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Meeting Report

Ninth Annual Sapporo Cancer Seminar

Cell Differentiation and Cancer Control[1]

Cancer cells are characterized by their being arrested at an incomplete stage of maturation, while retaining their capacity to proliferate. As a result, functionally incomplete cell populations accumulate, which give rise to the pathological entities designated as "cancer." The molecular lesions leading to maturation arrest occur in stem cells or in cells present at intermediate stages of the maturation path. However, experimental evidence gained *in vitro* and *in vivo* has shown that many such maturation-arrested cells retain their ability to differentiate when exposed either to natural differentiation-inducing factors present in sera or in cell- or organ-conditioned media or to a variety of other agents, including DNA-specific antitumor drugs, retinoic acid, and phorbol esters. Because maturation leads to cessation of growth, induction of differentiation constitutes a promising approach to the treatment of cancer. In keeping with this recognition, the Ninth Annual Sapporo Cancer Seminar focused on the molecular mechanisms that lead to the differentiation arrest of the cancer cell, the cellular processes that control the transition from proliferation to differentiation, and the clinical utility of differentiation-centered therapies.

Four opening lectures were presented, including one by F. Takaku (Tokyo University, Tokyo, Japan) delivered to the citizens of the host city Sapporo. He provided an overview on advances in the diagnosis and therapy of cancer. The remaining lectures were addressed to the conference participants. E. Mihich (Roswell Park Cancer Institute, Buffalo, NY) outlined therapeutic approaches for modifying the inappropriate gene expression that occurs in the cancer cell. Interference with transcription by means of antisense oligonucleotides, the use of nonpathogenic viruses for introducing new messages into the cancer cell, modification of the signal transduction pathway (for example, by inhibitors of protein kinase C or by structurally modified growth or differentiation factors), the use of biological response modifiers such as TNF-α[2] or TGF-β, and finally immunoaugmentation by drugs such as Adriamycin were among the possible approaches discussed. G. B. Pierce (University of Colorado, Denver, CO) explored the notion that the cancer cell follows the normal maturational process when placed in the appropriate embryonic environment. He showed that embryonal cancer cells with trophectodermal potential were killed in blastocele fluid, whereas those with embryonic potential were not; this observation indicates that programmed cell death occurs in the blastocyst, which has the capacity to eliminate cells, including tumor cells with trophectodermal potential. Cell killing was attributed to toxic levels of peroxides generated from polyamines by amine oxidase, an enzyme expressed in a developmentally regulated manner.

The fourth opening lecture was presented by D. Tarin (John Radcliffe Hospital, Oxford, United Kingdom), who discussed the control of tumor cell growth through differentiation induced by cytokines, hormones, retinoids, and antineoplastic agents. An approach that entails the modification of gene expression through suppressor genes received particular emphasis. That objective has been achieved experimentally through cell fusion and by chromosome or gene transfer.

Since diverse cell lines derived from cancer patients have been shown capable of differentiating upon treatment with a variety of inducers such as retinoic acid or DNA-specific agents, the clinical application of such agents by regimens that are geared toward noncytotoxic induction of differentiation rather than to cytotoxic cell removal appears merited. H. P. Koeffler (University of California, Los Angeles, CA) and Y. Yoshida (Kyoto University, Kyoto, Japan) each described trials using either 1α,25-dihydroxyvitamin D_3 (1α, 25$(OH)_2D_3$) or 1α-hydroxyvitamin D_3 in patients with myelodysplastic syndrome (preleukemia). Salutory responses were obtained, especially in Yoshida's trial in which nearly 40% of the patients showed improved blood counts. However, responses were transient, and they were related to the severity of the pretreatment cytopenia; the more severe that cytopenia, the less likely the improvement. Koeffler and his colleagues developed new vitamin D analogues which induce leukemia cell differentiation without causing hypercalcemia, the major toxic side effect. Among the compounds prepared, 1,25$(OH)_2$-16-ene-23-yne-D_3 was markedly effective against WEHI-3B cells carried in BALB/c mice.

