Symposium on “Retinoid Research and Clinical Application” Supported by the Japanese Cancer Association

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The Symposium on “Retinoid Research and Clinical Application” was held on November 10 and 11, 1989 at the Lecture Hall of the Foundation for Promotion of Cancer Research in Tokyo, within the framework of the activity of the Japanese Cancer Association. The organizers were Dr. Koichi Shudo, Faculty of Pharmaceutical Sciences, University of Tokyo, Dr. Yasutoshi Muto, First Department of Internal Medicine, Gifu University, and Hirota Fujiki, Cancer Prevention Division, National Cancer Center Research Institute, Tokyo. For preparation of this Symposium, we organizers first consulted Japanese scientists from various research fields about their interest in a Symposium on this subject, since this was the first meeting on retinoid research in Japan. Based on those discussions, we decided to extend invitations from the Japanese Cancer Association to more scientists than usual. That is, six presentations by invited foreign scientists were planned to be included in this Symposium. They were given by Dr. Pierre Chambon from France and Drs. Michael B. Sporn, Peter J. A. Davies, Richard C. Moon, Gregor Eichele and Gary Peck from the United States. In addition, there were 43 oral presentations from Japanese participants. The purpose of the Symposium was to impart and to discuss information concerning the present status of retinoid research at the international level. The meeting was divided into two parts; morning, for short presentations by the Japanese scientists, and afternoon, for three plenary lectures by the invited speakers each day. As scientific languages, Japanese was used in the morning and English in the afternoon. Over 300 participants were registered for two days. This number indicated the high level of current interest in research on retinoids.

Vitamin A is termed retinol and its derivatives are called retinoids in the literature. Figure 1 shows the interrelationship of retinoic acid, retinol and retinal and their biological roles. Studies on the relationships between retinol and cancer started in the 1920’s. The evidence that high doses of retinol can prevent cancer development stimulated the synthesis of new retinoids which are structurally different from retinol and also less toxic. The preventive and therapeutic effects of retinoids have been intensively studied since that time, leading to evidence that retinoids are possibly cancer chemopreventive agents. In addition, the study of the mechanisms of action of retinoids led to the discovery of their specific binding proteins, such as cellular retinol-binding protein (CRBP) and cellular retinoic acid-binding protein (CRABP). Furthermore, nuclear receptors for retinoic acid have recently been found. The evidence indicates that retinoic acid and retinoids will be useful tools to study gene expression during morphogenesis, chondrogenesis and differentiation.

Dr. Michael B. Sporn (National Cancer Institute, Bethesda, USA), who had first called a variety of synthetic derivatives of retinol “retinoids” in 1976 and is one of the pioneers in research on cancer chemoprevention, presented an overview of interactions between retinoids and transforming growth factor-β (TGF-β) and their applications in prevention of cancer. First, he reported the actions of TGF-β in inflammation, in formation of bone and cartilage, and in control of the immune system. Additionally, he mentioned results showing that retinoids and tamoxifen, an estrogen analog, prevent breast and skin cancers in experimental animals. The combination of retinoids and tamoxifen is now planned for clinical trials for prevention of malignancy in high-risk patients, as referred to in the talk by Dr. Richard C. Moon.

The link between the action of retinoids and the expression of tissue and epidermal transglutaminases was first reported in cultured mouse epidermal cells in 1980 by Dr. S. H. Yuspa and his associates. Dr. Peter J. A. Davies (Department of Pharmacology, The University of Texas Medical School, Houston, USA) presented a brief review of his studies on transcriptional activation of tissue transglutaminase gene expression by retinoids. A transcription “run-on” assay revealed that the gene was transcribed more actively in retinoic acid-treated cells than in the controls. Dr. Davies stated that since the basic mechanism of retinoid action seems to be analogous to that of the steroid or thyroid hormones, responsive genes presumably contain specific retinoid regulatory elements in their flanking DNA. The expression and activity of retinoic acid receptors (RAR) in normal

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Fig. 1. Biological roles of retinoic acid, retinol and retinal. [From reference: Pawson, B. A. A historical introduction to the chemistry of vitamin A and its analogs (retinoids). In "Modulation of Cellular Interactions by Vitamin A and Derivatives (Retinoids)," ed. L. M. De Luca and S. S. Shapiro, pp. 1-8 (1981). The New York Academy of Sciences, New York.]

and leukemic myeloid cells were studied. Finally, he suggested that both RAR-α and RAR-β mediate retinoid-induced changes in tissue transglutaminase gene expression.

Dr. Pierre Chambon (Institut de Chimie Biologique, Strasbourg, France) spoke about nuclear receptors for retinoic acid. The cDNAs for several nuclear receptors have two highly conserved regions, C and E, which correspond to DNA and ligand binding domains, respectively. The ligand binding domain can be regarded as forming a family of receptors for thyroid hormone, vitamin D₃ and retinoic acid. Based on these studies on nuclear receptors for the steroid and thyroid hormones, Dr. Chambon's group identified and cloned three human and mouse RARs-α, -β and -γ. He clearly demonstrated that RAR-γ gene plays a unique role in transducing retinoic acid signals at the level of gene expression during morphogenesis, chondrogenesis and differentiation of squamous epithelia. In collaboration with Dr. Chambon, Dr. Sumiharu Noji (Okayama University Dental School) reported the expression pattern of RAR genes during development of chick and mouse limbs. Dr. Takashi Ishikawa (Faculty of Medicine, University of Tokyo) reported cDNA cloning of human RAR-γ gene. The discovery of RARs provided a basis for understanding how retinoic acid acts on gene expression.

