peak were collected, after SDS－PAGE and silver staining, 70kD protein(by Bio－Rad protein standar) was found to be eluted at 17－270 mM NaCL gradient. After SDS－PAGE, the gel was electroblotted onto a nitrocelluse, the protein was probed with anti－HSP70 mAb as same as above and goat anti－mouse IgG alkaline－phosphatase－labled mAb. After being revealed, it was confirmed to be HSP70.

HSPs, called molecular chaperon, participate in folding, assembling and delivering proteins. Many researchers have found that tumor cells can express much more HSPs than normal tissue cells under physical condition, and these tumor cells have strong ability to survive from stresses. It indicates a poor prognosis of the tumor hosts. Hcaf is a cell line of higher metastasis, its biological behavior may be related to its higher expression of HSP70. Some researchers found that HSP－expressing tumor cells can be recognized and killed by effector T cells, we found that the number of HSP－expressing cells, the target cells to the effector cells, increased after stress, it may be conducive to immune therapy. Additionally, our study revealed that after the Con A－Sepharose and FPLC system affinity chromatography the HSP70 protein was pure enough for further research and utilization.

Expression of p53 Tumor Suppressor Gene and Proliferating Cell Nuclear Antigen (PCNA) in Adrenal Cortical Tumors and its Relationship to Biological Characters

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[Introduction] Tumors of the adrenal cortex express various endocrinological functions usually are known as primary aldosteronism, Cushing syndrome and rarely Li-Fraumeni syndrome which is autosomal dominant disease with multiple cancers in young adults and cancer-prone family. To detect p53 tumor suppressor gene in tumor tissue is clinico-pathologically important for early detection of cancers. The differential diagnosis of benignancy or malignancy is difficult based only on the routine microscopical studies, so that occasionaly a malignancy is based on the presence of metastasis. In this report, I examined a germline p53 mutations and PCNA using immunohistochemical and PCR-SSCP whether the expression is useful to differentiate benignancy and malignancy of an adrenal cortical tumor.

[Materials and Methods] Six cases of adrenal cortical tumors including 4 adenomas and 2 carcinomas were examined. Immunohistochemical stains for p53 and PCNA were performed by monoclonal antibodies. In addition, a germline p53 mutation was examined by PCR-SSCP method in six cases.

[Results] Every four cases of adenomas were negative for PCNA, but two cases of cancer were positive for PCNA by immunohistochemical stains.

Thus, immunohistochemical detection of PCNA was useful to determine benignancy and malignancy of the adrenal cortical tumors. All the six cases of adrenal cortical tumors (adenomas and cancer) were negative for p53 by immunohistochemical stains. However one of two case of cortical cancer showed germline p53 mutation in exon 6 by PCR-SSCP and one adenoma with aldosteronism expressed also p53 in exon 5. This findings suggest that a transition from cortical adenoma to carcinoma may exsist in some adenomas or that malignant potential is present in histologically benign adenoma.

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Poster 14

Construction of hIL2- perforin Recombinant Immunotoxin and its Bioactivity

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Background: Organ transplantation is a powerful approach to the treatment of various organ end-stage diseases. One of the problems associated with such a treatment is graft rejection. Since activated T cells are the major effectors mediating immunorejection. the strategy which selectively destroys activated, but not resting, T cells would be rational. It has been well known that activated T cells express high- affinity IL2 receptor on their surface, therefore it is a preferential targets for the activated T cell-oriented cytotoxic reagents. Although various strategies for blocking graft rejection have been developed by using IL2- receptor as target, bacterial- derived toxins (such as PE. DT) as effector molecules and mouse-derived mAbs as carrier, which are frequently used at present time, showed the numerous drawbacks in clinical use due to their unmunogenicity.

Purpose: The ideal immunotoxin would be a fully humanized, poorly immunogenic with a high -affinity for IL2 receptor and good cytotoxic potential toxins for activated T cells. We designed a humanized immunotoxin with human IL2 as carrier and perforin as toxin and the Bioactivity was assayed in vitro, Methods: By using genetic engineering technique, a hybrid cDNA coding for a fusion protein. between human interleukin 2 and a truncated human perforin was constructed and expressed in E-coli, the protein encoded by this cDNA containsed the entive interleukin2 sequence, fused at its C terminal to the bioactive part of human perforin in the N-terminal. Cytotoxicity of the immunotoxin was assayed by using CTLL2 cell line and IL2 activated T cells as targets, allogenic lymphocyte reaction was performed in the presence of IL2-perforin immunotoxin, more over, CTLL2 target cells incubated with IL2-perforin were neqatively stained and observed under electronmicroscope to display the morphological feature.

