Report on United States–Japan Cooperative Cancer Research Program: Melanoma and Skin Cancer—Biology and Comparative Features in the United States and Japan

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United States–Japan Cooperative Cancer Research Program: Melanoma and Skin Cancer—Biology and Comparative Features in the United States and Japan was held from November 13 to November 15, 1987, at the International Lecture Hall, National Cancer Center in Tokyo, Japan. This seminar was supported by the Japanese Society for Promotion of Science and the National Cancer Institute of the U.S. Nine participators from the United States, 20 from Japan, and one from the United Kingdom were involved. Besides these members, Dr. Robert W. Miller, National Cancer Institute, Bethesda, and Dr. Haruo Sugano, Cancer Institute, Tokyo, attended the entire meeting as the coordinators of the program. Other participators included 25 preregistered discussants.

OVERALL PROGRAM

A seminar reception was held on November 12, 1987, the night before the seminar, in the Togeki Sky Salon "Escargot" where approximately 50 people gathered and spent a pleasant evening meeting each other. The seminar started at 9:00 a.m., November 13, with introductory remarks by Dr. Atsuhi Kukita, Dr. Thomas B. Fitzpatrick, and Dr. Robert W. Miller. They stressed the importance of having the meeting, not only with two specific nations where the incidence, clinical types, course, and histopathologic types of melanoma are different, but also with the medical scientists and basic scientists who are worldwide leaders in their individual fields. The seminar dinner was held on November 14, with the honorable attendance of Prince Masahito and Princess Hanako.

The summation and closing remarks were made by Dr. Michael Wick, Dr. Kowichi Jimbow, and Dr. Haruo Sugano. Dr. Wick elegantly and concisely summarized the scientific merits of the whole meeting. Dr. Jimbow stressed the importance of medical collaboration between basic scientists and medical scientists in the study of malignant melanoma for the future in the United States and Japan. Dr. Sugano remarked on the important contributions of basic and clinical sciences in the field of dermatology by introducing Dr. M. Ota as an example of a best clinician, a best scientist, and a best man of letters in the field of dermatology in Japan.

SCIENTIFIC SESSIONS

The seminar was organized along multidisciplinary lines ranging from a study of the pigment cells of fish to human melanoma cells and clinical aspects. Specifically, topics were presented in the following five categories: i) epidemiology and comparative aspects, ii) the cellular and basic biology of melanoma, iii) pathogenesis, iv) biology of precursor lesions and early diagnosis, and v) methods of control and treatment.

Epidemiology and Comparative Aspects Clues to the pathogenesis of diseases can sometimes be found by comparing populations in which the disease is relatively rare with populations in which there is a high incidence. Dr. M. A. Tucker summarized the recent incidence rates of cutaneous malignant melanoma in three geographic locations (Table I).

She stressed that a) major host susceptibility factor is positive if there is a family history of melanoma; and b) if a family member has dysplastic nevi, he or she has an approximately 100% lifetime risk of melanoma.

Dr. Kukita and Dr. S. Ikeda implicated the unique location of the melanoma and nevi in the Japanese vs whites in the United States: in the pigmented races including black races, many melanomas occur on the unexposed areas, i.e., soles of the feet, whereas they are more commonly seen in the sun-exposed areas, e.g., upper back, in whites. Among the main arguments in terms of this racial difference are the following: 1) a remarkably low incidence of a high-risk group, i.e., patients with a family history of dysplastic melanocytic nevi, in the Japanese (Elder, Tucker, Fitzpatrick, and Jimbow); 2) a remarkably low incidence of familial malignant melanoma in the Japanese (Tucker, Miller); 3) low incidence of melanoma in the Japanese may be related to one or more of the following factors: a better DNA repair system (Fitzpatrick, Takebe, Carter), a better immune-surveillance mechanism (Tucker), age factor related to the exposure of children before a certain age, susceptibility of individuals to melanoma (Elwood).

It became clear that a low incidence of dysplastic melanocytic nevi (dysplastic nevus syndrome) in Japan vs a very high incidence of this syndrome in the United States may actually correlate well with the different incidence of melanoma in Japan and the United States.