Distinct from the molecular biological studies mentioned above, Dr. Yuichi Hashimoto (Faculty of Pharmaceutical Sciences, University of Tokyo) independently isolated the retinoid-specific binding proteins, RSBP-1 and RSBP-2, from HL-60 cells. These two proteins were later identified as the products (RARs) of RAR genes investigated by Drs. P. Chambon and R. M. Evans. It is noteworthy that the discovery of RSBPs stemmed from the chemical synthesis of "retinobenzoic acids," such as Am80, Am580 and Ch55, which was described by Dr. Hiroyuki Kagechika (Faculty of Pharmaceutical Sciences, University of Tokyo). It is well known that retinoids inhibit induction of ornithine decarboxylase (ODC) activity in mouse skin and bind to either CRABP or CRBP. However, as Dr. Mitsuo Ninomiya (Gifu University School of Medicine) reported, some of the retinobenzoic acids, such as Ch55, similarly inhibited induction of ODC in mouse skin, although they did not bind to either CRABP nor CRBP. These results strongly suggested that Ch55, for example, might bind to a binding protein other than CRABP and CRBP in cells. Using a binding assay with ³H-Am80 and ³H-retinoic acid, Dr. Hashimoto succeeded in the isolation of RSBPs (RARs) from nuclear and cytosolic fractions of HL-60 cells. The association constants of the RSBPs were 4.4 × 10¹⁰ M⁻¹ for Am80 and 2.4 × 10¹⁰ M⁻¹ for retinoic acid from Scatchard plots. Therefore, the presence of nuclear receptors for retinoic acid was shown from the study of proteins by Dr. Hashimoto as well as from that of genes by Drs. Chambon and Evans, as described previously.

Dr. Richard C. Moon (IIT Research Institute, Chicago, USA) reviewed chemoprevention of breast cancer by retinoids, retinyl acetate and 4-hydroxyphenyl retinamide (HPR). HPR reduced the number of tumors in mammary tumor virus-positive mice. The synergistic
The effect of retinoid and hormonal deprivation was more efficacious in prevention of N-methyl-N-nitrosourea (MNU)-induced mammary tumors than either modality alone. Furthermore, as described in the talk by Dr. Sporn, the combination of retinoid and tamoxifen inhibited the appearance of mammary tumors following surgical removal of the first tumor as well as it inhibited the growth of established tumors. Dr. Moon demonstrated that retinoids inhibited mammary ductal and end bud differentiation and proliferation induced by insulin and prolactin in organ cultures, accompanied with a decrease in DNA synthesis. Localization and metabolism of retinoids by the mammary epithelia are indicated to be of significance in the efficacy of the preventive action against breast cancer. It has been recently reported that eighteen human intervention trials are in progress; seven of the 18 are ongoing with retinol and retinoic acid, and one with HPR. Dr. Moore touched briefly upon the prevention trial of breast cancer in humans with HPR in Italy. It is intended to prevent tumor development of the contralateral breast in women who have had surgical dissection for breast cancer. Over 1,500 women are now involved in this study. In addition to retinoids, Dr. Hisataka Moriwaki (Gifu University School of Medicine) presented a short talk on chemoprevention of hepatocellular carcinoma with 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid (E5166). At present, E5166 is in phase II of a clinical study to prevent liver cancer in cirrhotics.

Dr. Gregor Eichele (Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, USA) talked about retinoic acid in relation to the pattern in the developing vertebrate limb. Morphogens modulated the program for determining the fates of cells in the process of embryonic development. Retinoic acid induces duplications in the developing chick wing and acts as a morphogen. In fact, the limb bud contained endogenous retinoic acid, which was distributed in a concentration-gradient manner, and had been synthesized from retinol. Dr. Eichele also showed that RAR transcripts are present in the early rudiment of limb buds. These results indicated that retinoic acid is a morphogenetic signalling compound locally produced in the early limb bud, and it is distributed through RARs in a concentration-gradient manner. Dr. Yuri Kabuto (National Institute of Neuroscience) reported the expression of CRABP Types I and II in the limb bud of chick embryo and in the central nervous system during early development. Dr. Koji Tamura (Tohoku University) presented the effects of retinobenzoic acids on the development of chick limb bud, showing that they were similar to those of retinoic acid.

Fig. 2. A photograph of the invited speakers, chairmen and organizers of the Symposium. In the first row, from left to right, Mrs. C. Eichele, Drs. R. C. Moon, P. J. A. Davies, G. Eichele, M. B. Sporn, P. Chambon and G. Peck. In the second row, from left to right, Drs. T. Sugimura, H. Fujiki, M. Hozumi, K. Shudo, Y. Muto and K. Hayasaka.
Dr. Gary L. Peck (Dermatology Branch, National Cancer Institute, Bethesda, USA) emphasized retinoids as therapeutic agents in dermatology. Synthetic retinoids, isotretinoin and etretinate, are effective in the treatment of a broad spectrum of dermatological diseases, cystic acne, psoriasis, Darier's disease and cutaneous disorders of keratinization. Dr. Peck described the clinical usefulness of the synthetic retinoids in cancer prevention and therapy for both cutaneous and internal tumors. In these cases, chronic maintenance therapy is needed. Acute side effects, systemic and chronic toxicities were discussed. Finally, he mentioned that the spectrum of retinoid-responsive diseases may not yet have been fully established, and studies on the synthesis of new compounds remain worthwhile. The clinical results with retinoids in Japan were not presented at this time.

In his concluding remarks, Dr. Muto stated that although retinoid research has a long history, its focus has recently dramatically changed from biochemistry to molecular biology. The roles of nuclear receptors for retinoids in the regulation of gene expression and embryonic development need to be further clarified. As a clinician, he stressed that new agents other than retinoids should also be developed for the purpose of cancer prevention. In this regard, a global collaboration is needed, as all the participants realized in the two days of this Symposium. It is hoped to hold a similar type of symposium or seminar again within the next two years.

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