Meeting Report

Recent Advances in Stress Signaling in Cancer

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Abstract

This meeting brought together some of the world’s leading scientists in the field of stress signaling, apoptosis, and cancer. This resulted in a productive interaction that updated our current knowledge on “Stress Signaling in Cancer.” It comes out that subtle disturbances in cell signaling can be associated with and even lead to cancer. As a corollary, it appears that correcting the signaling defects associated with cancer constitutes a new approach to the treatment and control of neoplastic diseases. The meeting also raised several questions that should be addressed. In particular, it is of the utmost importance to better understand the mechanisms that underlie the specificity of the cellular response with regard to different ligands. For example, why does Gadd45β prevent apoptotic cell death in response to tumor necrosis factor α, whereas it favors apoptosis after transforming growth factor β (E. De Smaele et al., Nature (Lond.), 414: 308–313, 2001; J. Yoo et al., J. Biol. Chem., 278: 43001–43007, 2003). Other questions concern the understanding of the crosstalk mechanisms between different stress and apoptotic pathways and how the strength and the position and timing of a signal may affect different pathways. The next few years of research in this field should be enlightening and fruitful.

Introduction

Cells respond to stress by activating signal transduction pathways, which culminates in the induction of homeostatic responses. The failure to adequately sense, generate, and transduce these signals contributes to neoplastic transformation, leading ultimately to resistance to cancer therapy. An International Union of Biochemistry and Molecular Biology symposium entitled “Stress Signaling in Cancer” was held July 25–27, 2003 at “Le centre de recherche en cancérologie de l’Université Laval” in Quebec City to discuss the signaling mechanisms involving the mitogen-activated protein (MAP) kinase (MAPK) pathways in the regulation of the stress response regarding apoptosis and cancer. This article reviews the major findings and future directions emerging in the field of stress signaling in cancer that were presented at the meeting.

Nutritional Stress and Cancer

The keynote address for the meeting was given by Dr. Jacques Pouységur of Nice, France, who summarized his latest work on “Nutritional stress and tumor angiogenesis.” He showed that, in response to hypoxia, tumors secrete angiogenic molecules such as vascular endothelial growth factor (VEGF)-A, which allows their rapid growth by promotion of tumor vascularization. The production of VEGF-A results from the hypoxia-induced activation of the transcription factor hypoxia-inducible factor (HIF)-1α. In cancer cells, the expression of VEGF-A is further regulated through oncogene- and growth factor-mediated activation of the extracellular signal-regulated kinase (ERK) MAPK cascade. This is because of ERK-dependent phosphorylation of HIF-1α and Sp1 transcription factors, which leads to the activation of the VEGF-A promoter. Additionally, the cellular protein level and activity of HIF-1α are critically regulated by the HIF prolyl-hydroxylases and factor-inhibiting HIF (FIH), the activities of which are strictly dependent upon the cellular concentrations of O₂ and 2-oxo-glutarate. Dr. Pouységur reported that HIF prolyl hydroxylase 2 was the key enzyme that triggered HIF-1α instability via the von Hippel-Lindau (VHL) protein degradation pathway during normoxia. Small interfering RNA-mediated ablation of both post-heparin plasma diaminel oxidase 2 and FIH (singly or simultaneously) was sufficient to activate hypoxia-inducible genes during normoxia. He hypothesized that HIF prolyl hydroxylase 2 and FIH represent potential tumor suppressor candidates that may contribute to tumor progression in a manner similar to that of VHL protein loss. The findings of Dr. Pouységur emphasize the role of the HIF-1α system as a master nutritional sensor and identify HIF-hydroxylases as potential targets against which novel therapies may be developed to regulate angiogenesis (1, 2).

p38 MAPK in Cancer

The p38 MAPK family is grouped into four isoforms. Stress-activated protein kinase 2 (SAPK-2)/p38 (herein called p38) refers to the α and β isoforms, whereas SAPK-3 refers to p38γ and SAPK-4 to p38δ. The p38 pathway is strongly activated by stressful stimuli, inflammatory cytokines, and VEGF. p38 is characterized by its dual phosphorylation site on a threonine-glycine-tyrosine motif in its kinase domain. The p38 pathway consists of membrane receptors or sensors that are connected, through adapter proteins, to small GTPases that are upstream of MAPK kinase (MKK) kinase, M KK, and the MAPK itself. M KK-3 and M KK-6 are two dual-activity kinases that are specific activators of p38. The M KK kinases are less characterized, whereas the small GTPases may involve Rac and Cdc42. Stimulation of p38 culminates in the activation of both nuclear and cytoplasmic targets. Major nuclear targets of p38 include the transcription factors activating transcription factor 2 (ATF-2), growth and DNA damage (GADD)-153, and Elk1, whereas MAPK-activated protein kinase 2 is a cytoplasmic substrate of p38 that leads to the phosphorylation of the small heat shock protein HSP-27 (heat shock protein of M, 27,000; see Ref. 3). The first session of the meeting was devoted to an update on p38 signaling pathways and on their role in cancer. Speakers of the session were as follows: Drs. Tak Mak (University of Toronto, Canada), Simon Rousseau (University of Dundee, United Kingdom), Angel Nebreda (European Molecular Biology Laboratory, Heidelberg, Germany), Albert Fornace (NCI, Bethesda, MD), Ettore Appella (NCI, Bethesda, MD), Jacques Huot (Université Laval, Québec, Canada), and Nathalie Rivard (Université de Sherbrooke, Canada).