Dr. Fitzpatrick and Dr. A. Sober proposed the necessity of public education for the early recognition of malignant melanoma. Finally, Dr. Elwood showed the natural history of cutaneous melanoma of individuals living in North America, Australia, and Europe. He also emphasized early diagnosis using professional and public education approaches. Identification of high-risk individuals, followed by early diagnosis or prevention by removal of precancerous lesions, is valuable for extremely high-risk groups.

The Cellular and Basic Biology of Melanoma This session was designed to clarify the regulatory factors responsible for proliferation and differentiation of cells in the epidermis, and to define

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Table I. Incidence Rates/100,000/Year

	Osaka, Japan	Seer, U.S.A.	New South Wales, Australia
Male	0.2	6.5	15.2
Female	0.2	6.3	19.1

the factors that might be relevant to prevention of skin cancer and melanoma.

First, Dr. S. Ito introduced a new method to quantitate the urinary melanogen, i.e., O-methyl derivatives of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. By this method, his research group was able to measure a 10- to 100-fold increase in the urinary excretion of "melanogene" in melanoma-bearing mice, as compared with normal C57BL mice. The question was raised whether such an excretion of urinary melanogen correlates with the content of eu/pheomelanin in melanoma tissue (Mishima, Jimbow). This analysis would provide, in the future, new information regarding the nature of the mixed content of eu/pheomelanin in melanoma tissues (Wick).

Tyrosinase is the enzyme responsible for the formation of melanin. Dr. T. Takeuchi, using an organ culture method and special substrains of mice (lethal yellow mice and recessive yellow mice), compared the biologic effect of DbcAMP and αMSH. Significantly, through this study, his research group has succeeded in defining the genetic control of the tyrosinase molecule for differentiation of pigment cells in hair follicles. The information provided is the first study to clarify the nature of the tyrosinase gene in "normal" tissue (Fitzpatrick). The biologic role of DbcAMP and αMSH to pigment cells was also tested in cultured goldfish by Dr. J. Matsumoto to clarify acquisition of phenotypic instability upon neoplastic transformation and the possible relationship between the stages of cytodifferentiation and susceptibility for neoplastic transformation.

Dr. M. Mihm contrasted the biologic characteristics of melanoma in Japanese cases based on his own review of 83 cases of melanoma and nevi in Japanese. He emphasized the importance of the histology of the acral lentiginous melanoma in situ depending on the anatomic regions affected by the neoplasm. Dr. Mihm also defined a subtle histologic change that would be the first sign in the development of melanoma in situ.

Dr. T. Kuroki introduced a new technique of explant outgrowth culture of pigment cells by which he could demonstrate a defect in UV-induced unscheduled DNA synthesis (UVS) in epidermal cells of xeroderma pigmentosum and binding and action of EGF and TPA on epidermal cells. He found, in contrast to pigment cells, essentially negative responses to TPA on epidermal cells.

Research groups headed by Dr. Y. Ishibashi and T. Hirone characterized a certain aspect in the nature of the precancerous condition in skin tumors. Dr. Ishibashi measured nuclear DNA content of epidermal cells for prokaryotic lesions using microfluorometry applied directly on paraffin sections. In contrast, Dr. Hirone and his associates showed a loss or reduction in the expression of antigen properties in melanoma tissues using various forms of polyclonal and monoclonal antibodies.