Results: Cytotoxic assay showed that the IL2-perforin could kill the CTLL2 cell line and IL2 acticvated T cells efficiently (specific lysis: 56%, 26%, respectively), and inhibit the allogenic mixed lymphocyte reaction significantly (30%). morphological change in the cell membrane of target cells killed by IL2-perforin immunotoxin was similar to that induced by natural human perforin, namly, forming characteristic, regularly distributed holes with diameter of 20nm on the membrane of killed cells

Conclasion: The results imply that the novel humanifed immunotoxin-IL2-perforin may be a promising agent for specific immunosuppression in humans.

POLYMERASE CHAIN REACTION ANALYSIS AND DNA SEQUENCING OF IMMUNOGLOBULIN HEAVY CHAIN GENE IN GASTRIC B-CELL MALIGNANT LYMPHOMAS IN CHINA AND IN JAPAN

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In order to see difference in molecular pathological features between Japanese and chinese gastric B-cell lymphomas (gBML), CDR3 region of immunoglobulin heavy chain gene (IgH) in 9 Chinese and 6 Japanese gBML cells and 2 Japanese extranodal B-cell lymphomas (eBML) was examined by means of polymerase chain reaction (PCR) and DNA sequencing of the PCR products. DNA was extracted from microdissected hematoxylin-stained gBML sections. Employing the DNA as templete DNA, PCR of a set of primers Fr3A and LJH was performed. Only in one high-grade MALT type case of Chinese gBMLs about 100bp long DNA was amplified. In Japanese gBML DNA amplification was seen in 5 cases except a case of early low-grade MALT type, revealing a band at about 100 bp length in 3 cases of high-grade MALT type and one case of eBML 3 cases and a brode band in 2 cases of low grade MALT type. In Japanese eBMLs immunocytoma showed two bands of amplified DNA and plasmacytoma did not. DNA sequencing of the amplified DNA revealing a band at about 100 bp length in one Chinese and three Japnese cases of gBML could be achieved only in two cases of Japanse gBML by dye-termination method. The DNA sequences indicated high homology of substances, against which antibody is ususally not produced in vivo. Consequently, in Japanese gBML, lymphoma cells of low-grade MALT type were thought to be neoplastic cells under the on-going somatic mutation in CDR3 region of IgH. And more Chinese gBMLs are thought to be under the on-going somatic mutation than Japanese gBMLs. The homology analysis of the DNA sequences read from the PCR products in two cases of Japanese gBMLs suggested that gBML occures in a microenvironment apart from the ordinary host immunity.

Poster 16

STUDY OF THE MECHANISM OF FOAM CELL FORMATION BY NORTHERN BLOT ANALYSIS

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Foam cell formation is the base of atherosclerotic plaque formation. So, the mechanism of foam cell formation is the key of pathogenesis of atherosclerosis. Now, it is one of interesting areas to study how the monocytes in blood and smooth muscle cells (SMCs) in the media of artery migrate to the subendothelial space, uptake lipids and become foam cells. Northern blot analysis is an important method in the molecular biology. In order to clarify the mechanism of foam cell formation in atherosclerosis, we used Northern blot analysis to investigate the expression of monocyte chemotactic protein-1 (MCP-1) mRNA in cultured vascular endothelial cells (ECs) and the influence of VLDL and oxidized VLDL (OX-VLDL) on expression of MCP-1 in ECs, to measure the levels of VLDL receptor mRNA expression in normal rabbit aortic wall and cultured SMCs.