Dr. Mak first introduced the importance of the stress-sensing mechanisms in cancer. He reported that ataxia telangiectasia mutated protein (ATM) is a major sensor of DNA damage. After activation by

Received 11/4/03; revised 12/15/03; accepted 1/5/04.

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Notes: This article reports on the International Union of Biochemistry and Molecular Biology satellite meeting on “Stress Signaling in Cancer” that was held in Quebec City in July 2003. The meeting was organized by Jacques Huot, Jacques Landry, Josée N. Lavioie, Normand Manceau, and Carl Séguin.

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DNA damage, ATM contributes to the phosphorylation of p53 on Ser15. This increases the stability of p53, brings the cells into the apoptotic pathway, or stops them in G1 or G2 phases of the cell cycle. Interestingly, checkpoint kinase 2 (Chk2) is also activated by DNA damage, which leads to phosphorylation of p53 on Ser20. This further contributes to the stabilization of p53. In contrast to ATM –/– mice and p53 –/– mice, the Chk2-null mice did not develop spontaneous tumors. Yet, tissues from Chk2 –/– mice show significant defects in DNA damage-induced apoptosis or S/G2 arrest. This indicates that ATM and Chk2 are not in the same pathway in the activation of p53 that leads to cell-cycle arrest or apoptosis. Dr. Mak also showed that the knockout of both the Brcal and Brca2 genes results in embryonic lethality and that the developing embryos show signs of a defective cellular proliferation associated with activation of the p53 pathway. The findings presented by Dr. Mak emphasized the intricacy of stress-sensitive pathways that govern the activity of p53 in response to DNA damage and illustrate the role of ATM protein in the process.

Dr. Nebreda also presented studies that support an important role for the MAPK kinase MKK-6. The specificity of the p38 isoforms activated by MKK-6 varies with cell types, the nature and the strength of the signals. He also reported that the activation of one isoform influences the activity of the others. By using gene-targeted inactivation, he generated p38α –/– mice, and he showed that p38α was required for the turnover of the MKK-6 mRNA. This establishes a negative feedback loop that shuts down the signal from the p38α pathway, but also from the other three p38 family members that are downstream from MKK-6. The inactivation of p38α is further associated with the down-regulation of the proapoptotic proteins Bad and Fas and with an up-regulation of the ERK survival pathway in p38α –/– cells. p38α –/– mice die embryonically at approximately E 10.5, mainly because of a defect in placental organogenesis, which emphasizes the essential role of this pathway in development. Dr. Nebreda also presented studies that support an important role for p38α as an inhibitor of the malignant transformation induced by oncogenes.

The identification of effectors downstream from signaling pathways helps to understand the functions regulated by these pathways. Dr. Rousseau discussed the SAPKs pathways that are involved in controlling the cell-cycle progression and the process of cancer. He looked for targets of the p38 pathway to understand its implication in cancer. He demonstrated that heterogeneous nuclear ribonucleoprotein A0/p38 is a novel target for MAPK-activated protein kinase 2, the kinase activated by p38, and he showed that this protein is implicated in posttranscriptional gene regulation. He also for the first time demonstrated that phosphorylation of NOGO-B on serine 20 is mediated through activation of the p38 pathway in stressed cells. Interestingly, NOGO-B, a myelin-associated protein, interacts with Bcl-2 in a p38-dependent fashion, which provides another clue in support of the hypothesis that the control of apoptosis is connected to the p38 pathway.