Interferons have been applied clinically in the treatment of several forms of cancer. L. Degos (Hospital Saint Louis, Paris, France) reported that almost all his patients with hairy cell leukemia responded to IFN-α. Full responses required up to 7 months of therapy and relapses occurred frequently after therapy was stopped. Degos presented some evidence that IFN-α interrupts an autocrine loop involving the stimulation of neoplastic lymphocyte proliferation by B-cell growth factor. Yoshida reported that IFN-γ was mildly effective in patients with myelodysplastic syndrome, and he suggested the use of combinations of IFN-γ and 1α,25$(OH)_2D_3$ in such patients, because of the synergistic effect these compounds display *in vitro*.

Based on the demonstrated ability of retinoic acid to induce the differentiation of diverse tumor cell lines *in vitro*, the clinical effectiveness of this agent has been explored further. Degos reported that the majority of patients with acute promyelocytic leukemia entered remission when treated with oral all-*trans*-retinoic acid. Remissions were durable, lasting for at least 8 months. Evidence was presented that the remissions were, in part, secondary to the induction of terminal leukemic cell differentiation. All-*trans*-retinoic acid appeared effective only in acute promyelocytic leukemia, a fairly mature leukemia. Based on the observation that retinoids could induce the differentiation of squamous carcinoma cells *in vitro*, R. Lotan (University of Texas, Houston, TX) presented clinical studies by his

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[1] The Ninth Annual Sapporo Cancer Seminar was held in Sapporo, Japan, July 5–7, 1989. The organizing committee of this meeting included M. Hozumi, Chairman (Saitama Cancer Center Research Institute, Saitama, Japan), A. Bloch (Roswell Park Cancer Institute, Buffalo, NY), H. Kobayashi (Hokkaido University, Sapporo, Japan), H. P. Koeffler (University of California, Los Angeles, CA), G. B. Pierce (University of Colorado, Denver, CO), M. Saito (Jichi Medical School, Tochigi, Japan), F. Takaku (Tokyo University, Tokyo, Japan), N. Takeichi (Hokkaido University, Sapporo, Japan), and D. Tarin (John Radcliffe Hospital, Oxford, United Kingdom).

[2] The abbreviations used are: TNF, tumor necrosis factor; TGF, transforming growth factor; IFN, interferon; ara-C, 1-β-D-arabinofuranosylcytosine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; IL, interleukin; MPO, myeloperoxidase; AFP, α-fetoprotein; 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3.

associate, Waun K. Hong, demonstrating that 13-*cis* retinoic acid was effective in the treatment of leukoplakia, a lesion that can progress to squamous cell cancer of the oral cavity. The retinoid was also effective in the adjuvant treatment that followed surgery for head and neck squamous cell carcinoma, decreasing relapse by 30% as compared to placebo-treated controls.

Like other DNA-specific agents, ara-C is capable of inducing the *in vitro* differentiation of myeloid leukemia cells, and the drug appears to be active in clinical use as well. Both Degos and H. Sawada (Kyoto University, Kyoto, Japan) showed that low doses of ara-C (10 mg/m^2/12 h, s.c.) were effective in the treatment of preleukemia and acute myelogenous leukemia. In acute nonlymphocytic leukemia, the overall response rate was 60%, median duration was 8 months, and median survival ranged from 16 to 22 months. In patients with acute myelogenous leukemia who are either elderly or have very hypoplastic bone marrows, survival appeared equal to that obtained with conventional high dose chemotherapy. K. Sanpi (Saitama Cancer Center, Saitama, Japan) and N. Tsuruoka (Showa University School of Medicine, Tokyo, Japan) gave examples in which differentiation therapy with either 1α(OH)D$_3$, plicamycin, or low dose ara-C was effective in the treatment of several patients with acute myelogenous leukemia, blast crisis of chronic myelogenous leukemia, or myelodysplastic syndrome.

