[CANCER RESEARCH 45, 2392-2394, May 1985]

Meeting Report

Fourth Annual Sapporo Cancer Seminar[1]

Viral Transforming Genes and Oncogenes—Origin, Structure, and Function

The long road toward an understanding of viral oncology continued at the Fourth Annual Meeting of the Sapporo Cancer Seminar, which dealt primarily with viral transforming genes and oncogenes. The Organizing Committee was chaired by K. Fujinaga (Sapporo, Japan) and included T. Osato (Sapporo, Japan), F. Rapp (Hershey, PA), K. Toyoshima (Tokyo, Japan), and P. K. Vogt (Los Angeles, CA).

The lectures and discussion concerned DNA-containing tumor viruses, retroviruses, and isolated human oncogenes. The definition of an oncogene was debated and centered around the problem of differences between transforming genes of DNA viruses and those carried by RNA viruses. It was generally agreed that the transforming genes of DNA viruses are required for vital functions in the replication of those viruses without the established need or isolation of cellular homologues. Conversely, RNA retroviruses can transduce transforming sequences (oncogenes) originally derived from cellular sequences; as such, they are not essential for virus replication.

With this as background, the transforming genes of DNA viruses and their role in human cancer were discussed by Rapp. For many of the DNA viruses, the region on the genome responsible for morphological transformation of cells in culture is known and resides in only a small portion of the virus genome. Paradoxically, the more complex the virus genome, the smaller is the region required for transformation. Newly derived evidence linking papilloma viruses with a variety of cancers, hepatitis B with primary liver hepatocellular carcinoma, and herpesvirus with multiple tumor species was described in conjunction with identification and known properties of the corresponding transforming genes. The role of the immune response was mentioned briefly, and the concept that the acquired immunodeficiency disease represents a convergence of retrovirus (HTLV),[2] DNA viruses (hepatitis B, cytomegalovirus, Epstein-Barr virus), and host surveillance (defective) was presented.

Work presented by Y. Gluzman (Cold Spring Harbor, NY), R. Henning (Ulm, Federal Republic of Germany), and N. Yamaguchi (Tokyo, Japan) led to the conclusion that no fewer than 2 functions of SV40 T-antigens are required for transformation and that one function probably involves binding to the normal cellular protein, the M_r 53,000 protein. However, transformation by complementary DNA of the T-antigen region (Gluzman) yielded cells unable to grow in soft agar; the complementary DNA failed to induce tumors when injected directly into newborn animals. The current feeling was that the specific function of the T-antigen, a M_r 82,000 glycosylated protein containing 708 amino acids, that is required for transformation remains obscure.

A number of groups reported on work with polyomaviruses [T. Benjamin (Boston, MA), T. Miyamura (Tokyo, Japan), and K. Segawa (Tokyo, Japan)]. Focus was on the middle T-antigen and the dissection of the functions leading to transformation. The interesting observation that middle T appears to play a role in assembly of virions would at least establish one of the functions of this important protein in virus replication (Benjamin).

A number of groups reported on studies of the early (transforming) region (E1a and E1b) of the human adenoviruses. The effect of site-specific methylation [W. Doerfler (Cologne, Federal Republic of Germany)], the influence of expression of E1a on plasma membrane and tumorigenicity [A. van der Eb (Leiden, The Netherlands)], the mutational effect on adenovirus type 12 [K. Shiroki (Tokyo, Japan)], and the use of adenovirus type 5 and type 12 recombinants [Y. Sawada (Stony Brook, NY)] were described. Transformation by adenovirus type 5 carrying adenovirus type 12 E1a and E1b was less efficient than that with either parental virus; regrettably, the tumorigenicity of the recombinant virus has not yet been checked.

T. Yamashita (Sapporo, Japan) reported that the transforming region of canine adenovirus lies between 15 and 25 map units; this result is the first to suggest that the left-hand end of the adenovirus genome is not invariably the transforming region.

D. Purtilo (Omaha, NE) summarized the many syndromes caused by Epstein-Barr virus in immunocompromised or deficient hosts. The cardinal sign, in the midst of many other immunological aberrations, is lack of antibody to the Epstein-Barr intranuclear antigen. Adding to the risk faced by these patients was a report by M. Okano (Sapporo, Japan) that B-lymphocytes in some genetically controlled immunological disorders are more sensitive to Epstein-Barr virus than are B-lymphocytes from noncompromised hosts.