Pathogenesis In this session, three major topics were discussed: 1) pigment cells and tumors in fish, 2) sun exposure and DNA damage, and 3) immunology and oncogenes in melanoma. Prince Masahito, Dr. T. Ishikawa, and Dr. Sugano demonstrated how the neural crest cells differentiate into pigment cells of fish, i.e., melanophores, erythrophores, and iridophores, and how these cells, when transformed, can be characterized, in addition to conventional histopathologic studies for the genetic/oncogenic expressions. In addition, Prince Masahito emphasized not only the fine structure of the pigment cells, but also transforming genes (ras gene) of the tumors. Several questions were raised regarding the nature of fish tumors; one of these questions was the difference in the site of point mutation to activate the ras gene (Wick and Carter). Following Prince Masahito's presentation, another topic of fish tumors was presented by Dr. I. Kimura, who reported on how environmental factors can activate or develop certain forms of pigmented tumors in fish. Dr. Kimura has shown that Nibe mitsukurii, feral fish inhabiting the shallow waters, produce pigmented tumors which probably reflect environmental pollution by carcinogens such as nifurpirinol, N-methyl-N'-nitro-N-nitrosoguanidine or 7,12-dimethylbenzanthracene. Removal of contaminated sediments from Kumano liver had reduced the incidence of tumors in Nibe fish at rates ranging from 40%–50% (1983) to 20% (1986).

Most of the U.S. participants were not familiar with the papers by Prince Masahito and Dr. Kimura. However, they indeed captured the interest of the attendants, and the two talks were highlights of this seminar, eliciting many questions. Among the questions asked were why the fish Nibe produced such a high incidence of pigmented tumors and how the incidence was so selective to pigmented tumors (Fitzpatrick, Mihm, Sober, Tucker, and Elder).

Dr. Carter and Dr. Ishikawa discussed DNA damage in skin cancer after exposure to ultraviolet (UV) irradiation. Dr. Carter stressed that chronic exposure to UV irradiation and a high total cumulative dose may be less deleterious than periodic bursts of large amounts of sun exposure that lead to severe sunburn. He also stressed that damage that is apt to be most cytotoxic is probably less effective as an inducer of skin cancer than more-subtle damage, which is tolerated but can initiate malignant transformation. Dr. Ishikawa, using polyclonal antibodies raised against DNA modified with carcinogen 4-hydroxyaminoquinoline 1-oxide or benzopyrene, succeeded in locating in situ DNA adducts on paraffin-embedded sections. He stressed that the intensity of staining correlates well with the level of unscheduled DNA synthesis. In answering several questions, he said that aged mice are deficient in DNA repair after UV exposure, and that intermittent UV exposure is carcinogenic to the melanocytes. Dr. Takebe and Dr. A. Oikawa discussed the nature of melanoma and other skin cancers in xeroderma pigmentosum (XP) patients. Dr. Takebe demonstrated several unique features of Japanese XP patients: a) 12% of skin cancers in Japanese patients with XP were malignant melanoma; b) their histopathologic subtype was lentigo malignant melanoma, while in U.S. patients with XP melanomas were mostly the superficial spreading subtype. He also indicated that the minimum age of melanoma development was three years, many patients being five years of age, and that eye melanoma can occur in XP patients, e.g., in one male it occurred at the age of 20. Dr. Oikawa presented a paper that was different from the one shown in the abstract. He discussed the response of unscheduled DNA synthesis (UDS) induced by UV irradiation in patients suffering from XP and showed that UDS-proficient XP cells reveal several unique responses to treatment with caffeine after UV irradiation: i) caffeine enhanced UV-induced sister chromatid exchange (SCE) dose-dependently and synergistically; ii) the enhanced SCE was not reduced by holding cells in a growth-arrested state; and iii) the SCE causing damage was retained in excision-proficient XP cells.

Immunologic aspects of malignant melanoma were discussed by Dr. J.C. Bystryn and Dr. M. Taniguchi. Dr. Bystryn discussed immunosurveillance and melanoma. He first showed the marked and specific increase in resistance to melanoma in animals (e.g., chimpanzee) by immunization to melanoma vaccines, and then emphasized that the vaccines can help subjects in melanoma-prone families or patients with dysplastic melanocytic nevi. Some of these melanoma vaccines are ready for control study. Dr. M. Tagawa and Dr. Taniguchi discussed the genomic cloning of DNA controlling expression of mouse melanoma antigen and transforming activity. They showed that pD2 (34.8 Kb) expresses M56 mouse melanoma determinants in human melanoma and possesses the transforming activity in NIH/3T3 cells.

