Eleventh Annual Pezcoller Symposium: Molecular Horizons in Cancer Therapeutics

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The goal of this symposium was to discuss information useful toward the development of new approaches in cancer therapeutics. New strategies that were discussed included: the development of specific DNA ligands and their evaluation as regulators of transcription, the stimulation of Ras GTPase as a basis for an anti-Ras chemotherapy, the clarification of the structure of CDK complexes with INK4 family factors toward developing inhibitors specific for cells that would affect pRB phosphorylation, the exploitation of the control of apoptosis with the p53 downstream involvement of caspase 9 function, a discussion of novel high throughput technologies for drug screening, and therapeutic developments based on the fact that tumor cells coopt fibroblast in making VEGF, which is then used for angiogenesis.

Immunological approaches to the development of new anticancer treatments were discussed with particular reference to antigen presentation and T-cell activation, the effects of IL-12, and the role of DCs. The therapeutic effectiveness of anti-CTLA-4 antibodies blocking the alternating function of this molecule on T-cell activation was related to tumor immunogenicity and also considered with reference to possible complicating effects on autoimmunity. The modulation of DCs by tumor and the role of stimulating and inhibiting cytokines were discussed; the converging immunotherapeutic approaches of increasing tumor “foreignness” and decreasing tumors-elicted suppressive cytokines were indicated. The possibility of developing therapeutic vaccine based treatments with DCs loaded with tumor RNA was illustrated as a practical approach. The immune mechanisms involved in the antitumor effects of IL-12 and the regulation of this cytokine were considered.

Mechanisms and relevant cross-talks among signal transduction pathways as well as cell cycle regulatory functions were discussed with reference to the possibility that novel treatments may be based on intervention on specific sites in this area of cell regulation. HGF and its receptor encoding gene, MET provided an example of functional regulation of invasive growth pathways. The disregulation of E2F and p53 in cancer cells and during adenovirus infection was discussed as was the cross-talks between pRB and p53 pathways in conditioning cytostatic versus apoptotic effects; the therapeutic utilization and mechanisms of ONYX-015 was also outlined. The clinical efficacy of an inhibitor of v-Ab1 tyrosine kinase, which also inhibits the function of the PDGF receptor in patients with Abl-positive chronic myelocytic leukemia, was discussed as an example of structure-based discovery and the development of selective protein kinase inhibitors. The potentiality and limitations of clinical trials of signal transduction inhibitors were reviewed with particular reference to the novel clinical methodologies, also involving surrogate markers, that are required in this new area of cancer therapeutics.

The role of P300/CBP in neoplastic signaling was illustrated with reference to coactivation of hypoxia-inducing factors and the expression of specific genes; interactions with cell cycle-dependent factors were identified; it was proposed that P300/CBP can operate both as oncprotein and tumor suppressor, similarly to E2F-1. The selective killing of transformed cells through derepression of E2F was indicated as a possible basis for intervention; a new peptide caused a marked increase of E2F by inhibiting the interaction of CyclinA/cdk2 with E2F and led to p53-independent apoptosis. The function of certain conformal mutants of p53 can be re-induced by acetylation of the COOH-terminus. The role of F-Box proteins in ubiquitin-dependent proteolysis and the down-regulation of E2F via a SCF–SKP2–dependent ubiquitination pathway at the S-G2 cell cycle interphase was indicated.

New strategies for the development of compounds of potential interest in cancer chemotherapy were discussed with particular reference to inhibition of specific gene functions and their control mechanism, to the modulation of factors affecting cell cycle and apoptosis, and to potential targets in the mechanisms of angiogenesis.

R. W. Burli discussed the regulation of transcription by synthetic DNA ligands as an approach carried out in P. B. Dervan’s laboratory toward developing small molecules that bind at subnanomolar concentrations to a specific DNA sequence in the human genome. Pairing rules have been developed to control the DNA sequence specificity of minor-groove-binding polyamides containing three aromatic ring amino acids, hydroxypropyle (Hp), imidazol (Im), and pyrrole (Py). An Im/Py pair recognizes G-C bp, whereas a Py/Im combination targets C-G bp. An Hp/Py pair distinguishes T-A from A-T bp. Using a simple hairpin molecular shape and a three-letter amino acid code, eight-ring pyrrole-imidazole polyamides achieve affinities and specificities comparable to those of DNA-binding proteins and have the potential to be specific for any desired DNA sequence. From the currently available polyamides binding 6-7 pairs, longer ones with increased specificity are being designed. In collaboration with T. A. Beerman at Roswell Park Cancer Institute, the targeting of polyamides to the ESX DNA binding domain within the HER2/neu promoter is being studied. The most active polyamide completely blocked ESX/DNA complexes, but it did not affect complexes of AP-2 factor on a site just upstream of the ESX site, thus providing an example of the specificity of the structures for target DNA sequences. This approach to DNA recognition could provide for the design of cell-permeable molecules for the control of gene-specific regulation in vivo.

