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MEETING REPORT

UICC Study Group on Basic and Clinical Cancer Research: Interrelations of Signaling Paths and What We Can Learn From Interfering With Them

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The purpose of these study group meetings, which are organized by the Tumor Biology Program of the International Union Against Cancer (UICC), is to establish a basis for possible clinical applications founded on molecular concepts. For this purpose, generally a few clinicians, pathologists, and epidemiologists are invited together with a core group of cell and molecular biologists. This time, based on the topic, mainly medical scientists and molecular biologists made up the majority of the participants. The meetings are of a particularly informal nature, to foster the exchange of ideas rather than to discuss data. It is for this reason that no book is published as a follow-up, but rather the present brief report. More detailed data can be requested from the participants directly. Their addresses are provided at the end of this report.

While the topics of all past meetings differed considerably, a substantial number of them dealt at least partially with transmembrane signaling and cytoplasmic signaling pathways to the nucleus [Burger et al., 1988; Burger and Croce, 1990; Levine and Burger, 1993; Burger and Folkman, 1994; Burger and Harris, 1995; Burger and Friend, 1996; Burger and Moses, 1997; Burger, 2000]. To promote signaling to the main theme of a meeting was therefore, high time. Over the years, many steps in signaling pathways have been characterized and entire pathways delineated. They revealed activating and inhibitory crosspoints, alternative and modifying pathways, soon bringing about a bewildering network from the cell periphery to the nucleus, and soon more sophisticated, as well as complicated, similar to the cellular metabolic charts known in the sixties and seventies. Very similar if not the same questions are now popping up in studying growth and metastasis as had to be dealt with in hormonal signaling earlier. What brings about specificity of response for instance? As in hormonal activation, the presence or absence of the receptor will decide on a cell's susceptibility to the extracellular signal. And as in hormone activation, the full availability of the subsequent cascade of events will also decide about the capability of a cell's response. Thus for growth response and metastasis submembranous and cytoplasmic elements have to be present and in a susceptible state (posttranslational modifications). This then together with the transcriptional apparatus in the nucleus will determine the outcome of the stimulus.

Many of the presentations at this meeting have provided clues to signaling pathway interactions. Many have questioned the simplistic concept we still have presently of signaling pathway interactions. It was generally agreed that different cancer types from different organs may considerably differ in their pathway aberrations. We will have to face the fact that even within one type of cancer, different signaling pathways can be modified and that this may still lead to similar phenotypes. What may complicate the predictability of the outcome of pathway disturbances is, however, the fact that quantitative approaches and cellular compartmentalization are barely taken into consideration so far and that all the arrows showing activation or inhibiton of a signaling step studiously put into pathway evaluation nowadays will remain to be assessed

Meeting was held at Woods Hole, Massachusetts, from June 17–20, 2001.

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in pool size and kinetic terms in the near future—a complication which will, however, not only call for more sophisticated technologies inlcuding biocomputation but which eventually will bring in more reliable interpretations into what is happening and particularly a better predictability of the pathway to malignancy and metastasis of a tumor cell.

Dr. R. Klausner opened the meeting with a general survey of cancer research, present day highlights and initiatives of the NCI (e.g., mouse gene collection resource, gene expression patterns of tumors), and present day short comings as well as its impact on the clinics and individual patients. There is no doubt that genomics, proteomics, and other technology improvements will lead not only to deeper insights into molecular mechanisms of the development of tumors but also to more rationally designed and hopefully more efficient therapeutical approaches. At present, signaling seems to be one of the areas where the expectations for gains in insights as well as in advances for therapeutic approaches are quite high. As pointed out by Klausner, in future studies of signal transduction in disease states, it has to be considered that cancer cells interact with a series of other normal cells (heterotypic biology) and that the cancer cells cannot only be studied in isolation (reductionist approach).

Ras and Related Signaling Pathways

Pathway interactions and particularly antagonization can be studied far easier in invertebrate model animals, and all those working on cancer aberrations in mammalian cells are impressed by the speed with which *C. elegans* geneticists can resolve such interactions.

Thus the induction of *C. elegans* vulval development involves the activation and action of a Ras protein. Dr. H.R. Horvitz found that the Ras pathway involved is antagonized by a set of more than 19 proteins, which include counterparts of the human proteins Rb, RbAp48, histone deacetylase, DP and E2F. Some of the other 19 proteins are also similar to human proteins, which we propose will prove to define the products of as yet unidentified tumor suppressor genes (TSG).