Finally, Dr. T. Sekiya discussed the oncogenes in pigment cell neoplasms in general. He found transforming activity in one of the melanoma cell lines, SK2 maintained in nude mice. The transforming gene was activated by a single point mutation at cordon 61 in the second axon. He further indicated that amplification of the C-H-ras-1 alle, in addition to the point of mutation, may play a role in the development of the tumor. Questions raised included whether

DNA changes specific to primary melanoma; whether it can be seen in other pigmented tumors, e.g., pigmented melanocytic nevi and dysplastic melanocytic nevi; and whether loss of chromosome 3 can also be seen in other tumors. These questions must be answered in the future.

Biology of Precursor Lesions and Early Diagnosis In this session the nature and significance of dysplastic melanocytic nevi and giant melanocytic nevi were discussed. Dr. Elder first summarized concisely the present status of dysplastic melanocytic nevi, e.g., clinical features, histopathologic diagnosis, and relationship to the development of malignant melanoma. He indicated that a useful, simple, clinical classification is divided into two categories: 1) high risk — dysplastic melanocytic nevi (DMN) with a personal or family history of malignant melanoma; and 2) low risk — no melanoma history. Clinical management should reflect this risk heterogeneity and should consist of patient education, periodic follow-up at intervals dependent on risk, and excision biopsy of lesions suspicious for malignant melanoma. He also indicated that future research should aim to define the significance of DMN as markers by means of epidemiologic case control and cohort studies, and as precursors by means of laboratory investigation of the mechanisms of tumor progression and the factors that promote or inhibit it. Dr. Jimbow then demonstrated the clinical features and characteristics of Japanese patients with DMN and their fine structural and immunohistochemical properties. Reports of DMN have increased in number in recent Japanese literature. Most of these cases, however, belong to a solitary, sporadic form, and only two of them appear to be familiar and/or associated with malignant melanoma. Although he did not specifically give an answer regarding the two questions of why such a low incidence of DMN occurs in the Japanese and why the incidence of DMN is high in white patients, the new methodology he brought about was the fine structural and immunohistochemical properties of DMN that were characterized by utilizing the monoclonal antibodies, MoAb HMSA. By demonstrating antigen protein reacting with MoAb HMSA, he could identify dysplastic melanocytes in situ on routine paraffin sections. It was also emphasized that the fine structural changes in synthesis and melanization of melanosomes appear to be unique to DMN; they may fill the gap of abnormal melanogenesis existing between cutaneous malignant melanoma and common melanocytic nevus.

Unique clinical features of giant pigment melanocytic nevus and malignant melanoma in Japan were demonstrated by Y. Hori and T. Arao. Dr. Hori first demonstrated the frequency with which giant pigmented melanocytic nevi progress to malignant melanoma. In the literature, it was estimated to be 2% – 31%; Lorentzen et al (1977) estimated a lifetime risk of malignant transformation of giant melanocytic nevi to be 4.6%, and Rhodes et al (1981) estimated it to be at least 6.3%. Dr. Hori also spoke of his experience with cases of congenital giant pigment melanocytic nevi that developed into malignant melanoma after abrasion of the nevi. Histopathologic and ultrastructural studies of nonmalignant nodules in giant nevi revealed that nevi cells were atypical and contained abnormal melanosomes. Dr. Arao showed an unusual case of a 32-year-old Japanese female who suffered from a giant pedunculate, heavily pigmented tumor occurring on the external genitalia.

Methods of Control and Treatment The aim of this session was first to summarize the clinical features of melanoma and skin cancer in the United States and Japan; second, to discuss the results of the treatment currently being used in both the United States and Japan; and third, to study the possibilities for development of rational and experimental approaches in the therapy. Dr. Sober compared the clinical characteristics of skin cancer and melanoma in Japanese and U.S. populations and defined the clinical and histopathologic features that are unique to melanoma found in the two groups. He emphasized that the ability to predict the outcome for an individual patient with melanoma is important not only to the prognostic interests of the particular patient involved but also to the design and interpretation of surgical and adjuvant therapy studies. He also stressed the importance of teaching for the general practitioners and subspecialties of medicine to detect early lesions of malignant melanoma. Questions brought about in his talk included whether the level of invasion or the thickness of the primary tumor is a more-accurate prognostic sign for malignant melanoma. His answer was that the tumor thickness is a much better indication than level of invasion for estimating the prognosis of malignant melanoma.