A. Wittinghofer discussed the GTPase reaction of Ras as a possible target for cancer chemotherapy. The GTPase of Ras is very slow and...
is stimulated 10^2-fold by GTPase activating proteins p120-GAP or neurofibromin. The structure of the Ras-GAP interaction was discussed with particular reference to the essential role of a crucial arginine that cannot be mutated without loss of action. It was found that GAP actively participates in the GTPase reaction by supplying an arginine into the active site. The positive charge of the arginine side chain contacts the phosphates and stabilizes the transitions state. Approaches to stimulate the GTPase reaction would be useful toward inactivating Ras and may lead to the design of anti-Ras drugs, although it is still to be proven that Ras inactivation will inhibit existing tumors.

E. D. Laue discussed the structure of the complex between CDK6 and p19^{INK4d}. CDKs are switched on and off at different times during the cell cycle. Two classes of CDKs are: (a) the Cip/Kip family including p21^{Cip1,WAF1}, p27^{Kip1}, and p57^{Kip2}, which inhibit all G1 and S-phase CDKs and are important in p53 and TGF-β-mediated cell cycle arrest; and (b) the INK4 family, which is specific for CDK4 and CDK6, and includes p16^{INK4A}, p15^{INK4B}, p18^{INK4C}, and p19^{INK4D}. The interactions of these factors with CDK4 and CDK6 regulate phosphorylation of the pRB. The three dimensional structure of p19^{INK4d} and of the p19^{INK4d}/CDK6 complex was determined. The p19^{INK4d}/CDK6 structure provided the first such structural information for a CDK/CDKI complex. The conformational changes induced by p19^{INK4d} seem to inhibit both productive binding of ATP and the cyclin-induced rearrangement of the kinase from an inactive to an active conformation. Binding of an INK4 family inhibitor would prevent binding of p27^{Kip1}, resulting in its redistribution to other CDKs. Critical residues involved in the interaction were identified. Clarification of the structure of the p19^{INK4d}/CDK6 complex may provide leads toward the design of inhibitors that might be specific for the cyclin d-dependent kinases.

S. Lowe discussed the control of apoptosis and senescence by cancer genes. He showed that oncogene signaling to p53 requires p19^{ARF}, for instance, EIA activates p53 but not in ARF-/- cells, in which EIA cannot induce ARF; in these cells, proliferation continues unchecked. ARF can synergize with DNA-damaging agents to promote apoptosis. Inactivation of the INK4a/ARF locus accelerates the development of Myc-induced B-cell lymphomas and reduces apoptosis consequent to reducing p53 function; these lymphomas are resistant to chemotherapy-induced death. Thus, disruption of the INK4a/ARF locus, similarly to loss of p53 function, represents a negative prognostic marker in human tumors. Caspase-9 and its cofactor Apaf-1 have no effect on activation of p53 by Myc but, in Myc-induced apoptosis, act as downstream components of p53. Indeed, Apaf-1 and caspase-9-deficient c-Myc-positive cells are resistant to apoptosis and susceptible to oncogenic transformation. It is likely that immediate p53 effectors converge on the Apaf-1/Pro-caspase 9 complex to facilitate caspase 9 activation. These observations may provide targets for the development of new chemotherapeutic intervention.

D. R. Wetmore described two proprietary solution phase affinity screening technologies, ATLAS (Any Target Ligand Affinity Screen) and SCAN (Screen for Compounds with Affinity for Nucleic acids), which enable high throughput drug discovery even for targets of unknown function and those incompatible with traditional screening technologies.

D. Fukumura outlined methodologies developed in R. K. Jain’s laboratory for the noninvasive monitoring of gene expression and function in vivo. Genetically engineered mice were developed to visualize gene expression, transparent windows (for example, dorsal chambers and cranial windows) to visualize tissues, and computer-assisted microscopy to quantify drug delivery and physiological functions continuously and noninvasively at high resolution in normal and tumor tissues in small animals. It was possible to show that the size of the holes (pores) in the walls of tumor blood vessels depends not only on the type of tumor but also on what organs these tumors grow in. Furthermore, these pores become smaller as tumors shrink during anti-angiogenic or anti-hormonal therapy. By using genetically engineered mice that emit green fluorescence wherever and whenever the VEGF gene is turned on, it was discovered that tumor cells coopt host fibroblasts into making VEGF. Hypoxia is sensed by cells through the intracellular molecule HIF-1. By deleting the gene for HIF-1 from cancer cells, it was shown that VEGF expression, oxygen level, and angiogenesis decreased in these tumors which surprisingly grew larger. These findings collectively suggest that: (a) the blood vessel wall can become resistant to drug penetration during tumor regression; (b) host cells should be considered as additional targets for anti-angiogenic therapy; and (c) tumors can become resistant to anti-angiogenic therapy if they have or develop HIF-1 mutation during the course of therapy.

Immunological approaches to the development of new anticancer treatments were discussed with particular reference to T-cells activation, effects of IL-12, and the role of DCs.