Methods: Human umbilical vein endothelial cells and rabbit aortic SMCs were cultured by collagenase digestion method. Confluent ECs were stimulated by VLDL or OX-VLDL. Total RNA from cultured cells and rabbit aortic wall was extracted by the acid guanidinium thiocyanate-phenol-chloroform method. RNA samples from the cultured cells and rabbit aortic wall were electrophoresed in 1.2 % agarose gels, transferred to a nylon membrane. Hybridizations were performed over night in the hybridization buffer, containing 10^6 cpm / ml of the ^{32}P labeled cDNA probes for human MCP-1 or for rabbit VLDL receptor. After hybridization the membrane was washed in the wash buffer at 57°C for 20 min three times. Autoradiography was performed with X-ray films at -70°C. The developed films were scanned by a densitometer.

Results: The level of MCP-1 mRNA expression in ECs stimulated by VLDL and OX-VLDL was increased. The effect of OX-VLDL was stronger than that of VLDL. VLDL receptor mRNA in rabbit aortic wall couldn't be detected by Northern blot analysis. But VLDL receptor mRNA in cultrued SMCs could be found. Using Northern blot analysis, we obtained satisfactory results. Quantitative analysis of specific mRNA expression is easy and accurate. The key to the achievment of Northern blot analysis is to prevent the degradation of RNA samples, and to transfer RNA from agarose gel to nylon membrane effectively.

Conclusion: (1) VLDL and OX-VLDL can stimulate vascular ECs to express MCP-1 mRNA. It suggests that VLDL and OX-VLDL play a role in monocytes migration. (2) Normal rabbit aortic wall can not express VLDL receptor mRNA, but cultured SMCs can. It suggests that SMCs may uptake lipoproteins through VLDL receptor and become foam cells. (3) Northern blot analysis is one of useful and advanced molecular biologic methods in studying of pathogenesis of atherosclerosis.

THE SIMULTANCE OBSERVATION OF APOPTOTIC NUCLEI BY TUNEL METHOD AND METHYL GREEN NUCLEI COUNTERSTING IN THE ABSORPTIVE EPITHELIAL CELLS OF RAT SMALL INTESTINE WITH CLSM.

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The usefulness and valuability of the methyl green (MG) nuclear counterstaining in histochemical staining have widely been appreciated. While we were observing MG counterstained sections by CLSM, it was incidentally found that a fluorescence ray was emitted through MG and was clearly detected by the CLSM. It was generally accepted fact that MG reacts specifically to the double strand (dbs)-DNA and this was experimentally proved in our laboratory (Umemura, S.). In the present report, MG stained nuclei of (I), the absorptive cells (terminal differentiating cells) of rat intestinal villi and (II) cells in rat intestinal crypts undergoing apoptosis, which was induced by Adriamycin (ADM) administration, were observed by a CLSM, and the changes of the intensity and distribution pattern of MG staining were precisely examined. The apoptotic cells of (I) also appreciated as slowly apoptotic cells. The apoptosis is thought to occur during the process of the terminal differentiation, which takes about 48 hours. To examine those apoptotic changes, TUNEL method, in which 3'ends of fragmented dbs-DNAs are end-labeled with histochemically detectable dUTP, was employed.

[Results and Discussion] In the observation of (I) cells, the most intense and diffuse MG staining was observed in the crypt regions (base of the villi). White those cells are moving up to the mid portions and tips of the villi in the process of differentiation, MG reacted substances are translocated to the peripheries of nuclei (heterochromatin regions) and the MG fluorescency in the heterochromatin regions was gradually weakened. On the contrary, TUNEL reaction products, which are solely localized in the heterochromatin regions, increased their amounts during the process. The heterochromatin regions to the site of the dbs-DNA degradation. In experiment (II), ADM damaged cell in the intestinal crypts showed typical "apoptotic bodies" which exhibited the intense TUNEL staining. In those cells, MG reacting substances are irregularly fragmented and diminished, and localized along the margins of TUNEL positive lesions. MG staining appears to reflect the amount of intact dbs-DNA rather accurately in those damaging cells.

