日本財団補助金による
1997年度日中医学協力事業助成報告書

－在留中国人研究者研究助成－

1998 年 3 月 12 日

財団法人　日中医学協会
理事長　中島章殿

Ⅰ. 研究者氏名　王　玉来

研究機関 信州大学医学部皮膚科学教室 研究指導者 斎田俊明 職名 教授

所在地〒390-0802 長野県松本市旭3－1－1 電話 0263-(35)-4600 内線 5274

Ⅱ. 過去の研究歴

１９９３年1月２８日より3月３１日まで、信州大学大学院医学研究科研究生（皮膚科学講座）として研修した。

そして、１９９３年4月1日より１９９７年3月３１日まで、信州大学大学院医学研究科入学、斎田俊明教授の指導を受けた。

Ⅲ. 過去の研究実績

Y.-L. Wang, H. Uhara, Y. Yamazaki, T. Saida. Immunohistochemical detection of CDK4 and $p14^{INK4}$ proteins in cutaneous malignant melanoma. British Journal of Dermatology 134(2):269-275,1996.

Ⅳ. 本年度の研究業績

(1) 学会、研究会等においての口頭発表（学会名・内容）

土肥庄二郎, 王　玉来, 木庭幸子, 斎田俊明：ワークショップ, 移植ヒト皮膚を用いる in vivo 発癌実験

第６１回日本皮膚科学会東部支部総会・学術大会, 松本, １９９７年１０月４, ５日.

H. Uhara, Y.-L. Wang, H. kubo, T. Saida: Invasive and *in situ* squumous sell carcinomas probably caused by chronic heat exposure, 19th World Congress of Dermatology, Sydney, Australia, June 15-20,1997.

(2) 学会誌等に発表した論文　無・有　（雑誌名・論文名）

福沢正男, 小岩原冬子, 王　玉来, 山崎自子, 河内繁雄, 斎田俊明：治療への反応性が悪かった若年男性の SLE, 皮膚科の臨床, ５２（１）４２～４５, １９９８.

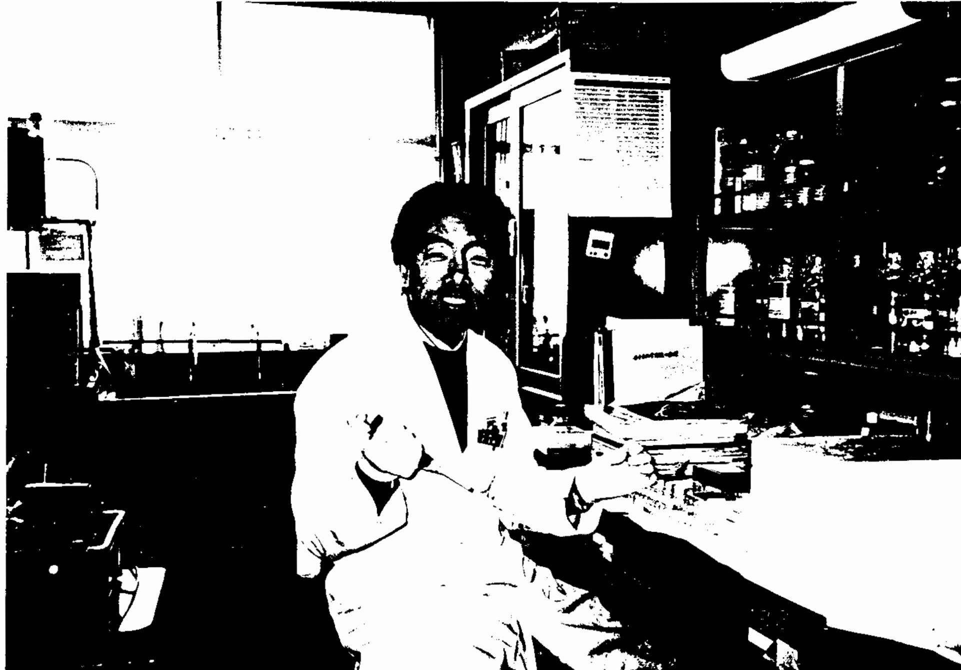
小岩原冬子, 王　玉来, 福沢正男, 山崎自子, 河内繁雄, 斎田俊明：B cell lymphoma：多発性の皮下結節としてみられ、血管壁に浸潤を示した１例, 皮膚のリンフォーマ, 印刷中, １９９８年.