The value of using the histopathological degree of tumor differentiation for judging prognosis was discussed by Tarin, who emphasized that this judgment is based upon the experience of the observer in assessing morphological criteria on a statistical basis. Accompanying incomplete cell maturation are metabolic alterations (*e.g.*, ectopic hormones, cachexia) that produce paraneoplastic syndromes and give rise to cell behavioral changes leading to metastasis. His experiments showed that the transfer of DNA from melanoma to nonmetastasizing fibrosarcoma cells resulted in metastasizing transfectants, raising the possibility that inappropriate activation of certain genes normally expressed only in migrating cells such as lymphocytes might play a role in establishing metastatic spread. J-C. Salomon (Centre National de Recherche Scientifique, Villejuif, France) expanded on the role of paraneoplastic events by suggesting that the excessive paraneoplastic production of normal or abnormal signal molecules constitutes an early event in cell transformation, and that by paracrine and/or by autocrine interactions these molecules modify tissue cell composition in favor of transformed cells.

Because of the potential therapeutic utility of differentiation-stimulating agents, the problem of acquired resistance to such agents was considered. R. E. Gallagher (Albert Einstein School of Medicine, New York, NY) did not observe any significant differences between wild type and 300-fold retinoic acid resistant HL-60 cells in either the uptake or metabolism of retinoic acid or in the expression of the retinoic acid receptor-α gene. N. Takeichi (Hokkaido University, Sapporo, Japan) demonstrated that a rat myelomonocytic leukemia cell line resistant to induction of differentiation by lipopolysaccharides was rendered more sensitive when the cells were treated with xanthine oxidase, a potential source of oxygens radicals. Similarly, the leukemogenicity of these cells, markedly suppressed in lipopolysaccharide-injected rats, was enhanced upon injection of superoxide dismutase. These results were interpreted to demonstrate that activated oxygen radicals produced by macrophages and neutrophils may be involved in the *in vivo* differentiation of these lipopolysaccharide-resistant cells.

The molecular mechanisms underlying the transition from cell proliferation to differentiation are largely unknown. Numerous papers addressed diverse aspects of this topic. A. Bloch (Roswell Park Cancer Institute, Buffalo, NY) showed that TNF-α and TGF-β, like tetradecanoylphorbol acetate, induce the differentiation of ML-1 human myeloblastic leukemia cells to monocytes. During the initiation of this maturation process, these three agents caused the rapid translocation of protein kinase C from the cytosol to the membrane. But differentiation did not occur until fetal bovine serum or a specific differentiation factor derived from human leukocyte-conditioned medium was supplied. This requirement indicated that the differentiation-inducing activity of TNF and TGF, like that of TPA, is related to establishing competence, whereas progression relies upon the action of specific differentiation-inducing factors.

The changes in gene expression that accompany induced cell differentiation formed the topic of numerous of the papers presented. Y. Ikawa (Tsukuba Life Science Center, Ibaraki, Japan) showed that in mouse erythroleukemia cells (SJ6) the down-regulation of c-*myb* is a prerequisite for commitment to erythropoietin-induced differentiation. The expression of this gene is transactivated by c-*jun*/AP-1, but this activation does not relate to the overexpression of c-*myb* in hematopoietic cells. Using M-1 murine myeloid leukemia cells and three variants thereof, T. Kasukabe (Saitama Cancer Center, Saitama, Japan) revealed that the more immature and leukemogenic the variant, the higher the level of c-*myb* that was expressed. Like c-*myb*, c-*myc* also appears to be involved in establishing commitment to differentiation. Thus, M. Obinata (Tohoku University, Sendai, Japan) reported that during commitment of mouse erythroleukemia cells, c-*myc* mRNA levels decreased markedly, and that transfection with an autonomously expressing c-*myc* vector abolished the ability of the cells to commit to terminal maturation.