A. Hurwitz described recent advances made in J.P. Allison’s laboratory toward the clarification of the relevance to autoimmunity and tumor immunotherapy of inhibitory signals interfering with T-cell activation. Activation of naïve T cells involves not only antigen recognition by the TCR but also costimulatory signals mediated by interaction of CD28 on the T cell with members of the B7 family on the antigen presenting cell. CTLA-4, a homologue of CD28 that binds B7 with high affinity, functions as an attenuator, effectively raising the threshold of TCR and CD28 signals required to attain T cell activation. Blockade of the inhibitory signals of CTLA-4 by administration of anti-CTLA-4 antibodies can exacerbate experimental autoimmune encephalomyelitis. In a variety of transplantable murine tumor models, the administration of anti-CTLA-4 antibodies achieves 65–100% tumor rejection. In other tumors, such as melanoma K1735, anti-CTLA-4 treatment results in significant retardation of tumor growth, but rejection is only rarely obtained. In some tumors, such as a mammary carcinoma (SM1) or melanoma B16BL6, anti-CTLA-4 administration has negligible effects. The effectiveness of anti-CTLA-4 treatment seems to correlate with the inherent immunogenicity of the tumor. Administration of anti-CTLA-4 along with irradiated, GM-CSF-expressing SM1 mammary tumor cells or B16BL6 melanoma results in rejection of established tumor. Vaccination of TRAMP mice with a combination of GM-CSF-expressing cells from a TRAMP-derived prostatic carcinoma cell line and anti-CTLA-4 at a time coincident with the appearance of frank carcinoma results in a significant reduction in tumor incidence and severity of disease. These data demonstrate that blockade of the inhibitory signals of CTLA-4 is a powerful means of enhancing T cell responses to tumors. The results also indicate a duality between tumor immunity and autoimmunity, and raises issues that must be addressed in the clinic.

A. Lanzavecchia discussed the failure of tumor cells to elicit stimulation of DCs. These professional antigen presenting cells capture antigens in peripheral tissues and migrate to secondary lymphoid organs where they stimulate naïve T cells. DC stimulation represents a critical requirement for the generation of the immune response. DCs use macrophagocytosis, mannose- and Fc-receptors for antigen capture and present antigens on both class II and I molecules. Using receptors characteristic of the innate immune system, immature DCs respond to inflammatory cytokines (TNF-α, IL-1), bacterial products (LPS), or viral infection (double-stranded RNA) by undergoing maturation. First, they synthesize MHC class I and class II molecules at higher rates while down-regulating endocytic activity. This results in the rapid accumulation of a large number of stable peptide-MHC complexes which are preferentially loaded with antigenic peptides. Sec-
ond, maturing DCs down-regulate receptors for inflammatory chemokines while up-regulating CCR7, a chemokine receptor that drives them to the afferent lymphatics and then to the T cell areas of the lymph nodes. Third, maturing DCs up-regulate adhesion and costimulatory molecules and produce cytokines such as IL-12 and IFN-α, thus acquiring enhanced T cell-stimulatory and Th1-polarizing capacity. Tumor cells make TGFβ and IL-10, which suppress DCs, and TNF which mildly stimulates them. In addition, tumor cells do not represent “danger signals” which would trigger a response. Increases of tumor “foreignness” and, decreases of tumor-elicited suppressive cytokines may represent converging approaches of immunotherapeutic value.

E. Gilboa outlined the possibility to carry out vaccination with DCs transfected with the mRNA content of tumor cells; this would extend the scope of vaccination to every cancer patient because RNA can be amplified from very few tumor cells. DCs pulsed with whole cell RNA from cancer of patients are capable of stimulating polyclonal CTL responses in vitro which recognize and lyse the patient’s own tumor cells. RNA can be successfully isolated and amplified from human colon cancer cells obtained by microdissection from a pathology slide, and DCs pulsed with the amplified RNA are able to stimulate a potent CTL response in vitro. Clinical testing of vaccination with RNA-transfected DCs is ongoing in patients with colon cancer and breast cancer.

G. Trinchieri discussed the proinflammatory and immune mechanisms involved in the antitumor effects of IL-12. IL-12 is produced by phagocytic cells and DCs in response to infectious pathogens, ECM components of inflamed tissues, and activated T lymphocytes expressing CD40 ligand. IL-12 induces IFN-γ by T cells and natural killer cells. The production of IL-12 is highly regulated and is down-regulated by IL-10, TGF-β, TNF, IFN-α/β and by cross-linking of Fc and complement receptors. IL-12 and IL-12-induced cytokines, create a microenvironment in which antigen-specific T cells are induced to differentiate to Th1 cells, whereas the differentiation of Th2 cells is prevented. IL-12 has a central role in the regulation of inflammation, innate and adaptive responses to infections, neo-angiogenesis, and resistance to tumors. It acts on tumor cells by two mechanisms, namely: (a) the inflammatory-type mechanisms which are mostly dependent on IFN-γ production and affect viability of tumor cells by inducing apoptosis, as well as by preventing angiogenesis and inducing hypoxia; and (b) induction of an antigen-specific T cell resistance to tumors that can be demonstrated in animals. Additionally, IL-12, via IFN-γ and nitric oxide production, also induces a potent, although transient, immunodepression.