Immunohistochemical Reaction of Anti-Fibroblast Monoclonal Antibody and Anti-Mononuclear Monoclonal Antibody in Experimental Thickening Carotic Artery Intima

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Thickening model of local intima of carotic artery of rabbits was made by feeding hyperlipid food after the injury to intima by injection of distilled water and air. Immunohistochemical stain of the thickening artery wall was performed with anti-fibroblast and anti-mononuclear monoclonal antibodies (MoAb). The results showed that the positive reaction of antifibroblast MoAb was observed in intima and media but the positive reaction of antimononuclear MoAb was only in intima. It was indicated that some of artery smooth muscle cells might have potential characteristics of fibroblast cells. This kind of smooth muscle cells might transform phenotypically and immigrate into intima and proliferate there when stimulated.

Key Words: arteries, intima, fibroblasts, macrophages, antibodies

The Study on c-myc Antisense RNA Inhibiting the Proliferation of Vascular Smooth Muscle Cells

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Objective: The proliferation and migration of vascular smooth muscle cells (VSMC) are the pathological basis of atherosclerotic formation and vascular restenosis after percutaneous transluminal coronary angioplasty (PTCA). The purpose of this research is to injure tbe vascular intima, mimicking the formation process of atherosclerosis and PTCA and to observe the effect of c-myc antisense RNA on preventing atherosclerotic formation and vascular restenosis.

Methods: The femur artery intima of 36 male Japanese white rabbits was injured by balloon sac and at tbe same time, c-myc antisense RNA, sense RNA(1mg per rabbit) and saline were injected into the blood. The rabbits were fed with hypercholesterol food and killed in 12 weeks. The femur artery was cut into 4 μm thick paraffin sections and stained for PCNA, actin and CD34 by s-p immunohistochemistry. The thickness of the intima was measured and the percentage of PCNA positive cells was counted by image analysis (LUZX).

Results: In tbe control group of sense RNA and saline, the intima became thick heavily, the formation of atherosclerotic plaque was obvious and the number of PCNA positive cells in the intima and the media significantly increased. The proliferating cells in the intima were actin positive and tbe endothelial cells were CD34 positive. But in the antisense RNA group. tbe thickness of tbe intima is only half of that of control group(219 μm) and the number of PCNA positive cells (30.5%) were significantly lower than that of the control group (63%).

Conclusion: c-myc antisense RNA could significantly inhibit the proliferation of VSMC and attenuated the development of atherosclerotic plaque and could be used to prevent the formation of atherosclerosis and vascular restenosis.

ULTRASTRUCTURE OF MYOCYTE IN TETRALOGY OF FALLOT AND THE OBSERVATION OF ITS Ca-ATPase ACTIVITY

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Introduction: Little was known on the ultrastructure of myocyte in tetralogy of Fallot and the cytochemical change of its Ca-ATPase. In this studies, samples were retrieved from 30 patients (2-30 years old) suffering from this disease and observed under transmission electron microscope (TEM), meanwhile, the activity of Ca-ATPase in myocyte was studied by enzyme chemical method.

Materials and Methods: Cardiac muscular tissues of right infundibulum were removed during the operation on the patients suffering from tetralogy of Fallot, cut into small blocks (1x 1 mm^3), and then treated with following two methods, respectively. 1) Transmission electron microscopic method; Tissue blocks were immersed in 2.5% Glutaraldehyde fixative for I hr., TEM samples were prepared as usual, and ultrathin sections were observed under JEM 1200 EX TEM. 2) Enzyme cytochemical method; Tissue blocks were fixed with 2% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M cacodylate for I hr., frozen sections in 10μm thickness were prepared for light microscope (LM), non-frozen in 40μm thickness for electron microscope (EM). The sections were then incubated in Ando's Ca-ATPase medium for 60min at 37°C , following that, the sections for LM was washed thoroughly and observed under LM, and those for EM were post-fixed in 1% OsO_4, and dehydrated in a graded series of alcohol, then embedded in Epon 812, ultrathin sections were observed under TEM.