The maturation of cells along different lineages is accompanied by differential gene expression. T. Sugimoto (Kyoto Prefectural University, Kyoto, Japan) examined N-*myc* and c-*src* expression during the neuronal or the Schwannian differentiation of neuroblastoma cells. Retinoic acid-induced neuronal differentiation of the cells was accompanied by a decrease in N-*myc* and an increase in c-*src* expression, whereas Schwannian differentiation, induced by bromodeoxyuridine, was characterized by a decrease in the expression of both N-*myc* and c-*src*. This finding was held to suggest that elevated c-*myc* expression is a general reflection of the undifferentiated phenotype, whereas c-*src* expression is associated with a specific maturational path.

The possible role the retinoblastoma gene (*RB*) may play in growth and differentiation was explored by J. D. Griffin (Dana-Farber Cancer Institute, Boston, MA). The *RB* gene product (Rb) is expressed in several lineages of human cells, including hematopoietic cells, where it may function as a cell cycle regulator, blocking the transition from G_0/G_1 to S. This block can be released through phosphorylation of Rb. Both lymphoid and myeloid cells contain *RB* transcripts, and while resting T- and B-lymphocytes express nonphosphorylated Rb, that gene product becomes phosphorylated after stimulation of the cells with mitogens. The amount and temporal appearance of the phosphorylated species correlates well with the entry of the cells into S. In contrast, monocytes and granulocytes express high levels of nonphosphorylated Rb, which remain nonphosphorylated even after mitogen stimulation, possibly reflecting a terminal differentiation state.

A gene (*MK*), the expression of which changes during the retinoic acid-induced differentiation of keratocarcinoma cells

was identified by T. Muramatsu (Kagoshima University, Kagoshima, Japan). The gene, which codes for a 15 Kd extracellular protein, is also expressed in the tissues of the 9-day mouse embryo, but in the day 15 fetus *MK* expression is restricted to the kidney. Since the process of differentiation entails profound morphological changes, the expression of genes involved in these alterations is expected to increase as well. K. Nagata (Kyoto University, Kyoto, Japan) demonstrated that the differentiation of M-1 myeloid leukemia cells to macrophages is accompanied by a pronounced increase in vimentin mRNA as well as in vimentin filaments.

Numerous of the studies reported at the seminar dealt with the isolation of new cytokines and with their ability to induce cell differentiation. M. Hozumi (Saitama Cancer Center Research Institute, Saitama, Japan) reported on the isolation of a differentiation-inducing glycoprotein (D-factor) of molecular weight of 40,000–50,000 from the conditioned medium of Ehrlich ascites cells, and the purification of a growth inhibitory factor of molecular weight of 25,000 and a differentiation-inhibitory factor of molecular weight of 68,000, from the conditioned medium of a differentiation-resistant clone of M-1 murine myeloid leukemia cells. Each of these factors elicited the corresponding specific response in myeloid leukemia cells. In a similar vein, I. Olsson (Lund Hospital, Lund, Sweden) described the isolation of a factor from mitogen-stimulated lymphocytes and from some T-lymphocyte lines, which inhibited the growth of leukemic and normal progenitor cells but caused HL-60 promyelocyte differentiation. The factor differed from TNF, but displayed some antigenic relationship to lymphotoxin. All three cytokines bound to a single receptor molecule with an apparent M_r of 70,000 which turned over continuously without recycling, and was down-regulated by protein kinase C. T. Sato (Asahi Chemical, Fuji, Japan) purified a M_r 50,000 factor from the medium of THP-1 human monocytic leukemia cells conditioned with TPA or with retinoic acid. This cytokine was found to stimulate myeloid cell differentiation to macrophage-like cells. Finally, T. Tanaka (Kyushu University, Kyushu, Japan) showed that a factor(s) present in a rat adipocyte cell line is capable of inducing myelomonocytic leukemia cell differentiation.

