[CANCER RESEARCH 51, 745–747, January 15, 1991]

Meeting Report

Tenth Annual Sapporo Cancer Seminar[1]

Recent Topics in Cancer Research

The Tenth Sapporo Cancer Seminar was held immediately following the annual meeting of the JCA[2] which was organized in Sapporo by Dr. H. Kobayashi, the 1990 President of that Association. Instead of the specialized focus which has traditionally characterized the format of these seminars, this year a few areas of oncology were chosen for review because the Seminar was in celebration of the tenth anniversary of this series of outstanding international oncology meetings.

I. J. Fidler (Houston, TX) reviewed recent advances in the biological therapy of cancer metastasis. A successful treatment should circumvent the heterogeneity of tumor cell populations and should minimize or overcome resistance. Macrophages activated to become tumoricidal through interactions with liposomes of appropriate composition containing certain immunomodulators appear to be effective in coping with both of these problems.

The multiple physiological functions of macrophages were briefly reviewed. It was emphasized that macrophages can be activated by muramyl dipeptide analogs, as well as by interferon, and recent studies with MTP-PE, a muramyl dipeptide derivative, were discussed. This agent, like certain cytokines, can be best administered encapsulated into liposomes, this resulting in greater selectivity of macrophage activation. Macrophages activated by liposomes containing MTP-PE and interferon are capable of killing both sensitive and drug-resistant tumor cells. The physicochemical requirements of liposomes for optimal activity were briefly reviewed as were the experimental conditions leading to maximum therapeutic effects in mouse metastatic models. It was recognized that inadequacy of macrophage to tumor ratios and of efficiency of killing may represent therapeutic limitations, the former suggesting that reduction of tumor burden by other modalities of treatment may usefully precede macrophage-based treatments. The possibility of achieving therapeutic synergisms through combinations of cytokines was also stressed.

The outstanding results achieved with MTP-PE in liposomes in the treatment of osteogenic sarcomas in dogs and the initial promising results obtained in children with osteogenic sarcoma were reviewed. After appropriate Phase I trials including monitoring of macrophage functions and of other immune parameters, MTP-PE in liposomes was tested in children selected according to strict eligibility criteria including the development of pulmonary metastasis despite surgical eradication of primary tumor and adjuvant treatment with chemotherapy and radiotherapy. Following surgical excision of recognizable lung metastasis the MTP-PE treatment was given twice a week for 3 months: in 9 of 9 children a radiological shadow was seen at the end of treatment which was caused by a granulomatous reaction with only some residual tumor cells.

L. A. Liotta (Bethesda, MD) reviewed studies on the regulation of the expression of the metastatic phenotype by positive and negative regulatory gene products. Five proteins have been identified in his laboratory in recent years which may each affect different phenomena in the metastatic process. These are: (*a*) the laminin receptor, with high affinity for laminin, which is usually augmented in human carcinoma cells as compared to nonmalignant epithelium cells and which is regulated in breast cancer by hormones; (*b*) type IV collagenase, a metalloproteinase which cleaves type IV collagen and is increased in metastatic breast and colon cancer cells: (*c*) TIMP2, a newly identified protein which is a natural inhibitor of type IV collagenase; (*d*) the autocrine motility factor which acts through a pertussis toxin-sensitive transducer pathway and results in pseudopodia protrusion. Urinary levels of these cytokines in bladder cancer patients were significantly correlated with tumor invasion and disease stage and grade. A carboxyaminoimidazole derivative (L651582) was found to block the action of these cytokines and to reduce cell motility; (*e*) the gene NM23 was identified for which mRNA levels are decreased in mouse and human tumor cells with high metastatic potential. The product of NM23, an M_r 17,000 protein, was found to be highly conserved and to be essentially identical to the awd protein involved in *Drosophila* development. Discussed in some detail were the inhibition of type IV collagenase by TIMP2, the possible functions of NM23, and recent studies with L651582. It is of interest that TIMP2 also inhibited angiogenesis in the chicken chorioallamtoid membrane system and thus may play a role in decreasing tumor-related angiogenesis.

Based on a large body of information acquired, it was concluded that NM23 product can act as a suppressor factor at the level of transduction of signals required for the metastatic process. The agent L651582 inhibited both motility and proliferation of a number of different types of cell lines, including melanoma, prostate, bladder, breast, and colon. *In vivo* L651582 inhibited metastasis of the sarcoma i.m. and of the HT29 human colon tumor line in nude mice. This effect is seen not only in a preventive mode but also in the treatment of advanced metastatic disease.

G. L. Nicolson (Houston, TX) discussed the diverse factors that condition the metastatic process with particular emphasis on those which determine organ preference of metastasis. Many examples of organ preference of metastasis are found in human cancer and in experimental tumor models. For instance, human cutaneous melanoma metastasizes to many different sites, whereas ocular melanoma disseminates essentially only to the liver. Organ specificity has multifactorial determinants. Invasion is affected by the activity of tumor cell surface-specific degradative enzymes; various tissues can contain inhibitors of these enzymes, while normal host cells such as fibroblasts can contribute enzymes like collagenases which favor tumor cell invasion. Organ-derived chemotactic factors and tumor auto-

Received 8/30/90; accepted 11/1/90.