In regard to signal transduction, mechanisms and relevant cross-talks among pathways as well as cell cycle regulatory functions were discussed with particular reference to the possibility that novel treatments may be based on intervention on specific sites in this area of cell regulation.

P. Comoglio outlined an example of the functional regulation of signal transduction pathways for invasive growth. HGF, also known as “scatter factor,” is a mesenchimal cytokine that triggers a unique biological program leading to “invasive growth.” This phenotype results from the integration of apparently independent biological responses to HGF. In a spheroid model system, HGFs, but not EGFs or PDGFs, cause scattering and morphogenesis. HGF is also very strongly angiogenic in a rabbit cornea model. The HGF receptor is encoded by the oncogene MET which features unique signal transduction properties inasmuch as its cytoplasmic tail contains a two-tyrosine multifunctional docking site that binds multiple SH2-containing intracellular signal transducers. Invasive growth results from the concomitant activation of Ras (growth), PI-3K (scattering), and STAT (cell polarity and morphogenesis). MET activates all pathways. Specific amino acid replacements affect individual pathways. Knockouts involving either HGF or MET result in embryonic lethality because of alteration of growth and oriented migration in several tissues and placenta. A new human gene family, encoding large transmembrane proteins, Sexplexins share homologies with the HGF receptor extra-cellular domain. These molecules are receptors for Semaphorins, which in mammalian cells control cell-cell repulsion. MET, Semaphorins, and Plexins seem to belong to the same gene superfamily. MET is found overexpressed and amplified in a number of human metastatic tumors such as renal cell carcinoma and tumors of the larynx. A direct genetic connection between this oncogene and human cancer has been recently established by the finding of the germ line and somatic mutations in the receptor catalytic domain.

F. McCormick discussed the disregulation of E2F and p53 in cancer cells and during adenovirus infection. E2F activity is misregulated in the majority of cancer cells, through the loss of Rb or p16INK4A, or through elevation of cyclin D1. Likewise p53 function is impaired, through loss of p14 ARF, which is strongly induced by E2F, amplification of mdm2, or mutation of the p53 gene itself. Similar changes occur during adenovirus infection of resting, differentiated normal cells. E1a binds Rb and up-regulates E2F, and E1b binds p53 and inhibits its function. In both situations, E2F activation is essential and could lead to p53 activation, resulting in growth arrest or apoptosis. Adenovirus mutants of E1a or E1b could grow efficiently in cancer cells because these proteins are dispensable in these cells but do not grow in normal cells, in which the Rb and p53 pathways are intact and E1a and E1b are essential. ONXY-015 lacks the E1b 55k gene and, therefore, cannot neutralize p53 or carry out other 55k-dependent functions and replicates with varying degrees of efficiency in most cancer cells, probably because the p53 pathway is defective in these cells. In HCT-116 colon cancer cells in which p53 is wild type but p14 ARF is not expressed, infection with ONXY-015 fails to induce p53, presumably because of the lack of p14 ARF, but in primary, normal epithelial cells, p53 induction is seen. In contrast, wild-type adenovirus degrades p53 in both HCT-116 and primary cells. ONXY-015 has entered clinical testing in head and neck cancer, ovarian cancer, pancreatic cancer, and gastrointestinal metastases to the liver. A synergistic interaction between ONXY-015 and chemotherapy has been observed in the clinic.

N. Lydon outlined the structure-based discovery and the development of selective protein kinase inhibitors. The conservation of structural features within the ATP-binding cleft initially led to the belief that drug specificity would be impossible to achieve for ATP-competitive molecules. This dogma has now been clearly dispelled with the discovery of inhibitors of the epithelial growth factor receptor and Abl kinases. CGP 57148B is an inhibitor of V-Abl tyrosine kinase, which also inhibits the function of the PDGF receptor. Although Abl-positive cell lines are markedly inhibited by this agent, src-positive cell lines are not. This compound is now in clinical trial and is very effective in patients with Abl-positive CML. Recently, crystallographic information has revealed significant differences in the ATP-binding cleft between individual kinases, providing a molecular basis for inhibitor specificity. By focusing on the example of TCR-kinase, by virtual computer screening based on crystallographic knowledge, a library of about 2,000,000 compounds gave about 5,000 virtual hits from which combinatorial chemistry was initiated to identify lead agents; such methods have enabled a number of small molecular ATP-competitive protein kinase inhibitors to be tailored for selectivity against a number of kinase targets.