As mentioned by Dr. Mak, two critical control mechanisms that mediate cellular responses to stress involve the tumor suppressor p53 and cell-cycle checkpoints. Dr. Fornace presented his findings showing the role of the p38 kinases in regulating both p53 and G2 cell-cycle checkpoint control. He showed that the p38 kinases are strongly and rapidly activated by stresses. In fact, p38 is an important component of the “braking system” that prevents the growth of cells after exposure to genotoxic or oncogenic stresses. p38 relays stress signals by stimulating p53 and inhibiting Cdc25B, which can cause a damaged cell to undergo growth arrest or apoptosis. In some cell types, normal p38 signaling to p53 is attenuated in Gadd45a –/– mice, such as in UV-irradiated skin and in fibroblasts expressing oncogenic Ras. Interestingly, Gadd45a –/– mice exhibit genomic instability and Gadd45a mutations are found in some human tumors. Dr. Fornace put forward the hypothesis that p53, p38, and Gadd45 have tumor suppressor-like properties, whereas proteins that oppose this pathway, such as and Cdc25B and the novel Wip-1 phosphatase, act as oncogenes.

Oncogenic Ras activates p53-dependent transcription in human cells. To be fully active, p53 must be posttranslationally modified. In particular, Ser33 and Ser46 are phosphorylated by p38 in response to UV, and phosphorylation of Ser46 is important for the induction of apoptosis. In H-rasV12-transformed human cells, p53 is phosphorylated at Ser33 and Ser46, suggesting a possible activation of p38 by Ras. In a screen for human p53 gene targets, Dr. Appella has found that Wip1 is induced by genotoxic and nongenotoxic stresses in a p38-dependent manner. Interestingly, retrovirus-mediated Wip1 overexpression prevents phosphorylation of p53 at Ser33 and Ser46, abrogates Ras-induced apoptosis, and leads to premature senescence. In vivo, inactivation of p38 by gene targeting or by Wip1 overexpression leads to tumor formation after injection of EIA+Ras-expressing mouse embryo fibroblasts into nude mice. The Wip1 phosphatase gene, PPM1D, is amplified and over-expressed in 11% of human breast tumors with wild-type p53, which suggests that it is a candidate proto-oncogene. PPM1D also complements other growth-promoting oncogenes including NEU1, which is amplified and over-expressed in ~50% of breast tumors. Because both Wip1 and p38 act on cellular targets other than p53, Dr. Appella concluded that several other pathways, distinct from p53, may contribute to tumor initiation or progression in cells that over-express Wip1.

The last two talks of the session addressed the role of p38 in regulating and sensing cytoskeleton alterations. Dr. Huot showed that in endothelial cells, oxidative stress induces a quick coactivation of p38 and ERK MAPK. Activation of the p38 pathway by H2O2 results in phosphorylation of HSP-27 and in an increase in actin polymerization, whereas activation of the ERK pathway triggers the phosphorylation of tropomyosin 1. Upon phosphorylation, tropomyosin 1 colocalizes with actin stress fibers. Inhibiting the ERK pathway results in a misassembly of focal adhesions. This phenomenon, coupled with the p38-mediated increase in actin polymerization, is associated with membrane blebbing and with a loss of the endothelial layer integrity, a permissive event leading to transendothelial migration of cancer cells. ML-7, an inhibitor of cell contractility, inhibits the phosphorylation of tropomyosin and blocks the formation of stress fibers and focal adhesions, which results in membrane blebbing in the presence of H2O2. Dr. Huot proposed that phosphorylation of tropomyosin 1 downstream from ERK contributes to the formation of actin filaments, increasing cellular contractility and promoting the assembly of focal adhesions. These findings indicate that the cytoskeleton remodeling induced in response to oxidative stress is regulated by the integrated activation of the ERK and p38 pathways. The results point to a mechanism that explains the protective function of the ERK pathway during stress.

E-cadherins are transmembrane glycoproteins that function as Ca2⁺-dependent cell-cell adhesion molecules that are linked to the actin cytoskeleton via catenins. In the absence of extracellular calcium, epithelial cells become rounded and there are no more cellular contacts. Dr. Rivard presented evidence that E-cadherin-mediated cell-cell contact triggers p38 cascade activation in a phosphatidylinositol 3'-kinase-dependent manner. She demonstrated that phosphatidylinositol 3'-kinase is recruited to and activated by E-cadherin-mediated cell-cell contacts in confluent epithelial cells and this activation is essential for the integrity of adherens junctions and p38 activation. Afterward, she demonstrated that hDlg (human disc large), an homologue of disk-large tumor suppressor that interacts with
adeno-matous polyposis coli, is associated with phosphatidylinositol 3'-kinase and E-cadherin at the sites of cell-cell contacts. Reduction of hDlg expression levels by small interfering RNA not only severely alters adherens junction integrity, but also prevents the recruitment of p85 phosphatidylinositol 3'-kinase to E-cadherin-mediated cell-cell contact and inhibits p38 activation. In conclusion, hDlg, because of its interaction with adeno-matous polyposis coli, may play an intimate role in the processes regulating proliferation and cell polarity/differentiation in response to E-cadherin-mediated cell contact in epithelial cells (17).