Ⅴ. 今後の研究計画および希望

今後は前癌病変を含めて、様々な良性と悪性皮膚腫瘍について同じ方法を用い検討し、アンドロゲン受容体遺伝子のクローナリテイといった１側面から、即ち腫瘍の単一性という面から再評価することを目的として調べていきたい。

Ⅵ. 研　　究　　報　　告（日本語、又は英語で書いてください。 4,000字以上で記載してください。別紙可）

別 紙



Ⅶ. 指導教官の意見

王　玉来君は貴協会からの在留中国人研究者研究助成を受け、「X染色体不活性化の検出による皮膚前癌病変におけるモノクローナリテイに関する研究」というテーマで研究し、その結果を研究報告（別紙）としてまとめることができました。これから、投稿する予定です。本研究におけるモノクローナリテイの検出法は、開発されてからまだ3年と日も浅く、皮膚腫瘍についてはメラノーマ転移巣1例について行われている以外報告はありません。今回の研究は皮膚前癌病変について一部の結果を得ることができましたが、今後は前癌病変を含め症例数を増やして、様々な良性と悪性皮膚腫瘍について同じ方法を用い検討し、アンドロゲン受容体遺伝子のクローナリテイといった1側面からの見方ではあるとしても有益な情報となりうると思われます。

最後に、日中医学協会からの研究助成に厚くお礼申し上げます。

信州大学医学部皮膚科学教室　教授　斎田俊明

Clonal analysis by the study of X chromosome inactvation in melanoma and preneoplastic tissues of the skin

Abstrct

Recently,the gene for the androgen receptor has been shown to be a highly polymorphic locus in which methylation of DNA correlates with inactivation of one or the other X homologue. Analysis of clonality by X chromosome inactivation has proved useful in the study of neoplastic and preneoplastic tissue. we performed clonal analysis on malignant melanomas and preneoplastic lesions of females as well as keratoacanthoma, epithelioma, lymphomatoid papullosis and dysplastic nevus. PCR amplification was performed to generate fragments surrounding the highly polymorphic CAG trimetric repeat in exon I of the androgen receptor gene, using DNA extracted from frozen tissues or paraffin-embedded archival sections as templates. Of a total of 40 tumors analyzed, 34 cases showed heterozygosity(85%) and were therefore informative for clonal analysis. Monoclonal composition of the tumors was suggested in a total of 26 of 34 cases, including 13 melanomas, 7 keratoacanthomas,2 epithelioma, 4 lymphomatoid papullosis and none dysplastic nevus. However, polyclonal composition was observed in two melanomas, in other containing mutation in p16 gene. Our studies demostrated the utility of PCR amplification of highly polymorphic repetitive sequences for analysis of patterns of X-chromosome inactivation. This approch is practial for the analysis of clonal cell composition in a high proportion of bothformalin-fixed and frozen archval tissues.

Introduction

Analysis of clonality by X chromosome inactivation has proved useful in the study of neoplastic and preneoplastic tissue(1,2) as well as in the study of female heterozygotes of X-linked diseases, especially the immunodeficiency syndromes(3). Although analyses ofchromosome deletions, gene rearrangements, or patterns of oncogene activation have been widelyused, the most consistently hibrmative marker ofclonal composition of tissues is the cellular pattern of x-chromosome inactivation.

During the process of embryogenesis in the female, either the maternally derived or paternally derived X-chromosome in each cell is randomly and permanently inactivated(4,5). The choice once made is stable through subsequent cell cycles(6). Thus,normal tissues in females are composed of cellular mosaics differing in which of the two X chromosomes has been inactivated. By contrast, in neoplasms of females, there is a uniformity in the pattern of X-chromosome inactivation which indicates clonality of the cel1ular composition. (1)

Application of polymerase chain reaction(PCR) for analysis of clonality was first targeted to protions of the PGK-1 gene(7). The increased sensitivity of the PCR-based approach made it theoretically feasible for the first time to use DNAs derived from archival tissue collections. RFLP analysis of PCR products has been used for the analysis of clonality in solid tumors including breast lesions (8). This approach, however,is also severely limited by the relatively low level of heterozygosity in the amplification target, permiting anadysis of clonality in only ~30% of cases.