Dr. F. McCormick reminded the participants that although Ras proteins play a direct, causal role in cancer, attempts to target Ras directly have not been successful and attention has now turned to downstream targets. Drugs based on the Raf-MAP kinase pathway have entered clinical trials, and targets in the PI kinase pathway, such as PKB/Akt are being screened. The latter pathway regulates cell survival, suggesting that inhibitors would cause cell death. Inhibition of the Raf-MAP kinase pathway causes growth arrest, as a result of decreased expression of cyclin DI and destabilization of Cdk4. In cancer cells that retain p53, inhibition of Raf-MAP kinase increases p53 levels and sensitizes those cells to killing by DNA damaging agents. Drugs in the Ras pathway may, therefore, cause apoptosis in cancer cells and merit clinical evaluation.

Dr. C.J. Marshall is investigating the interrelationships between signaling through Ras and Rho family GTPases in cancer. Several lines of evidence indicate upregulation of Rho family signaling in tumors. His work demonstrates interactions between Ras and Rho signaling. Rho signaling suppresses the ability of Ras to induce the cell cycle inhibitor p21Waf1. In turn, Ras signaling suppresses Rho signaling to stress fibre formation. This creates conditions permissive to cell motility.

Ras is also linked with the PI3-kinase pathway as discussed by Dr. L.C. Cantley. Phosphoinositide 3-kinase (PI3K) is activated by growth factors and hormones and produces lipids that activate downstream signaling pathways, including the AKT/PKB protein ser/thr kinase. The PTEN tumor suppressor gene encodes a phosphatase that keeps the PI3K pathway in check by dephosphorylating the lipid products of PI3K. The role of genes that encode PI3K subunits in tumor growth due to loss of PTEN is being addressed by gene deletion studies in mice.

p53

Dr. A.J. Levine opened a new window on the interactions of the Wnt-1 pathway with the p53 pathway. While screening for genes regulated by the Wnt-1-beta catenin pathway, he discovered a Wnt-1 induced secreted protein (WISP-1). This protein has all the attributes of a potentially important paracrine signaling molecule with additional functions. The WISP-1 protein was shown to activate the AKT protein kinase which in turn blocks p53 mediated apoptosis.

P53 can be activated by DNA damage or oncogenes to induce cell cycle checkpoints, cellular senescence, or apoptosis. Using the

Eμ-myc transgenic lymphoma model, Dr. S.W. Lowe has shown that disruption of apoptosis downstream of p53 was sufficient to recapitulate the effects of p53 loss during lung lymphoma development and that the aneuploidy occuring in p53 mutant lymphomas did not contribute to lymphomagenesis. In contrast, disruption of both apoptosis and a senescence-like arrest were required to recapitulate the effects of p53 loss in promoting resistance to DNA damaging anticancer agents.

Cell Cycle Pathways

The fact that the ubiquitin/proteasome pathway can steer cell cycle signaling was earlier shown by Dr. W. Krek and delineated in detail. The F-box protein SKP2, the substrate-specific receptor of SCF^{SKP2} ubiquitin-protein ligase, is emerging as a central component of a signaling network controlling the progression of mammalian cells from quiescence to proliferation. Signals conveyed via the PI3kinase-pathway allow the rapid accumulation of SKP2 which, in turn, targets key regulators of mammalian G1 phase progression such as the tumor suppressor p27 for ubiquitin-mediated proteolysis. Based on Dr. W. Krek's pioneering work, it has now become clear that disruption of components of this signaling network, including SKP2 can lead to loss of growth control underlying the development of various forms of human cancer.

Cyclin D1 is overexpressed in the majority of human breast cancers. Dr. P. Sicinski found that mice lacking cyclin D1 are resistant to breast cancers driven by the Ras and Neu oncogenes, while being sensitive to mammary carcinomas induced by the Myc and Wnt-1 oncogenes. His results raise the possibility of an anti-cyclin D1 therapy for a subset of human breast cancers.

Regulation at DNA Transcription, DNA Replication, and the Chromosomal Level

Control of cell proliferation and differentiation by the Max transcription factor network was discussed by Dr. R. Eisenman. The Myc oncoprotein functions as a component of a network of transcription factors (the Max network) comprised of Myc family proteins, Mad family proteins as well as Mnt and Mga—all of which form heterodimers with the small bHLHZ protein called Max. Heterodimers between Mad and Max bind DNA and repress transcription, while Myc–Max dimers activate transcription at specific binding sites. Thus, the Max network is a transcriptional switching system and the balance between Myc and Mad appears to act as determinant of cell proliferation and differentiation. Recently, using both Drosophila and mammalian cells, Eisenmann has shown that Myc and Mad predominantly influence cell growth (i.e., cell size) through modulation of expression of gene targets involved in metabolism, translation, and ribosome biogenesis. Deregulation of Myc in many tumors may result in unlimited increases in cell mass, which in turn, drive secondary genetic changes, which lead to frank neoplasms.