K. Yanagi (Ibaraki-Ken, Japan) suggested that the morphological transforming region of HSV-1 (0.32 to 0.42 map unit) leaves no sequences in the cell lines established and therefore follows the "hit-and-run" theory previously postulated for transformation by HSV-2. However, it now seems probable that a small amount (~300 base pairs) of HSV-2 DNA is integrated during transformation and more sensitive probes will be required to establish sequences of HSV-1 which may or may not integrate during transformation.

K. Hirai (Bohseidai, Japan) and M. Nonoyama (St. Petersburg, FL) agreed that the transforming region of Marek's disease virus rests in the internal repeat sequences of the long (L) region of the virus genome. This region shows expanded heterogeneous sequences in the DNA from attenuated (nononcogenic) virus, and this forms the basis for future studies designed to evaluate differences between oncogenic and nononcogenic Marek's disease virus.

Vogt introduced retroviruses with an overview focusing on the common properties of *onc* genes: origin from a eukaryotic ge-

[1] The Fourth Annual Sapporo Cancer Seminar Symposium was held in Sapporo, Japan, August 29 to 31, 1984. The chairman of the meeting was K. Fujinaga.

[2] The abbreviations used are: HTLV, human T-cell leukemia virus; HSV, herpes simplex virus; EGV, epidermal growth factor; FSV, Fujinami sarcoma virus; LTR, long terminal repeat.

Received 12/27/84; accepted 2/5/85.

nome; potential for oncogenicity if expressed in the appropriate cellular environment; evolutionary stability suggesting essential functions in the normal cell; and coding capacity for a protein. Retroviruses make use of oncogenes in 2 mechanisms of carcinogenesis: transduction of an oncogene into and expression in a normal cell and activation of a cellular oncogene through the regulatory sequences of a provirus inserted in the vicinity of that cellular proto *onc* locus. *onc* genes appear to have primary normal functions in the control of cell growth. Their number must be limited, but it is not clear whether the more than 20 presently known *onc* genes represent the majority of these potentially carcinogenic cellular sequences. A search for new rapidly transforming viruses may uncover additional *onc* genes. Vogt gave a brief characterization of recent avian sarcoma virus isolates and of the avian acute leukemia virus, S13. Another new rapidly leukemogenic avian retrovirus, avian erythroblastosis virus H, was discussed by T. Yamamoto (Tokyo, Japan) and S. Kawai (Tokyo, Japan). It represents a second independent isolate of the known *onc* gene *erb* B. Since avian erythroblastosis virus H is able to induce erythroblastosis in fowl, the *erb* B gene alone is sufficient to cause this disease. It codes for a membrane-associated glycoprotein the synthesis and glycosylation of which have been followed in great detail. A sequence analysis of *erb* B shows that it belongs to the *src* gene family, although it has no overt protein kinase activity. The *erb* B gene had previously been found to be homologous to the gene for the EGF receptor. Sequence comparisons with related genes and deletion mutant analysis of the *erb* B gene itself distinguish 3 domains: a glycosylation-membrane anchorage domain; a kinase-related domain; and an erythroblastosis-inducing domain. The nucleic acid sequence homology between the *erb* B gene and EGF receptor is also reflected in immune cross-reactions between the EGF receptor and the *erb* B glycoprotein. *erb* B is amplified and shows enhanced expression in several human tumor cell lines. M. Shibuya (Tokyo, Japan) presented a deletion analysis of the transforming functions in the *fps* gene of FSV. The 5′ portion of the FSV *fps* appears nonessential for transformation, although a deletion of this portion reduces pathogenicity. This observation is in accord with an analysis of the genome structure of avian sarcoma virus PRCII, which lacks sequences that make up the 5′ portion of FSV *fps*. In recent years, *onc* genes have attracted far more interest than have tumor-specific surface antigens. The 2 may be related, however, as is indicated by the presence of a cell surface antigen common to and specific for cells transformed by the *onc* gene *src*. Cells transformed by *fps*, *fes*, or *abl* do not show this antigen (N. Kuzumaki, Sapporo, Japan). The multiplicity of Friend virus strains makes sequence analysis particularly important, especially in the region of the transformation-specific M_r 155,000 glycoprotein gene that contains ecotropic and xenotropic sequence elements. A comparison of the M_r 155,000 glycoprotein sequence of the K-1 strain with the 502 strain presented by Y. Ikawa (Tokyo, Japan) suggests that the 2 transformation-specific genes derive from independent recombination events between xenotropic and ecotropic components.