Recently, a polymorphic short tandem repeat(STR) has been identified in the X-linked human androgen receptor gene (HUMARA) (9). This sequence, consisting of the trinucleotide [CAG]n, shows heterozygosity in ~90% of the general population(10). Using DNA from hamster/human hybrid cell lines,as well as the DNA from human tissues, these workers were able to show that methylation of Hha I sites within the HUMARA ampnfication target directly correlates with X-chromosome inactivetion. Thus, based on the high levelof heterozygosity and the presence of adjacent methylation sensitive restriction endonuclease sites, the HUMARA STR is an appropriate target for the determination of X-chromosome inactivation.

In the current study, we use PCR amplification of the HUMARA STR for analysis of patterns of X-chromosome inactivation in malignant melanomas and preneoplastic lesions of females as well as keratoacanthoma, epithelioma, lymphomatoid papullosis and dysplastic nevus. The method is informative in a high proportion of cases, permitting determination of clonality in both fresh and archival tissue specimens.

Materials and methods

Tissues

A total of 40 tumors, including 15 malinant melanomas, 9 keratoacanthomas, 4 epithelioma, 7

lymphomatoid papullosis and 5 dysplastic nevus,were obtained from the Department of Dermatology at the Shinshu University Hospital in Japan. No initial chemotherapy was performed bebre tumor excision. Surgically removed tissues were sampled for histopathological diagnosis, and the remaining tissue was frozen or paraffin embedded (10% formalin fixed) for extraction of DNA. DNA was extracted from frozen samples following the procedures previously described(10). Sections of paraffin-embedded tissues staines with hematoxylin and eosin were examined under the microscope, and normal and neoplastic regions were identified using standard morphological criteria. A single adjacent unstained section 10 um thick was used for DNA extraction. DNA was extrcted from sections following the procedures previously described(11).

PCR

One microgram of DNA extracted from frozen tissue was digested with l0 U of Hha I(Toyobo,Japan) in l0 ul of digestion buffer recommended by the manuhcturer. After incubation at 37C for l2 h, digestion was teruninated by incubating the digestion mixture at 95C l0min. From this mixture, 2 ul of DNA were used as a template for the PCR reaction. DNAs extracted from selected areas of paraffin-embedded archival sections were also digested with 5 U of Hha I in l0 μl of digestion buffer at 37C for l2h. After heat inactvation, the DNAs were reextracted with phenol, precipitated with ethanol, and resuspended with PCR reaction mixture.

Primers sequences were (5' to 3') TCCAGAATCTGTTCCAGAGC and TGGGGAGAACCATCCTCACC. Two μl of each DNA reaction mixture were added to a 50 μl reaction containing 100 ng of each primer, standard PCR reaction buffer(Toyobo,Japan), 200um concentration of each dATP,dTTP,dGTP, and 20um dCTP. The tubes were heated to 80℃, then 0.1 μl [α-^{32}P]dCTP(6000Ci/mmol) and 0.25 unit Taq polymerase were added. Fortycycles of ampliufication were carried out using cycling paramenters of 95℃ for 15s, 64 ℃ for 30s, and 72℃ for 30s. Two to 5 μl of each reaction were added toan equal volume of formamide containing 0.1% bromophenol blue and 0.1% xylene cyanol, loaded onto a 7.2 M urea- 6.5% polyacrylamide gel, and electrophoresed at 60W for 3.5 h. Gels were dried and autoradiographed for 12-48 h at room temperature using Fuji X-ray film. PCR amplification was also performed from DNA extracted from white blood cells or from histologically normal tissue from each case.

Results

PCR amplification was performed to generate fragments surrounding the highly polymorphic CAG trimeric repeat in exon l of the androgen receptor gene, using DNA extracted from frozen tissues or parafnn embedded archival sections as templates. PCR amplmcation was successful from all the samples used. There was basically no difference in terms of the success rate between frozen matehal and parafnn embedded matehal. PCR amplincation from undigested DNA revealed that 34 of 40 cases(85%) were heterozygous in this locus and were, therefore, informative for analysis of clonality(Table l). In 34 cases that showed heterozygosity, two bands were identified conresponding to the amplification products of each X-chromosome. For each case, the intensity of the signals for both alleles was almost identically to each other and there was no difference between the PCR product from histologically normal tissue or from WBCs and that from the tumor(Fig 1).