[1] The Sapporo Cancer Seminars are held in Sapporo, Japan, under the sponsorship of the Cancer Seminar Foundation presided over by Dr. T. Yamazaki (Sapporo). The Tenth Seminar was held on July 6, 1990 and was chaired by Dr. H. Kobayashi (Hokkaido University, Sapporo).

[2] The abbreviations used are: JCA, Japanese Cancer Association; AACR, American Association for Cancer Research; HPV, human papilloma virus; MTP-PE, muramyl tripeptide phosphatidylethenolamine; gp90, M_r 90,000 glycoprotein; PGP, M_r 170,000 membrane glycoprotein; MDR, multidrug resistance; TIMP2, tissue inhibitor of metalloproteinases.

crine motility factors also play a role in the specificity of the metastatic process.

Adhesion preference mechanisms between organ endothelium cell surfaces and tumor cells were emphasized. gp90 was identified on B16 melanoma clones showing brain colonization preference and this molecule was always present on stable clones but was lost in unstable clones which had also lost the ability to colonize preferentially in brain. Nevertheless, antibodies against gp90 did not affect tumor cell implantation. It would appear that gp90 may have a role in determining the capacity of melanoma cells to grow in the brain microenvironment after adhesion and invasion may have been mediated by other factors. The possibility that gp90 may be a member of a transferrin-like receptor family was considered. An antibody against gp90 inhibited growth differentially with different cell clones and in direct proportion to their brain metastatic potential and had no effect on cells from the parent line. This antibody also competed with legitimate transferrin for binding to its receptor. Significant relationships between transferrin and brain metastatic potential were also observed in Fischer rats bearing mammary tumors.

Brain-metastasizing cells were stimulated only by conditioned medium from brain endothelial cells, not from liver endothelial cells. It would seem that certain tumor cells have the ability to respond specifically to the endothelial cell-derived growth factor of an organ and that this response is instrumental in the determination of that organ-specific metastasis.

H. zur Hausen (Heidelberg, Federal Republic of Germany) discussed the importance of DNA viruses in human neoplasia. Cancer of the uterine cervix is one of the major types of cancer and appears to be associated with HPV. A variety of HPV genotypes can be found associated with cancer. The virus is present in nonproliferating as well as proliferating cells of certain tumors. Following *in vitro* induction of transcription of HPV-transforming sequences by dexamethasone, the immortalized cells develop tumors in nude mice. In this system modification of HPV transcriptions can be correlated with tumor development consistent with the role of HPV in neoplastic transformation. Amplification of persisting tumor-virus DNA by viruses, particularly the herpes group, plus associated mutagenic events, may contribute to the increased risk for neoplasia; inhibition of these phenomena by helper-dependent parvoviruses and consequent reduction in oncogene expression may provide a new mechanism of suppression of oncogenesis.

K. W. Knizler (Baltimore, MD) discussed the molecular correlates of the progressive clinical and histopathological changes which characterize the evolution of colorectal tumors. These molecular changes include protooncogene activation and elimination of tumor suppressor gene function. The information presented clearly indicated that genetic changes can be identified which result in changes of the regulatory balances between functions expressed by certain oncogenes and by tumor suppressor genes, changes which are consistent with the evolution of certain malignancies.

J. J. Yunis (Philadelphia, PA) discussed the possible prognostic significance of recurrent chromosomal abnormalities and oncogene defects in follicular lymphoma. In 82% of 102 patients studied and also in 19% of 54 patients with diffuse large cell lymphoma, a characteristic chromosome translocation with gene rearrangement was noted. Other recurrent chromosomal defects were found during the evolution of the disease. It is expected that other recurrent breakpoints will be seen in follicular lymphoma and that sequential molecular alterations may be found which would characterize a specific human malignancy.

M. Foti (Philadelphia, PA) discussed the history of the AACR, its goals and operations, with emphasis on relationships to the Japanese community of oncologists and the JCA. The AACR is the only scientific society for cancer research in the Americas that encompasses basic research as well as clinical investigations and epidemiological studies: one of its major goals and responsibilities is to promote *avant garde* cancer research with added emphasis on areas linking laboratory and clinical research. In order to foster these activities, the AACR aims at facilitating scientific communications through the publication of scientific journals, the scheduling of an annual multidisciplinary meeting and of small conferences on specialized subjects, and the continuous exploration of new means of information dissemination and exchange (including electronic publishing). Additional goals include fostering international collaborations among scientists through the organization of joint international conferences and the activities of the corresponding membership in the AACR.

The journal *Cancer Research* was founded in 1941 and is the most cited oncology journal in the world. In 1990, 38% of published papers are expected to originate from abroad with 11% coming from Japan. Also in 1990, a new journal, *Cell Growth & Differentiation*, was started in response to the scientific needs of the membership and the community of oncologists at large. In the near future, a journal on cancer epidemiology and prevention will also be launched.

A series of special conferences has been started to provide a focused forum for *avant garde* discussions for members of the AACR and other interested scientists in the international community of basic oncologists.