The biological effects of a variety of cytokines formed the topic for various papers. N. A. Nicola (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) demonstrated that the same cytokine can cause growth or differentiation depending on the target cell upon which it acts. Thus, a cloned leukemia inhibitory factor induced the differentiation of M-1 myeloid leukemia cells, stimulated the proliferation of DA-1 myeloid cells and inhibited the differentiation of embryonal stem cells. Receptor analysis showed no ostensible differences in its binding properties, but differences in the cellular processing of the receptor complexes may provide for specificity. In a similar manner, Y. M. Vasiliev (All-Union Cancer Research Center, Moscow, USSR) demonstrated that the phorbol ester TPA, a potent inducer of cell differentiation, caused two types of morphological change depending on the nature of the target cells, fibroblasts *versus* epitheliocytes. Y. Eto (Ajinomoto Co., Kawasaki, Japan) reported that the erythroid differentiation factor which has sequence homology with TGF-β and is identical with the gonadal protein activin A, stimulated hemoglobin accumulation in MEL cells *in vitro* and *in vivo*. This differentiation event was accompanied by a decrease in tumor cell growth. K. Kishi (Nigata University, Nigata, Japan) found that recombinant murine interleukin 4 was capable of stimulating the formation of granulocyte/macrophage colonies from hematopoietic precursor cells. The ability to elaborate such factors in an autocrine manner renders cells, which require them for growth or differentiation, independent of an external source. K. Hara (Jichi Medical School, Tochigi, Japan) reported that transfection of the murine IL-3 gene resulted in the autonomous growth of an IL-3-dependent murine myeloid cell line (NFS-60). The activation of cytokine-producing cells can significantly affect the growth or differentiation potential of cells dependent on such cytokines. T. Hirano (Osaka University, Osaka, Japan) showed that oil- or pristane-induced granulomatous tissue in BALB/c mice produced IL-6 which, in turn, stimulated the growth of IL-6-dependent plasmacytoma. The inference derived from this observation was that chronic inflammation, as exists for instance in rheumatoid arthritis, may be accompanied by the enhanced production of IL-6 resulting in the consequent formation of IL-6-dependent plasma cell neoplasias. Inversely, a decrease in cytokine levels may equally affect cell function. As suggested by K. Miyazono (University of Tokyo, Japan), the decreased production of TGF-β in patients with aplastic anemia and pure red cell aplasia may contribute to the pathogenesis of their bone marrow aplasia. This cytokine was shown to be secreted by human platelets as a latent high-molecular-weight complex, consisting of TGF-β, an NH_2-terminal remnant of TGF-β precursor and a TGF-β-binding protein.

By modifying the amino acid composition of cytokines, their biological properties can be significantly altered. K. Takeda (Showa University, Tokyo, Japan) demonstrated that by changing selected amino acids in TNF, the binding affinity of the derived molecules was extensively altered, resulting in some instances, in increased biological activity. One possible mechanism by which cytokines may regulate growth and differentiation was disclosed by Y. Fujii (Hokkaido University, Sapporo, Japan) who demonstrated that TNF-α, produced by macrophages derived from differentiation-induced ML-1 human myeloblastic leukemia cells, is capable of regulating the formation of monocytes from such ML-1 cells. Low concentrations of TNF-α stimulated, whereas high concentrations inhibited the differentiation of the myeloblasts to the monocyte stage.

Not only cytokines but a variety of other agents have the capacity to stimulate cell differentiation in a rather specific manner. M. Saito and S. Kitagawa (Jichi Medical School, Tochigi) found that synthetic amphipathic sialo compounds such as sialocholesterol and sialodiglyceride were potent inducers of HL-60 granulocyte differentiation, α anomers being more potent than β anomers. A correlation was shown to exist between the type of ganglioside that is present on the cell surface and the maturation stage reached by the cells. During TPA-induced differentiation of HL-60 cells along the monocyte/macrophage path, the ganglio series ganglioside G_{M3} was found to be increased. This ganglioside, when added to the culture medium, induced HL-60 cell differentiation toward the macrophage stage. Similarly, neolacto series gangliosides were observed to be increased during retinoic acid-induced HL-60 cell differentiation and these gangliosides, when added to the culture medium, proved capable of stimulating the granulocytic differentiation of HL-60 cells. Retinoic acid-resistant HL-60 cells showed a markedly decreased content of neolacto series gangliosides, but they were still sensitive to induction of differentiation when these gangliosides were supplied in the culture medium. The therapeutic potential of these observations is worthy of exploration.