Genomic DNAs extracted from frozen samples or from paraffin-embedded archival sections were next digested with methylation -sensitive restriction enzyme Hha I, before PCR amplification. Predigestion of DNA wth Hha I permitted PCR amplmcation only from the methylated(inactivated) allele. Hha I pretreatment of genomic DNA from normal tissue or WBCs, ffolowed by PCR amplincation of AR, yielded two product bands, and the intensity of the two bands was almost identical(Fig 1). By contrast, PCR amplmcation from Hha I degested tumor DNA derived from 26 of 34 heterozygous cases after digestion with Hha I generated either a singde product band or showed significant reduction in the intensity of one of the two bands. There was, agian, no difference in terms of the success rate of PCR amplfcation between frozen material and parafin-embedded material. These observations indicated that these 26 tumors are of clonal composition. By contrast, the intensity of the two

product bands were almost identical in two melanoma, in other we previously showed a CC--TT mutation in exon 2(codons 57/58) of p16.

Discussion

The present study demonstrates the technical feasibility of clonality analysis by PCR amplincation of short tandem repeats. The use of PCR based methods permitted the use of both frozen and formalin-fixed tissues, making the approach practical for application in both recent and archival tissue collections. Previous PCR-based methods have been directed to aportion of the PGK-l gene, showing heterozygousity in only 30% of cases(12,13), precluding the widespread application of the method. However, analysis of repetitive sequence polymorphisms, as described in the current articles, showed heterozygosity in>90% of cases. Thus, PCR amplification of the AR target is a practical appproch for the analysis of clonality in a high proportion of cases from women.

Of the 13 melanomas, 7 keratoacanthomas,2 epithelioma, 4 lymphomatoid papullosis in which monoclonal expansion was suggested by the present study. But none of the dysplastic nevus was suggested.Unique characteristics of nevus cells,such as growing, intradermal dropping,might represent the retention of capability in oncogenic evolution, and should not be regrarded as a manifestation of a neoplastic nature.

Polyclonal composition was observed in two melanoma, which evaluated in the present study were also analyzed for the presence of p16 mutations. The other case, exhibied a CC--TT tandem base mutation in p16 (S. Dohi et al, unpubulished data). Inactiation of the p16 gene can occur by mutation at the majoority of codons in the highly conserved regions of the gene. If the tumor consists of cells derived from more than one progenitor cell and if alteration of these gene is essential for the tumorigenesis, each clone should have mutations in the p16 gene and/or other genes independently.

Insummry, PCR amplification of a highly polymorphic repective sequence of the X-linked human androgen receptor gene can be used for the investigation of clonal composion in fresh and archival formalin-fixed tissues. Common neoplastic and preneoplastic tissues of the female skin, including malignant melanoma, keratoacanthoma, epithelioma and lymphomatoid papullosis were shown to have a uniform pattern of X-chromosome inactivation, consistent with their full malignant potential.

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Table 1. Analysis of clonality by polymerase chain reaction amplification of short tandem repeat

Histologic diagnosis	total cases	Heterozygous	Monoclonal
malinant melanoma	15	15	13
Keratoacanthomas	9	7	7
Epithelioma	4	2	2
Lymphomatoid papullosis	7	5	4
Dysplastic nevus	5	5	0
Total	40	34	26