D. Livingston discussed the role of P300/CPB in neoplastic signaling. P300 and CBP are large, signal-integrating nuclear molecules that have at least four sets of biochemical functions: (a) they act as coactivators for numerous DNA binding transcription factors; (b) one
domain of both proteins (CH2) is an intrinsic histone acetyl transferase which acetylates certain targets such as p53. (c) p300/CBP also interact with at least two extrinsic histone acetyl transferase proteins (p/CAF, src-1) and a multiprotein complex containing RNA pol II and itself, presumably while participating in a higher level of transcriptional control through integration of signals coming from disparate pathways; and (d) the NH2-terminal segments of p300/CBP contain sequences that resemble HPV E6 and participate in the control of MDM2-mediated degradation of p53 through the binding of the CH-1 region to mdm2. The CH-1 NH2-terminal domain, like CH2, is a cysteine/histidine rich region and operates as a contact center for the coactivation of HIF-1. HIF-1 activation represents a main line of defense of normal cells against the presence of an hypoxic state. HIF-1 function is likely important for stimulating tumor angiogenesis in solid tumors with hypoxic segments. Therefore, the HIF-1/CH-1 interaction is a potential site for antitumor therapeutic attack. A tumor-suppressing function for CBP was detected in CBP +/+ mice. There is an increased incidence of tumor development in patients with Rubinstein-Taybi syndrome, the origin of which is a single germ line-inactivating mutation in CBP. A cell cycle-dependent interaction of p300 with a nuclear protein, SYT, was also detected. p300-SYT complexes can be detected only in G1-confluent cells. In conclusion, p300 and CBP are at the center of a network of complex signal generation that seems to contribute to tumor and transformation suppression. At least the interaction of HIF-1/CH-1 interaction, it also has the potential of contributing to cancer progression. Thus, one might speculate that, like E2F-1, p300/CBP can operate both as oncogene(s) and tumor suppressor(s).

W.G. Kaelin considered the selective killing of transformed cells through derepression of E2F. A replication-defective adenoviral vector was developed in which a reporter gene was placed under E2F control. In a rat glioblastoma model, this vector gave rise to reporter activity in tumor tissue, but not normal tissue. Thus, derepression of E2F-responsive promoters can be measured in vivo and can be potentially exploited in gene therapy of cancer. E2F is increased in cancer cells and can induce both cellular proliferation and apoptosis. The latter occurs via both p53-dependent and p53-independent pathways. Tumor cells may be uniquely sensitive to E2F agonists. E2F DNA-binding activity is down-regulated by cyclin A/CDK2. In collaboration with Novartis, a cell-membrane-permeable peptide was developed that blocks the interaction of cyclin A/CDK2 with E2F1; because tumors often have inactive pRb, such agents should result in a marked increase in E2F. Indeed these peptides, but not mutant derivatives thereof, induced p53-independent apoptosis in a wide variety of tumor cell lines but not in nontransformed cells. Deregulation of E2F1 was sufficient to render cells sensitive to the apoptotic effects of the cyclin/CDK2-inhibitory peptides. Thus, deregulation of E2F, in conjunction with loss of cyclin/CDK2 activity, leads to cell death.

M. L. Avantaggiati described the role of acetylation in the regulation of the activity of wild-type and tumor-derived p53 protein. Dr. Avantaggiati showed that the DNA-binding and transcription ability of certain types of p53 mutants (i.e., conformational mutants versus structural mutants) can be re-induced both in vitro and in vivo through acetylation of p53 COOH terminus by the acetyltransferase P/CAF. This is the first example of rescue of the activity of cancer-derived p53 proteins throughout a physiological signal. Strikingly, P/CAF protein levels are found down-modulated in a spontaneously occurring tumor cell line harboring a missense mutation of the p53 gene. Ectopic expression of P/CAF therein inhibits growth and restores responsiveness to signals activating p53. Viewed as a whole, the data indicate that at least a set of mutations of the p53 gene could be relatively well tolerated in a genetic background wherein P/CAF signaling is intact. Thus, the extent of p53 acetylation determined by the activity of one or more intracellular acetyltransferases may be a direct determinant of oncogenesis.

W. Krek discussed the role of F-Box proteins in the ubiquitin-mediated proteolysis in cell cycle control. The rate-limiting step in this protein degradation pathway is the polyubiquitination process, which requires an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and, because protein ubiquitination must be highly selective, a third component—the E3 ubiquitin protein ligases. According to the F-box hypothesis, F-box proteins function as interchangeable adaptors that link a core ubiquitin ligase, the Skp1-Cdc53/CUL-1 complex, with a wide array of substrates destined for destruction. The role of the human F-box protein p45SKP2 in cell cycle control and transformation has been investigated in this laboratory. p45SKP2 has been identified as a cyclin A/CDK2 associated protein required for S phase progression. p45SKP2 associates with human CUL-1 and the ubiquitin-conjugating enzyme CDC34. Given the fact that p45SKP2 forms SCF complexes, human cell lyse-activated p45SKP2 exhibits associated ubiquitination-promoting activity in conjunction with CDC34. p45SKP2 can first be detected at the transition between the G1 and S phases; then it increases during S-G2 and drops sharply as cells proceed through M phase. The formation of the SCFp45SKP2 complex is, at least in part, determined by the periodic expression of p45SKP2. SCFp45SKP2 may function in the cell cycle-regulated destruction of E2F-1; the timing of p45SKP2 accumulation coincides with the timing of E2F-1 destruction. p45SKP2 and E2F-1 interact with each other in vitro and in vivo and disruption of this linkage resulted in a reduction in E2F-1 ubiquitination. p45SKP2 must have other targets, too, because its role in E2F-1 regulation cannot explain its requirement in the G1-S phase transition. p45SKP2 is commonly overexpressed in transformed cells, and this is accompanied by a redistribution of cyclin A/CDK2 complexes from p21 CDK-inhibitor-bound form to p45SKP2-containing complexes. Expression of p45SKP2 in quiescent cells induces S phase entry, allows the generation of cyclin A-kinase activity, and promotes the degradation of p27Kip1 CDKIs. It can be postulated that p45SKP2 and p27 are in a balance that determines active E/A CDK2 (entry into the cell cycle) or inactive E/A CDK2 (exit from the cycle). The discovery of F-box proteins and SCF E3 ubiquitin ligases represents an important advance in understanding the mechanisms of selective and regulated proteolysis in cell cycle control. New therapeutic approaches may specifically target the molecular interactions and the biochemical pathways in which specific SCF E3 ligases and their regulators participate and that are changed in tumor cells.