Insertional activation of the resident *myc onc* gene is found in avian lymphoid leukosis, with the majority of cases showing a deleted provirus integrated upstream from the 2 cellular *myc*-coding exons and in the same transcriptional orientation (W. Hayward, New York, NY). Activated cellular *myc* transcription then starts with the promoter in the 3′ retroviral LTR. Splicing to the first cellular exon makes use of a cryptic splice donor site or of the regular splice donor site of the first noncoding exon of the cellular *myc* locus. Enhanced *myc* expression is also regularly seen in Burkitt lymphomas as a result of chromosome translocations, mostly between chromosomes 8 and 14. Of particular interest is an analysis of the Ramos cell line which shows that all 3 exons of c-*myc* remain intact after the translocation. In this case, activation of *myc* seems to be the result of increased gene expression rather than of an alteration in the gene itself. Differences found between viral and cellular *myc* genes were emphasized by T. Papas (Bethesda, MD); these differences may have functional significance. Since the NH_2-terminal amino acid sequences of *myc* proteins synthesized from normal or insertionally activated genes have not yet been determined, the possibility of truncation or elongation of the known coding domains remains open. *myc* appears also to be activated in certain rapidly dividing cells. An example is the regenerating rat liver (K. Hayashi, Tokyo, Japan). Activation of cellular *myc* in regenerating rat liver is under precise temporal control and appears to be repressed by a protein, possibly the *myc* protein itself.

The role of *onc* genes in chemically induced tumors was illustrated by S. Sukumar (Bethesda, MD) with rat mammary carcinomas caused by nitrosomethylurea. In all of these tumors, the H *ras* gene is activated by a single-point mutation in the 12th codon and becomes transforming in transfection assays using NIH 3T3 cells. The reproducibility of this activation in an experimental system is striking and in contrast to the generally low incidence of altered *ras* genes in human primary tumors. T. Sekiya (Tokyo, Japan) has cloned the activated *ras* gene from a human melanoma and has shown a mutation in codon 61, one of the preferred mutation sites for *ras* activation. A survey of human stomach cancers showed NIH 3T3 cell-transforming activity in the DNA of about 8% of the primary growths. This activity was not due to a member of the *ras* gene family (M. Terada, Tokyo, Japan).

HTLV, the first human retrovirus, probably plays an etiological role in adult T-cell leukemia. Its genome lacks homology to human DNA and therefore appears not to carry a cell-derived *onc* gene. It also does not integrate in a defined area of the host genome, making it unlikely that a cellular *onc* gene is activated by insertion of the provirus. Thus, by elimination, the oncogenic determinant of HTLV appears to be a viral sequence. A candidate for such a sequence is the pX region located 3′ to the *env* gene. It is a sequence unique to HTLV and related viruses, contains several open reading frames, and does not code for a virion protein. M. Yoshida (Tokyo, Japan) reported pX expression in HTLV-infected cells. The pX protein appears to be responsible for a specific activation of the HTLV LTR that results in enhanced transcription from the HTLV promoter as demonstrated with a molecular construct containing the HTLV LTR in front of the chloramphenicol acetyltransferase gene. Presumably, the pX protein could also increase transcription from specific cellular promoters. M. Hatanaka (Kyoto, Japan) provided a clue as to the origin of the pX region with data that suggest homology to rodent DNA. The expression of the pX region is also affected by deletions that are frequent in HTLV proviruses. Thus, similar to the products of some known *onc* genes, the pX region may also be expressed as a fusion protein with *env* sequences at its NH_2-terminal (Hatanaka). M. Miwa (Tokyo, Japan) has carried out sequence comparisons between HTLV I and HTLV II showing a

remarkable conservation of the pX region in contrast to the considerable divergence in viral structural sequences. Y. Hinuma (Kyoto, Japan) suggested that among the cellular genes that may be increased in their expression by a product of pX could be the gene for the interleukin 2 receptor. Interleukin 2 receptors are found amplified in certain HTLV-infected cells and may play an important role in the leukemogenic process.

The meeting ended with a general discussion that concentrated on the problems of defining the transformed cellular phenotype and explaining activation of cellular proto *onc* loci; the question raised was: is the change that turns an innocuous and presumably life-sustaining gene into an oncogenic determinant a change in gene expression or one in the coding sequences and thus in the function of the corresponding protein?

Fred Rapp[3]
Department of Microbiology
The Milton S. Hershey Medical Center
The Pennsylvania State University
Hershey, Pennsylvania 17033

Peter K. Vogt
Department of Microbiology
University of Southern California
School of Medicine
Los Angeles, California 90033

[3] To whom requests for reprints should be addressed.