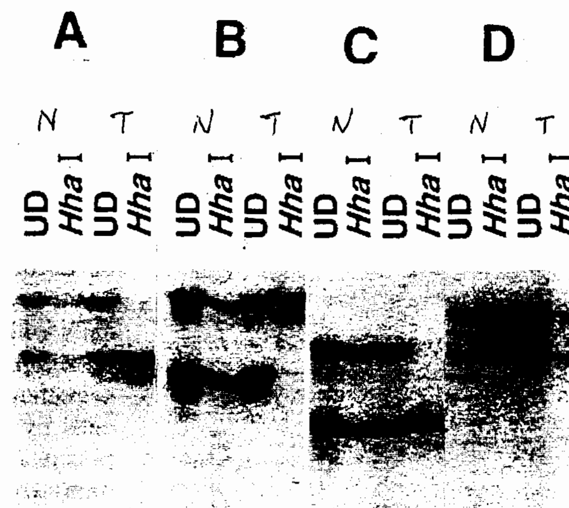

Fig 1. Demostration of clonal composition of a tumor using apolymerase chain reaction(PCR)-based method. Genemic DNA derived from histologically normal tissue or while blood cells(N) or tumor(T) was digested with methylation-sensitive restriction enzyme Hha I, and PCR amplification was performed, targeting a highly polymorphic CAG repeat in exon 1 of the androgen receptor gene, using [32p]end-labeled primer as one of the primers. PCR amplification was also performed on undigested genemic DNA. PCR products were electrophoresed on a 7.2% denaturing polyacrylamide gel. A-C: PCR product from Hha I digested template derived from the normal tissue(N-Hha I),as well as that from undigested template derived from the normal tissue(T-Hha I),yielded two bands, and the intensity of the two bands was almost identical.By contrast,PCR amplification from Hha I digested tumor DNA(T-Hha I) generated either asingle fragment or two fragment with one signal significantly reduced.(A:a case from melanoma; B:a case from keratoacanthoma; C:a case of Lymphomatoid papullosis) By contrast,the intensity of the two bands was almost identical in the PCR product from Hha I difested tumor DNA(T-Hha I) derived from one melanoma(D).

Print Post Approved: 381667/00473

ISSN 0004-8380

Australasian Journal of Dermatology

VOLUME 38 • SUPPLEMENT 2 • JUNE 1997

ABSTRACTS

OF THE

19TH WORLD CONGRESS OF DERMATOLOGY

15-20 JUNE, 1997
SYDNEY AUSTRALIA

0004-8380(199702)38:1;1-G

b
Blackwell Science

A 81-year-old Japanese woman was referred to our clinic for evaluation of the nodule of the face that had developed during preceding several years. Physical examination disclosed 10 × 7 mm, elliptical, dark brown to black nodule on her left nasojugal furrow. Histological examination revealed multiple, dilated, thin-walled blood vessels throughout the dermis. These blood vessels were lined by a single layer of endothelial cells. The lumen contained blood and eosinophilic serous materials. By immunchistochemical staining these endothelial cells were positive for CD34, Facor VIII-related antigen and thrombomodulin, confirming that the nodule is vascular tumor.

This case implies that cutaneous hemangioma is one of the skin lesions to be differentiated in the clinical diagnosis of pigmented BCC of the face.

2374 Classical Kaposi's sarcoma: Low dose interferon alfa treatment

E. Tur, S. Brenner. *Dept of Dermatology, Sourasky Medical Center, Tel Aviv, Israel*

No definitive cure is known for Kaposi's sarcoma. All treatments are palliative and have only temporary efficacy. High doses of interferon alfa are needed for treating AIDS-related Kaposi's sarcoma, whereas low doses are generally ineffective. We have previously shown a beneficial effect of low-dose interferon alfa in the treatment of 4 patients with classical Kaposi's sarcoma. The present report summarizes the results of low-dose interferon alfa treatment of 10 patients with classical Kaposi's sarcoma.

Ten patients, 56–96 y old were treated: 9 men, 1 woman, 3 of whom had an associated malignancy. Subcutaneous injections of interferon alfa, 2–6 million units, were given 3–6 times a week, for 4–12 months.

In 9 out of the 10 patients, initial response was noted after 3–13 weeks of treatment. This was manifested by a reduction of lesion size and fading of color, leading to a partial resolution. Remission was achieved after 4–6 months, for a duration of 8–45 months. Recurrences were retreated, with additional remissions after only 5–8 weeks. Side effects included fever and fatigue, which were overcome by dose reduction.

There are responders and non-responders. Within 3 months of treatment a response should be noted when such a response is about to occur. The extent of the disease is not a predictive factor, as our 3 patients with widespread lesions and an associated malignancy had a favorable response. There were no indications for age, gender, duration of the disease or previous treatments giving a clue. Whereas in AIDS-related Kaposi's sarcoma patients become refractory to IFN, this was not observed in our patients with classical Kaposi's sarcoma. Moreover, unlike AIDS-related Kaposi's sarcoma, continuous treatment was not needed in classical Kaposi's sarcoma.