S. B. Kaye discussed the potentiality of signal transduction inhibitors in cancer therapeutics and the initial clinical evidence substantiating it. Experimental data indicate that such an approach generally leads to cytosis rather than cytotoxicity, and, in most cases, continued treatment is necessary, with experimental tumor regrowth often seen after the cessation of treatment. Cell cycle arrest via p21 or p27 could also reduce the efficacy of antiproliferative chemotherapy. Whereas cell cycle arrest may increase resistance to certain drugs, it may also favor apoptosis. Agents in preclinical and clinical development include inhibitors of farnesytransferase, CDKs, members of the PKC family, and certain tyrosine kinases, for example, those specific for EGFR receptors, VEGF receptors, and PDGF receptors. Several of these agents are synthetic small molecules, although there is one example in the clinic of a naturally occurring compound, bryostatin (derived from a marine invertebrate) with complex effects on PKC. Direct down-regulation of gene expression by sequence-specific antisense oligonucleotides with phosphodiester backbone has led to agents active against PKCα and c-raf-1-kinase. In the latter case the efficacy of paclitaxel against human cervical cancer cells has been...
markedly potentiated. Transmembrane growth receptor signal inhibition can be achieved with specific monoclonal antibodies. Herceptin, a humanized anti-HER2/neu monoclonal antibody, has been extensively tested in women with HER-2/neu-positive breast cancer. Its greatest therapeutic effect was evident in combination with doxorubicin or paclitaxel. In most cases, cytotoxic chemotherapy relies for its effectiveness on a series of intracellular signals that lead to apoptosis of tumor cell. Experimental models strongly suggest that a range of aberrant cell signals play a crucial role in resistance to chemotherapy. Various signal inhibitors can lead to experimental reversal of drug resistance. Phase I trials of signal transduction inhibitors pose the requirement for clinicians to reThink trial design, whereby evidence that the drug has the desired effect at the putative target should become the primary end point, rather than normal tissue toxicity. This leads to the requirement that surrogate end points be identified and proper assays developed that could be used in clinical trials to assess the potential value of a new signal transduction inhibitor. Furthermore, Phase I trials need to address two different applications: (a) the use of these drugs as single cytostatic agents; and (b) their use in combination with chemotherapy.

In addition to the oral presentations summarized above, several posters that concerned areas of research germane to the topics of the Symposium were part of the meeting, as indicated below.

G. Cassinelli presented data on cellular responses to taxanes with different antitumor activity. The effects of Taxol and IDN5109, a novel Taxol analogue, were compared on the IGROV-1/Pt1 ovarian carcinoma cell line, which is characterized by high sensitivity to taxanes in vivo. The relative cytotoxic activity of the two taxanes correlated with their ability to promote tubulin polymerization, mitotic arrest, and apoptosis. After 24 h of exposure, IDN5109 at the concentration of 0.1 μg/ml, induced the appearance of phosphorylated Raf-1 and Bcl-2 proteins concomitant with an accumulation of cells with a G2-M DNA content. At the same concentration, Taxol did not appreciably affect cell cycle progression or Raf-1 or Bcl-2 phosphorylation. In contrast, the extent of p34^cdc2 dephosphorylation was found to be comparable in cells exposed to Taxol or IDN5109. The strong phosphorylation of Raf-1 and Bcl-2 in response to IDN5109 could reflect a more effective cell death signal.

M. Cioffi described the serological detection of anti-p53 antibodies by Elisa; serum anti-p53 antibodies could represent a new and sensitive tool for the detection of preneoplastic lesions. Human p53 was produced, and purified p53 and control extract were used to develop an ELISA method. Serological analysis of anti-p53 antibodies offers advantages related to simplicity of analysis, no need for tumor tissue, and the possibility of following the fate of antibodies during treatment of the patient.