Dr. K. Ishihara summarized the present status of melanoma treatment by interferon, interleukin-2, and other biologic response modifiers (BRM). In Japan many BRM have been applied, in addition to chemotherapy of malignant melanoma. These include interferon (IFN), interleukin-2 (IL-2), tumor necrosis factor (TNF), living BCG, MY-1, WPG (CWS of Bifidobacterium bifidum), OK-432 (Picibanil, originating hemolytic streptococci), bestatin, forpfenicinol, etc. Among IFN-α, -β and γ, IFN-β showed better results for the treatment of melanoma than IFN-α. Prolongation of survival time by the administration of IFN-β was observed. His opinion was that efficacy of IFN-γ appears to be lower than IFN-α and -β.

Dr. DuPont Guerry discussed the immune-based therapy of malignant melanoma. He summarized the current approaches to the therapy of melanoma using immune-based tactics in the following ways: 1) nonspecific immune stimulation (e.g., with microbial products) or suppression of "suppressor" mechanisms (e.g., with low-dose cyclophosphamide); 2) "active" vaccination with autologous or allogeneic tumor cells, tumor-associated antigens, and anti-idiotypic antibodies; 3) "passive" therapy with naked or adorned monoclonal antibodies of at least relative specificity for melanoma; 4) the use of a heterogeneous group of BRM comprised of various cytokines including the interferons, interleukin-2, and tumor necrosis factor; and 5) autologous cellular therapy with lymphokine-activated killer (LAK) cells or tumor infiltrating lymphocytes (TIL).

The current knowledge of the experimental chemotherapy for malignant melanoma was discussed by Dr. Wick and Dr. Mishima. Dr. Wick first introduced the reducing agent as the controller of replication in malignant melanoma. The rationale for carrying out this approach of selective chemotherapy relies on unique cellular target; nonessential function to host; nontoxicity; and complementary action with conventional agents (differentiation vs gross). He also introduced a new experimental chemotherapeutic agent, i.e., 3,4-dihydroxybenzylamine, by which he found a marked inhibition of melanoma cells in vitro and in vivo (growth inhibition being 70% in 3,4-dihydroxybenzylamine and 48% in dopamine). He also indicated that this new agent can be a project for the phase I clinical study.

As the last topic of this session, Dr. Mishima discussed selective thermal neutron capture therapy and diagnosis of malignant melanoma using its specific metabolic activity. The new compound which he introduced is ^{10}B1-BPA. After introducing the theory and rationale of this chemoradiotherapeutic agent, he showed the sufficient ^{10}B accumulation in inoperable human melanoma lesion and its disappearance by a 10-min neutron irradiation. He also indicated the possibility of performing successfully the first clinical trial.

SEMINAR SUMMARY

Malignant melanoma has now become a major international health problem, affecting all nations with increasing incidence and mortality. It may be epidemic in the western world and of increasing importance in the eastern countries. The foremost basic biology in both melanoma and nonmelanoma skin cancer has been carried out primarily by Japanese and U.S. scientists. The present conference involving these two nations has therefore brought together leaders in the field from both countries and has provided a new level of communication that serves to enhance progress in this significant area. During this seminar, recent advances regarding the contribution of basic knowledge of normal melanocytes to the study of melanoma and oncogene structure, function, immunology of malignant melanoma, as well as new therapeutic modalities, have been discussed for their potential application to malignant melanoma. Special scientific advantages to better clinical management in Japan and the United States resulting from this meeting include increased

communication among the investigators and establishment of collaborative efforts between the Japanese and U.S. investigators attending this seminar. This development of mutual collaboration should greatly increase the pace at which fundamental basic observations are being made, as well as their potential application to clinical progress.

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APPENDIX I.

Members and Participators

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