2375 Prevalence of actinic keratosis in Japan

K. Naruse, M. Ueda, T. Nagano, T. Suzuki, S. Harada, K. Imaizumi, S. Watanabe, M. Ichihashi. *Kobe University School of Medicine, Kobe, Japan*

Most of the epidemiological studies on skin cancer that have been conducted to date have addressed the incidence in light-skinned Caucasians. To determine the prevalence rate of skin cancer and actinic keratosis (AK) on sun-exposed body sites of Japanese in Japan, we examined the skin of 4736 people during health examinations. The study was undertaken in Kasai City, Japan, which had a population of 52837 in 1992, where participants in a regional health examination were seen by dermatologists. The final diagnosis was made histopathologically. Participants were also interviewed by means of a questionnaire. A total of 36 cases of AK and two of basal cell carcinoma were identified, to give a prevalence of 413.4 per 100,000 for AK. The prevalence among outdoor workers was significantly higher than that of indoor workers. Furthermore, when the participants were classified into three Japanese skin types, the prevalence of AK among people of skin type I, who are sensitive to UV irradiation, was significantly higher than that among people of skin type II and III, who are less sensitive.

2376 Invasive and in situ squamous cell carcinomas probably caused by chronic heat exposure

H. Uhara, Y.-L. Wang, H. Kubo, T. Saida. *Shinshu University School of Medicine, Matsumoto City, Nagano, Japan*

We report five cases of squamous cell carcinoma (SCC) and/or SCC in situ developed on the shins that had been chronically exposed to heat of "KOTATSU", a traditional type leg-warmer generally used in Japan. The patients, 4 females and one male, were elderly Japanese, ranged from 75 to 85 years in age. All the patients had multiple brownish hyperkeratotic lesions, 1 to 20 mm in size. Erosive or crusted nodules were also observed in some cases. Reticular erythematous pigmented lesions of erythema ab igne were detected in 2 of the 5 cases. Histopathologically, keratinocytes in the lower epidermis showed various degrees of nuclear atypia, suggesting the diagnosis of thermal keratosis or SCC in situ. Frank dermal invasion by atypical keratinocytes was observed in 4 cases. Elastica-van-Gieson stain revealed thickened collagen bundles and diminished elastic fibers in the upper and middle dermis. By contrast, markedly increased elastic fibers were detected in the lower dermis. These changes were compatible to those recognized in damaged skin by chronic irradiation of infrared rays.

In conclusions, chronic exposure of the shins to heat of the traditional leg-warmer used in Japan should be added to the list of causes of cutaneous SCC.

2377 Simultaneous chemotherapy and radiotherapy for cerebral metastases from malignant melanoma

J. Ulrich[1], C. Rosin[1], G. Gademann[2], K.-H. Kühne[1], H. Gollnick[1].
[1] *Department of Dermatology & Venereology, University of Magdeburg School of Medicine, Magdeburg, Germany,* [2] *Department of Radiotherapy, University of Magdeburg School of Medicine, Magdeburg, Germany*

Rationale: Patients with cerebral metastases from malignant melanoma in the presence of additional extracerebral metastases have a particularly poor prognosis. Hitherto, in cases of primary inoperable disease palliative radiation therapy was usually chosen. Furthermore remissions of cerebral metastases from malignant melanomas under chemotherapy or combined chemo-immunotherapy have rarely been reported. Not until the introduction of the cytostatic fotemustine (being able to cross the blood-brain-barrier) have remissions been demonstrated. In the present paper, we report our clinical results of the combination therapy with fotemustine and irradiation in patients with metastatic melanoma.

Patients and Methods: Since 1993, 9 patients have been treated with the combination therapy. The treatment protocol was as follows: 100 mg/m^2 fotemustine were given as intravenous infusion once a week for 3 weeks. After an interval of 4 weeks without any treatment, the same infusion was given monthly until the disease eventually progressed. Simultaneously, the patients received either focal irradiation (up to 60 Gy) or a total brain irradiation (up to 39 Gy).

Results: In our 9 patients treated to date, we observed 4 CR, 2 PR, 2 NC, and 1 PD with a mean survival of 24.1 weeks (95% CI 20.9; 27.3) with respect to their brain metastases. There were few side effects, including thrombocytopenia and leukopenia, as well as alopecia, with quite acceptable overall tolerance.

Conclusions: Though limited by the small number of patients treated, the present study suggest a therapeutic synergism of the combined chemo-radiotherapy. This treatment may add a well tolerable option to the armamentarium for the palliative, conservative therapy of cerebral metastases from malignant melanoma.