M. Gunzer indicated that antigen-specific T-cell activation results from repetitive interactions of T cells with DCs when a 3-D collagen matrix is used as the cell environment. On the single-cell level, long-lived antigen-presenting cells-T-cell interactions were detected. In the close distance to DCs, a strong induction of T-cell migration through the collagen lattices was observed without the need of physical DC-T cell contact. Large clusters of DCs and T cells, as detectable in liquid culture, were completely absent within 3-D lattices; yet, an antigen-specific activation of T cells was achieved within 3-D matrices as judged by: (a) T-blast formation; (b) up-regulation of CD25; and (c) observable cell divisions. T cells were seen to migrate along the entire surface of one DC and to make repetitive contacts to the same DC or sequential interactions with different DCs. DCs themselves were able to contact several migratory T cells simultaneously. The analysis of many hundreds of individual DC-T cell contacts yielded a mean interaction time of 10–15 min, but, occasionally, long-term contacts of several hours were also observed. In vivo, the need for repetitive contacts could result in a threshold number of antigen-laden DCs within a lymph node, which would lead to low zone tolerance or licensing several DCs for further activation of cytotoxic T cells.

R. Fähræus outlined the inhibition of αvβ3 integrin-mediated cell spreading on vitronectin by the tumor suppressor protein p16^{Nk4a}. The effect of p16^{Nk4a} on cell migration and especially its effect on αvβ3-dependent cell spreading were studied. Expression of full-length p16^{Nk4a} blocked αvβ3 integrin-dependent cell spreading on vitronectin, but it did not affect non-αvβ3-dependent spreading on collagen IV. Similarly, synthetic peptides derived from p16^{Nk4a}, p18^{Nk4c}, and p21^{Cip1/Waf1} [which can be delivered directly into cells from the tissue culture medium and block CDK4 and CDK6 activity at concentrations that inhibit cell cycle progression in late G1] also inhibited αvβ3-dependent spreading on vitronectin. These peptides had no effect on non-αvβ3 integrin-dependent spreading on collagen IV, laminin, and fibronectin. G{α}S arrest induced by L-mimosine or by the inhibition of CDK2 activity did not affect cell spreading. The CDK6 protein was found to suppress p16^{Nk4a}-mediated inhibition of spreading and was localized to the ruffling edge of spreading cells, which indicated that CDK6 is the target for the CDKIs and that CDK6 is involved in matrix-dependent cell spreading. Thus, a novel G1 CDK-associated integrin regulatory pathway that acts upstream of αvβ3-dependent activation of PKC and a novel function for the p16^{Nk4a} tumor suppressor protein regulate matrix-dependent cell migration.

B. S. Kubens showed that colon carcinoma cells SW480 can go through the cell cycle while they migrate within a three-dimensional matrix in contrast to previous beliefs that cell migration and proliferation do not occur simultaneously. The cells stop migration immediately before cell separation for up to about 1 h. The finding suggests that the cells go from G0 to early mitosis while they migrate. Colon carcinoma cells are able to go through all phases of the active cell cycle while they move within the matrix. As a result of an adjuvant chemotherapy, the number of already migrating and at the same time proliferating cells would be reduced, and the establishment of metastasis in distant organs could also be diminished.

P. Michieli demonstrated that mutant MET-mediated transformation is ligand-dependent and can be inhibited by HGF antagonists. Mutations in the genes that encode for Met, Ret, and Kit receptor tyrosine kinases invariably result in increased kinase activity and in the acquisition of transforming potential. The role of HGF in mutant Met-mediated cell transformation was studied. The transforming potential displayed by mutant forms of Met found in human cancer is entirely dependent on the presence of HGF. Moreover, mutant Met-induced transformation of NIH3T3 cells can be inhibited by HGF antagonists and increased by HGF stimulation. An engineered Met receptor that contains an oncogenic mutation but is impaired in its ability to bind HGF completely loses its transforming activity. These results suggest that cell transformation is dependent on ligand-induced receptor dimerization. Moreover, mutant Met-driven tumor growth depends on the availability and tissue distribution of active HGF, and this provides proof-of-concept for the treatment of mutant-Met-related pathologies by HGF-antagonizing drugs.

S. Cavassa showed that HGF modulated cell adhesion during invasion of ECM. HGF stimulates epithelial cells to execute a program known as invasive growth, which combines proliferation and migration through the ECM. The ability of MDA-MB-231 carcinoma cells to invade in vitro reconstituted basement membranes as well as isolated ECM components was studied. MDA-MB-231 cells express high amounts of matrix metalloproteases thus displaying strong basal invasive activity. HGF did not further increase protease activity but promoted cell adhesion, migration and invasiveness on several base-
ment membrane components, including laminin 1 and 5, and stromal proteins, such as fibronectin and vitronectin. HGF promotes cell adhesion to laminins via β1 and β4 integrins and to fibronectin and vitronectin via β1, β3, and β5 integrins.