New analogues of retinoic acid were prepared by K. Shudo (University of Tokyo, Japan). Among these, a terephthalic

anilide and a chalconecarboxylic acid were potent inducers of HL-60 cell differentiation. These agents were not bound to cellular retinoic acid-binding proteins, but to retinoid receptors present in the nucleus. In a similar manner, T. R. Breitman (NIH, Bethesda, MD) observed that, in HL-60 cells, retinoic acid retinoylated a nuclear protein with a molecular weight of 55,000–60,000, the extent of retinoylation correlating with the differentiation response that was obtained. The differentiation-inducing ability of $1\alpha,25(OH)_2D_3$ was further demonstrated by T. Kuroki (Institute of Medical Sciences, University of Tokyo, Japan) who showed that the vitamin stimulates the differentiation of epidermal keratinocytes, and that psoriatic epidermal keratinocytes were resistant to the inhibition of DNA synthesis caused by this metabolite. C. Miyaura (Showa University, Tokyo, Japan) reported that $1\alpha,25(OH)_2D_3$ as well as interleukin 6 are potent inducers of M1 mouse myeloid leukemia cell differentiation to macrophages. While IL-6 was produced by M-1 cells following differentiation, the presence of IL-6 antibody did not prevent the $1\alpha,25(OH)_2D_3$-induced M1 cell differentiation. The retinoic acid induced differentiation of HL-60 cells was shown by H. Henmi (Sagami Research Center, Sagamihara, Kanagawa, Japan) to be inhibited by pertussis toxin, whereas the toxin did not affect differentiation induced by IFN-γ, lymphotoxin, granulocyte-macrophage-colony-stimulating factor, or cholera toxin. Upon treatment with pertussis toxin, a M_r 21,000 membrane protein was found to be ADP-ribosylated, suggesting that this membrane protein may play a role in the retinoic acid-induced signal transduction.

Among other agents shown capable of inducing differentiation is the antibiotic trichostatin A, which T. Beppu (University of Tokyo, Japan) demonstrated to be a potent inducer of erythroid differentiation. The effect of 8-chloro cyclic AMP on the differentiation of human gastric carcinoma cell lines was examined by A. Takanashi (Hiroshima University, Hiroshima, Japan). Treatment with the agent decreased the type I and increased the type II regulatory subunits of the cyclic AMP-dependent protein kinase, the type I subunit being considered to be involved in cell growth, the type II subunit in cell differentiation. K. Sasajima (Nippon Medical School, Tokyo, Japan) evaluated the effect of alkyl glycerolipid, an inhibitor of phospholipase A_2, on human lung adenocarcinoma A549 cells. He showed that the growth of these cells was inhibited and the appearance of mucus cell morphology stimulated by this agent. Because evaluation of the differentiation-inducing capacity of diverse agents are generally carried out in the presence of sera which contain multiple growth and differentiation factors, the possible interactions between the test agents and these natural factors in producing a biological effect need to be kept in mind.

The identification of effective differentiation inducers depends largely upon the adequacy of the *in vitro* and *in vivo* model systems applied, and new cell lines and animal systems that have the potential for serving as improved models are continuously being developed. Y. Honma (Saitama Cancer Center, Saitama, Japan) described a carefully defined murine model involving M-1 leukemia cells and the syngeneic SL murine host. Using this model, he showed that the combination of $1\alpha(OH)D_3$ with daunomycin can prolong the survival time of the leukemic host in excess of that achieved by the agents administered alone. A human T-cell differentiation model that lends itself to the analysis of factors regulating T-cell ontogeny and function was described by T. Ariyasu (Fujisaki Cell Center, Okayama, Japan), and M. Asashima (Yokohama City University, Yokohama, Japan) reported on a new cell line derived from a *Xenopus laevis* tumor. Finally, H. Kobayashi (Hokkaido University, Sapporo, Japan) discussed a rat fibrosarcoma cell line (KMT-17) that lends itself to studies on the expression and shedding of tumor-associated antigens.