2378 Adjuvant chemotherapy for malignant melanoma with DTIC

J. Ulrich[1], A.-K. Ulrich[1], C. Garbe[2], U. Ellwanger[2], K.-H. Kühne[1], H. Gollnick[1]. [1] *Department of Dermatology & Venereology, University of Magdeburg School of Medicine, Magdeburg, Germany,* [2] *University of Tübingen School of Medicine, Tübingen, Germany*

Rationale: The adjuvant immuno-and chemotherapy for malignant melanoma is still a matter of scientific controversy. Whilst few studies have reported positive effects of such therapy in patients with stage I and II disease, in stages with loco-regional metastases there seems to be an advantage for the patients treated.

Patients and Methods: In this retrospective study, 365 patients with melanoma stages Ia through IIIb received an adjuvant therapy with 5 courses of DTIC (200 mg/m^2) after surgical removal of the primary tumour plus elective, or therapeutic lymph node dissection. The patients' survival rate was analysed according to Kaplan & Meier. In addition, 323 of the patients were compared with untreated controls from the Central Malignant Melanoma Registry of the German Dermatological Society.

Results: The mean follow-up of the patients was 5 years. The median survival (95% Confidence interval) was as follows: 140.8 (131.5–150.1) months for patients with stage Ia and Ib, 148.4 (134.2–162.7) months for patients with stage IIa, 92.0 (49.6–134.4) months for patients with stage IIb disease, 137.0 (0–309.4) months for patients with stage IIIa disease, and 34.0 (0–70.9) months for patients with stage IIIb disease, respectively. Matched pair analysis failed to demonstrate a statistically significant advantage for patients treated with DTIC with respect to both relapse-free interval and overall survival.

Conclusions: The present data suggest that the adjuvant mono-chemotherapy with DTIC does not provide a prognostic advantage for patients with malignant melanoma thus confirming the findings of large randomised trials. In adjuvant situations, therefore, the therapeutic efficacy of interferons as well as cytokines should rather be elucidated.

The 61st Annual Meeting of the East-Japan Dermatological Society
October 4-5, 1997, Matsumoto

第61回日本皮膚科学会東部支部
総会・学術大会

プ ロ グ ラ ム
抄 録 集

会期：平成 9 年10月 4 日(土)・ 5 日(日)

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TEL　0263-34-7100
FAX　0263-34-7101

事務局：
信州大学医学部皮膚科学教室
〒390　長野県松本市旭3-1-1
TEL　0263-37-2647
FAX　0263-37-2646

3．移植ヒト皮膚を用いる *in vivo* 発癌実験

土　肥　庄二郎
（信州大、諏訪中央）
王　　玉　来、木　庭　幸　子、斎　田　俊　明
（信州大）
瀧　澤　好　廣
（長野松代総合）

発癌機構を解明することは、癌の治療や予防に重要な意義を有する。近年、分子生物学的手法が急速に発展し、発癌機構の解明も日進月歩の勢いで進んでいる。しかし、これまでの発癌研究は *in vitro* を対象としたものが多く、*in vivo* の研究はマウスなどの実験動物の組織を用いる研究が主体であった。そのため、実際にヒトの臓器でどのように癌が発生し、進展していくかについて未解明のところが多い。

皮膚は直視下に実験操作を加えることができ、観察も容易であることから発癌研究において恰好の研究対象となり得る。これまでヒト皮膚を実験動物に移植した実験モデルは、ヌードマウスにおいてもヒト皮膚を長期間生着させておくことが不可能であったために、開発が遅れていた。しかし、SCID マウスの発見により、ヒト皮膚の *in vivo* 実験モデル作製の可能性が出てきた。上山らが作出した nude-scid マウスや beige-scid マウスへヒト皮膚を移植すると比較的容易に、しかも一年以上もの長期間にわたり、その3次元的構築を保持したままに生着させ続けることが可能となった。

我々は、これらの SCID 系マウスを利用してヒト皮膚 *in vivo* 実験モデルを作成し、発癌に関するいくつかの研究を行ってきた。ヒト前癌病変皮膚の移植実験では、ケラチノサイト系腫瘍の前癌症（Bowen 病や日光角化症など）と非ケラチノサイト系腫瘍の carcinoma *in situ* 病変（乳房外 Paget 病や悪性黒色腫の色素斑部）との間で、植皮片内の腫瘍細胞の生着率に明らかな差異がみられた。また、beige-scid マウスへ移植した Bowen 病の1検体で SCC への進展が確認された。移植ヒト皮膚への化学発癌剤の塗布および中波長紫外線照射による発癌実験では、一部の前癌病変移植片などにおいて発癌の進展をうかがわせる所見が認められた。