J. Subjeck outlined the effects of fever-like WBH on host antitumor responses and immunological activity. Temperatures in the physiological range (i.e., febrile temperatures) were not observed to be damaging but, conversely, were observed to lead to biologically relevant responses. Such relevant changes required several hours of exposure and were recognized as being similar to those arising during a natural febrile episode. In mice exposed to mild WBH, changes in lymphocytes included alterations in the organization of the spectrin-based cytoskeleton and formation of uropods, activation and reorganization of several PKC isoforms, and induction of heat shock proteins. Moreover, hsp70 is found to directly interact with spectrin and PKC in an ATP-dependent manner, which suggests a role for hsp70 as a molecular chaperone in the fever-induced cytoskeletal alterations. The impact of fever-like WBH on tumor growth was examined in vivo. Treatment with mild WBH caused a small but significant anti-tumor effect in both SCID mice bearing human tumors and BALB/c mice bearing syngeneic tumors. This effect was a result of lymphocyte and/or natural killer cell activity as indicated by increased apoptosis of tumor cells after heating, increased numbers of leukocytes in the tumors, and the inhibition of tumor cell apoptosis by specific inactivation of immune cell functions. Damaging effects were not obtained in normal tissue or normal tissue vasculature. This response and its interaction with the organisms’ immune system may provide a new means of cancer therapy in conjunction with appropriately defined immunotherapy.

B. Leyland-Jones described the identification by comparative genomic hybridization of chromosomal imbalances associated with platinum drug resistance. The most consistent chromosomal abnormalities involved regions of 4q and 6q. Chromosomal aberrations that were specifically associated with clinical resistance to cisplatin were defined. The high frequency of 12p amplification characteristic of testicular germ cell tumors was confirmed, and a high frequency of deletion of 13q was observed. Deletion of 4q was the most consistent change associated with intrinsic resistance; acquired resistance was frequently associated with the amplification of 8q. These aberrations may indicate the position of genes involved in clinical resistance.

G. Damia described the serine-15 phosphorylation of p53 induced by DDP but not by Taxol in human colon carcinoma cell line HCT 116 and ovarian cancer cell line A2780. At equitoxic concentrations, both drugs induced increases in p53 levels in both cell lines; these increases were associated with an increased transcription of waf1 and mdm2 with DDP being the stronger inducer. In the two ataxia telangectasia-minus (ATM) cells used, DDP induced phosphorylation of serine-15. The ATM-related protein seemed to remain as the most likely candidate kinase for activation by DDP.

Appendix

The Program Committee consisted of the cochairs, Drs. Giulio Draetta (European Institute of Oncology, Milan, Italy), Antonio Lanzavecchia (Basel Institute for Immunology, Basel, Switzerland), Alex Matter (Novartis Pharma Inc., Basel, Switzerland), and Frank McCormick (University of California, San Francisco, CA).

In addition to the Program Committee members, invited participants included: M. L. Avantaggiati (Roswell Park Cancer Institute), R. Burli (California Institute of Technology, Pasadena, CA), P. Comoglio (Institute for Cancer Research, Turin, Italy), M. Eck (Dana-Farber Cancer Institute), D. Fukumura (Massachusetts General Hospital, Boston, MA), E. Gilboa (Duke University Medical Center, Durham, NC), J. Howard (Institute for Genetics, Cologne, Germany), J. Huberman (Roswell Park Cancer Institute), A. Hurwitz (University of California, Berkeley, CA), W. Kaelin (Dana-Farber Cancer Institute), S. Kaye (Beatson Laboratories, Glasgow, United Kingdom), W. Krek (Friedrich Miescher Institute, Basel, Switzerland), E. Lave (University of Cambridge, United Kingdom), B. Leyland-Jones (McGill University, Montreal, Quebec, Canada), N. Lydon (Kinetis Pharmaceuticals, Inc., Medford, MA), S. Lowe (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) P-G. Pelicci (European Institute of Oncology), E. Repasky (Roswell Park Cancer Institute), A. Simpson (Ludwig Institute for Cancer Research, Sao Paulo, Brazil), G. Trinchieri (Wistar Institute, Philadelphia, PA), D. Wemore (Scriptgen Pharmaceuticals, Inc., Waltham, MA), A. Wittinghofer (Max Planck Institute for Molecular Physiology, Dortmund, Germany), P. Workman (Cancer Research Campaign Center for Cancer Therapeutics, Belmont, Sutton Surrey, United Kingdom).

The posters were presented by G. Cassinelli (Istituto Nazionale Tumori), M. Cioffi (Istituto di Patologia Generale e Oncologia, Naples, Italy), M. Gunzer (Institute of Immunology, Witten, Germany), R. Fähræus (Cancer Research Campaign Laboratories, Dundee, United Kingdom), B. Kubens (Institute of Immunology, Witten, Germany), P. Michieli (Institute for Cancer Research, Turin, Italy), J. Subjeck (Roswell Park Cancer Institute), S. Cavassa (Institute for Cancer Research, Turin, Italy), G. Damia (Istituto di Ricerche “Mario Negri,” Milan, Italy).
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Enrico Mihich and David Livingston


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