By virtue of the very universal nature of cancer research, the AACR has moved from a rather insular position in the early part of the century to a very international posture reflected in its international membership, the organization of binational symposia, and scientific interactions with our sister societies abroad. Much progress in this respect has been achieved in relation to the JCA and it is expected that these interactions will continue and intensify. Embodied in the mission of the AACR, the relationship with the JCA will include: extensive and on-going communication between the Boards of Directors of AACR and JCA, collaboration and communication among scientists, publication of excellent research articles in our respective journals, and commitment to future joint conferences.

A. V. Gudkow (Moscow, USSR) discussed the molecular mechanisms of MDR in animal and human cells. The importance of this area of study was stressed because MDR is a major obstacle to achieving successful cancer chemotherapy. A classical type of MDR is associated with the increased expression of PGP which in man is encoded by the *mdr1* gene. Currently, the configuration of PGP, which functions as an ATP-dependent pump, is being elucidated and hypotheses are being verified about the mechanisms by which this pump can act on a relatively large number of heterogeneous drugs. Enhancement of transcription and/or amplification of *mdr* genes may result in the MDR phenotype. Amplification was seen in rodent tumor cell lines in culture but was demonstrated in only two cases of mouse tumors, selected *in vivo* for resistance. In rodent cells *mdr* genes are amplified within long amplicons varying in structure in different cell lines. Amplification of the *mdr* gene has never been found in human tumor cells. Rather, increased

transcription of *mdr1* is responsible for the increased expression of PGP. The mechanisms of transcription from the two promoters of *mdr1* genes were discussed.

R. Y. Micetich (Edmonton, Alberta, Canada) discussed the potential usefulness of site-specific drug delivery in cancer therapeutics. The importance of selective drug concentration at the desired target site was stressed as a basis for selective toxicity. The usage of antibodies as vectors for selective drug delivery was outlined with particular emphasis on monoclonal antibodies, chimeric antibodies, and truncated antibodies as ligands in drug conjugates.

E. Mihich, (Buffalo, NY) pointed out that the main objective of cancer chemotherapy, namely to develop agents that are specific for cancer, has not yet been reached. However, in some cases success has been achieved in developing agents that are sufficiently selective against tumors as to be useful in the clinics. Another main objective is to prevent or overcome resistance, which, combined with the limited degree of selectivity of antitumor action of the available drugs, provide the major obstacles to achieving successful chemotherapy of cancer. Despite the fact that a cellular target that would provide a unique site for specific intervention has not yet become available, with rapidly increasing knowledge of the molecular mechanisms of control of normal and malignant cells and the factors conditioning tumor growth, exciting new opportunities are becoming available to pursue novel approaches in medicinal chemistry and biochemical pharmacology toward developing more selective, and hopefully soon, specific anticancer agents.

Gene transcription occurs through the action of several proteins; it is conceivable that it may become possible to interfere with their synthesis or function. Certain hormones have been shown to have differential effects on the transcription of the different genomic functions they regulate, implying a diversity in transcription and indicating specificities that may be exploited for therapeutic intervention. After the mechanisms of tumor suppressor genes are understood, it may become possible to develop agents mimicking tumor suppressor gene control mediators and thus institute a new type of "gene-oriented" therapy. Another approach to specific interference with gene expression may be provided by antisense agents.

Membrane signal transduction, taken as a whole, offers multiple sites of intervention which are being actively pursued, but the question is still open whether any of the components of this process may offer sites for specificity of antitumor action.

Efforts are being made to develop treatment based on the induction of terminal differentiation of tumor cells and these may hold a promise for therapeutic specificities and, therefore, are worthy of intensive pursuit.

Resistance may be classified as natural or acquired following exposure to drugs. As shown by the example of methotrexate, acquired resistance may be related to different mechanisms. Natural resistance to a drug may also be related to the metabolic requirements of drug action in target cells. For example, resistance to 5-fluorouracil may be due to the presence of inadequate pools of reduced folates such that the formation of the tertiary complex of 5-fluorodeoxyuridine monophosphate, thymidylate synthetase, and 5,10-tetrahydrofolate is inadequate to ensure inhibition of thymidylate synthetase or duration of this inhibition sufficient to cause cell growth inhibition. Indeed, the administration of leucovorin can partially reverse natural resistance to 5-fluorouracil, also in patients with colorectal cancer and with other neoplasias. As discussed above, MDR may be related to overexpression of PGP, as also demonstrated in fresh tumor samples from cancer patients. Nevertheless, diversity of mechanisms is a prominent characteristic of MDR and their elucidation is a prerequisite for developing potentially effective therapeutic intervention aimed at MDR elimination.

In conclusion, this symposium clearly met the standards of excellence established during the past 10 years and provided for stimulating discussions of new leads and opportunities in the management of cancer, both through therapeutic means and through prevention based on increased knowledge of the mechanisms of development and regulation of neoplasia.

Enrico Mihich
Grace Cancer Drug Center
Roswell Park Memorial Institute
Elm and Carlton Streets
Buffalo, New York 14263