The incomplete stage of maturation that is characteristic of the cancer cell is associated, as it is in normal immature cells, with the presence of specific markers of that stage of differentiation. A number of reports dealt with this developmental aspect. M. Yamada (Osaka University, Suita, Japan) demonstrated that MPO is synthesized in HL-60 but not in differentiated HL-60 cells, and that differentiated HL-60 can release intracellular MPO, whereas HL-60 cells cannot. The concentration of extracellular MPO may, therefore, be useful as an indicator of the type and the number of immature cells present in normal and leukemic subjects. Using a series of monoclonal antibodies to various cytoskeletal proteins, G. A. Bannikov (Academy of Medical Sciences, Moscow, USSR) showed that the expression of these proteins changed in a coordinate manner during human epidermal development. In human tumors of the skin and mammary gland, the expression of these proteins followed fetal-like patterns. The expression of neurofilaments, reported by M. Sato (Tokushima University, Tokushima, Japan) to occur in a neoplastic human salivary intercalated duct cell line, may be a similar reflection of impaired development. Y. S. Kim (University of California, San Francisco, CA) observed that the differences in maturing phenotypes obtained upon treatment of diverse colon cancer cell lines with sodium butyrate were reflected in the distinct patterns of CEA-specific mRNAs that were expressed. The possibility that the synthesis of differentiation markers may be affected by cell contact was raised by G. I. Abelev (Cancer Research Center, Moscow, USSR). He showed that AFP, produced by fetal but not by mature hepatocytes, is reexpressed in hepatocytes surrounding necrotic areas caused by exposure to carbon tetrachloride. Similarly, the transfer of mouse hepatocytes into sparse culture produced a sharp increase in the level of AFP. That increase was suppressed in dense cultures, leading to the suggestion that intracellular contact mediates AFP-synthesis. That cell contact may affect signal transduction related to growth and differentiation was also suggested by the studies of A. Ichihara (University of Tokushima, Tokushima, Japan). He demonstrated that hepatocytes in close contact remained in G_0, whereas loss of contact led to their moving to G_1. A hepatocyte growth factor present in rat platelets stimulated their further progression into S. Renewed cell contact, resulting from cellular proliferation, caused a return of the cells to G_0. Neonatal hepatocytes grew autonomously in culture, but upon contact with adult hepatocytes they stopped growth and proceeded to differentiation. IL-1 and TGF-β also inhibited the growth of neonatal and of adult hepatocytes, but these cytokines did not affect the proliferation of several hepatoma cell lines.

Because signal transduction constitutes a critical component of differentiation-induction, the nature of the enzymes involved in this process is being actively examined. M. Oishi (University of Tokyo, Tokyo, Japan) explored the effect some inhibitors of protein tyrosine kinases exert on MEL and F9 cell differentiation. Herbimycin A effectively induced the differentiation of these cell lines, whereas genistein and ST 638 induced differentiation only when the cells were simultaneously treated with DNA-specific agents such as mitomycin C. These results were suggested to indicate that the phosphorylation and dephosphorylation of protein tyrosine kinases form part of the differentiation process. A number of oncogenic viruses encode proteins which possess tyrosine-specific protein kinase activity. These include $p60^{src}$ and $p^{130\ gag\text{-}fps}$. Y. Uehara (National Institute of

Health, Tokyo, Japan) explored the mechanism by which herbimycin inhibits these enzymes, and found that binding of the antibiotic to reactive sulfhydryl groups led to their inactivation.

The diversity of topics addressed during the conference attested to the central position the process of differentiation holds in cell biology and particularly in cancer cell biology. The information presented made it apparent that elucidation of the molecular mechanisms which control differentiation will contribute significantly toward the solution of the cancer problem.

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