Results: 1) Change of ultrastructure of myocyte; Hypertrophy, atrophy and degeneration could be observed in the myocytes. Deformed sarcolemma appeared as villi-like protrusion, bundles of myofibrils arranged in disorder, or got dissolved. Transverse tubule and sarcomere varied in size, correspondingly, the malposition of I band and M line occurred, and I band arranged closely. The denatured intercalated disk twisted extremely, some of them aggregated densely, some dissolved and broke into fragments. Many denatured mitochondria which distributed among broken myofibrils or cytoplasm dissolution area, appeared as vacuoles or semilune, and different shape of breaches were seen on it sometimes; the cristae of mitochondria were found to break up. Nuclei became deformed, nuclear membrane depressed deeply to form incisurae, nuclear protrusion and nuclear baggage were evident. Heterochromatin were seen to pile up under nuclear membrane. A number of lipofuscin granules dispersed around the perikaryon. Plenty of lysosomes distributed among bundles of myofibrils, proliferation and degeneration of the wall of capillary could be seen, small processes stretched out of the inner surface of endothelium, collagenous fiber in mesenchyme proliferated obviously and disturbed in arrangement. 2) Change of enzyme cytochemistry; Reaction products of Ca-ATPase were observed as brown precipitation of lead sulfate, which distributed on the sarcolemma, in a part of cytoplasm and on the wall of capillary. The activity of Ca-ATPase on mitochondria and sarcolemma was much lower than that in normal myocytes, however, increased evidently in the sarcoplasm.

Discussion: Anoxia of myocyte induced by tetralogy of Fallot resulted in the obstruction of oxdative phosphorylation, which lead to denaturation of mitochondria, and the content and activity of Ca-ATPase decreased, excessive contraction of myocytes made I band dense. Affected by mechanic load of blood dynamics, myofibrils were disturbed in homolateral arrangement. The decrease of ATP produced by denatured mitochondria resulted in abnormality of Ca pulp, and the overload of Ca in sarcolemma finally caused the series changes of ultrastructure.

Enzyme cytochemical study on nematolysosome (NLY) in cell of Betz in cerebrum under high-voltage electron microscope

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We once reported the presence of nematolysosome in the neurons of central nervous system in rat and guinea pig. This study investigated nematolysosome in cell of Betz in cerebrum by means of ACPase enzyme cytochemistry and high-voltage electron microscope. Materials and methods: Wistar rats were used as experimental animals under anesthesia with ether, perfusion fixative through heart were carried out with 2% formaldehyde and 0.25% glutaraldehyde in 30mM PIPES buffers for 15 min, the cerebrum were removed immediately and cut into small block, which were immersed with same fixative for 60min at 40°C, and then washed through with the same buffers, sections in 50 μm thickness were prepared by microslicer, then were incubated with Gomori's ACPase medium for 80 min at room temperature. For control tests, some sections were incubated in the medium without substrate, after cytochemical reaction, the sections were rinsed with the same buffer, the samples for the observation of transmission electron microscope were prepared as routine. Some specimens were cut into 2-3 μm thick sections, recovered on Formvar-coated double grids, observed under a high-voltage electron microscope (Hitachi H-1250M) operating at 1000KV. Result: The reaction product of ACPase activity was found as black electron dense precipitation on the spherical lysosomes and nematolysosomes, Nematolysosomes were distributed near the nuclei of the cell of Betz. ACPase activity was also seen on flat sac of Golgi complexes, it sometimes becomes difficult to distinguish nematolysosome from flat sac of Golgi complexes with ACPase activity. However, the ACPase-positive cisterae of Golgi apparatus with the shape of paralleled lamella were always found near the other ACPase negative cisternae, and they are often a little thinner than nematolysosome, nematolysosomes tend to extend along thread structures, sometimes, they were cut off and presented discrete segregations, which look like bamboo. Discussion: Up to now, it was little known about nematolysosomes, we used many methods in order to demonstrate the morphology of nematolysosomes. For example: PIPES buffers substituted for cacodylate buffers; and applying a fixative of lower concentrated glutaraldehyde is helpful for the detection of ACPase activity; DMSO addition to the fixative could help to overcome the latency of ACPase activity; the increase of section thickness also decrease the difficulty caused by ultrathin section for the observation of nematolysosome, 2-3 μm thickness of section under high-voltage electron microscope will be advantageous to describe wholly the morphology of nematolysosomes, we once reported the distribution of nematolysosome in Purkinje cells of cerebellum, the results were similar to this study. The function of nematolysosome may be related to the transport of materials in neuron. From this findings, the nematolysosome was formed from the Golgi complexes the same as spherical lysosomes. Whether the function is related to the transport of neuron transmitter or not, it needs to study further.

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