Regulation of DNA replication and origin was delineated by Dr. T. Orr-Weaver. Analysis of the regulation of DNA replication in the Drosophila ovarian follicle cells reveals that Rb/E2F/DP acts to limit the initiation of replication. There is a complex between Rb/E2F/DP and the origin recognition complex (ORC), raising the possibility that Rb limits origin activity directly, rather than through transcriptional targets. A new component of the replication initiation complex has been identified and called double parked (DUP) or Cdt1, and this protein may be key in limiting replication initiation to once per S-phase.

The importance of proteins at the plus end of the microtubule (plus-end-tracking proteins) for controling microtubule dynamics and the formation of attachments, for example at the kinetochore, was discussed by Dr. D. Pellman. Data was presented, showing that in yeast, one of these proteins is not essential for viability in haploids, but becomes essential in cells of increased ploidy. The implication of this finding for cancer therapeutics is obvious.

Proteasomes do also control the proper chromosomal separation in mitosis as shown by Dr. J.M. Peters. The separation of sister chromatids at the onset of anaphase is mediated by a proteolytic cascade that involves the anaphase-promoting complex (APC), a ubiquitin protein ligase, and the protease separase. Activation of this pathway is controlled by the spindle assembly checkpoint, a signaling mechanism that monitors bipolar attachment of chromosomes to the mitotic spindle. Dissecting the APC-separase pathway and the spindle assembly checkpoint may be important both for uncovering defects that cause chromosomal instability in tumor cells and for understanding

and optimizing the effects of anti-mitotic cancer drugs.

Strategy to Selectively Kill Tumor Suppressor Deficient Cells

Human epithelial neoplasms typically harbor mutations of specific TSG, and restoration of TSG function is sufficient to inhibit tumorigenesis in model systems. Thus Dr. W.G. Kaelin proposed that possible approaches to treat cancer would be on one hand to develop small molecules that either mimic a critical biochemical activity of a given tumor suppressor protein product or on the other hand small molecules which selectively kill cells in which said biochemical activity is missing. Dr. W.G. Kaelin's approach is somewhat similar to that of Dr. F. McCormick's, who could kill tumor cells selectively with a virus, namely by first identifying genes which are synthetically lethal to TSGs of interest. He then provided an example: the von Hippel-Lindau (vHL) TSG, which is inactivated in most kidney cancers, regulates the stability of the Hypoxia-inducible factor (Hif) transcriptional regulator. Small molecules that inhibit the Hif targets VEGF and TGFα are currently being tested as therapies for vHL−/− tumors, and Dr. Kaelin's group is now screening for small molecules that selectively kill vHL −/− cells and not isogenic, vHL, controls.

Metastasis

Cell adhesion receptors and ligands play important roles in cell behavior. They signal through many of the same pathways as do growth factor receptors and affect cell proliferation, survival and differentiation as well as cell adhesion, shape, polarity, and motility. Plausible arguments suggest that there may be as many as 2,500 adhesion-related genes in the mammalian genome. Adhesion changes are clearly involved in invasion, and metastasis and DNA array screens offer a valuable approach to uncovering them. A screen of 10,000 genes by Dr. R. Hynes revealed a "top set" of 32 consistent changes, around half of which were in adhesion genes. These include rhoC which regulates cell motility and is shown to be causal in enhancing metastasis. Other interesting changes occur in fibronectin that may regulate cell survival or proliferation and angiogenesis as well as IQGAP which may regulate cell−cell adhesion. Further analyses along these lines should uncover other adhesion-related changes in the various steps of invasion and metastasis.

The cytokine TGFβ causes cell-cycle arrest, apoptosis, and various other responses in normal cells through a pathway that often suffers inactivation or degeneration in human cancer. Building on his knowledge of how this pathway works and is integrated with the signaling networks of the cell, Dr. J. Massagué discussed his recent work on how breast cancer cells selectively lose growth inhibitory responses to TGFβ, and how they acquire TGFβ-dependent bone metastasis activity. Downregulation of myc and upregulation of the Cdk inhibitors p15INK4b and $p21^{Cip1}$ constitute an integrated program of TGFβ cytostatic gene responses, and disruption of this program through specific loss of myc downregulation is seen in certain human breast cancer cells. Deprived of its cytostatic arm, the TGFβ pathway in these tumor cells is now directed to support metastasis through mechanisms that Massagué is currently trying to elucidate.

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APPENDIX A

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