JNK MAP Kinase in Cancer

The c-Jun NH$_2$-terminal kinase (JNK) family of MAPK contains three isoforms: JNK-1 (SAPK-$\gamma$, SAPK-1c), JNK-2 (SAPK-$\alpha$ or SAPK-1a), and JNK-3 (SAPK-$\beta$ or SAPK-1b). All of the isoforms are activated by various types of chemical and physical stresses. The activities of JNK are regulated by dual threonine and tyrosine phosphorylation involving the threonine-proline-tyrosine motif in the activating loop of their kinase domain. The direct upstream activators of the JNKs are MKK-4 and MKK-7. Ten protein kinases phosphorylate and activate MKK-4/7, including transforming growth-factor-$\beta$-activated kinase-1 (TAK-1), the kinase encoded by the tumor progression locus 2 gene (TPL-2), and apoptosis signal-regulating kinase 1 (ASK-1). Signaling specificity inside the JNK pathways is provided by scaffolding proteins, such as JNK interacting protein 1, that specifically interact with MKK-7 and JNK. Upstream of the MKK kinases enzymes are the small GTPases Cdc42 or Rac1. Once activated, the JNKs phosphorylate their substrates, such as cJun, JunD, and ATF-2, as does cFos, can associate to form the transcription complex Elk1, on serine/threonine residues. Phosphorylated cJun, JunD, and JNKs phosphorylate their substrates, such as cJun, JunD, ATF-2, and Elk1, on serine/threonine residues. Phosphorylated cJun, JunD, and ATF-2, as does cFos, can associate to form the transcription complex AP-1 and induce the expression of several genes bearing the AP-1-recognition sequence (3). The signaling mechanisms by which the JNK pathways modulate neoplastic transformation were addressed by Drs. Silvio Gutkind (NIH, Bethesda, MD), Ping Lu (Harvard Medical School, Boston, MA), Ze’ev Ronai (Mount Sinai, New York), Guido Franzoso (University of Chicago, Chicago, IL) and Zigang Dong (University of Minnesota, Austin, MN).

Dr. Gutkind examined the proliferative signaling that emanates from G-protein-coupled receptors (GPCRs) and showed that some of them like the thrombin protease-activated receptor 1 behave as ligand-dependent oncogenes that activate the stress pathways. He found that although GPCRs activate ERK through protein kinase C (PKC) and G$\beta y$ subunits acting on Ras, they induce c-jun expression independently of ERK. This led him to discover that GPCRs stimulate JNKs and that Rac1 and Cdc42 link GPCRs to this stress pathway. He also found that a unique repertoire of signaling molecules links transforming GPCRs to ERK-5 and to a network of SAPKs involving JNKs, p38-$\alpha$, p38-$\beta$, and p38-$\gamma$. In turn, the activation of these kinases converges to the activation of transcription factors that regulate the c-jun promoter, thereby stimulating c-jun expression. Interestingly, the expression and activity of c-jun are also regulated by RhoA, and Jun proteins are integral components of the transforming pathway initiated by RhoA. Hence, the Rho GTPases are important transforming factors, and part of the effects of these factors relies on their role in coupling GPCRs to stress pathways (18, 19).

Dr. Lu discussed his discovery of Pin1, the prolyl isomerase that specifically isomerizes the phosphorylated Ser/Thr-Pro bond and induces conformational changes that regulate protein function. The WW domain of Pin1 is essential for mediating the specific interaction between Pin1 and its substrates. Pin1-induced conformational changes have profound effects on protein function and in cell signaling and cancer. Pin1 is over-expressed in many human cancers, with its levels correlating with cyclin D1 levels in human breast cancer. In fact, Pin1 positively regulates cyclin D1 function at the transcriptional level via multiple oncogenic pathways. Pin1 binds c-Jun phosphorylated on Ser63/73-Pro motifs by activated JNK or oncogenic Ras and thereby cooperates with them to increase the transcriptional activity of c-Jun toward cyclin D1. Pin1 also contributes to the up-regulation of cyclin D1 through posttranslational stabilization. Consistent with these findings, deletion of Pin1 in the mouse results in many phenotypes resembling cyclin D1-null mouse phenotypes. Pin1 is an E2F downstream target gene, the overexpression of which is essential for the Neu/Ras-induced transformation of mammary epithelial cells, whereas inhibition of Pin1 might suppress the transformed phenotypes. Thus, Pin1 overexpression functions as an important catalyst that amplifies and translates multiple oncogenic signaling mechanisms during oncogenesis. In contrast, inhibition of Pin1 provides a unique opportunity for disrupting oncogenic pathways and, therefore, become an appealing target for novel anticancer therapies. Dr. Lu also showed that the knock out of Pin1 is associated with an Alzheimer disease phenotype. All these findings clearly illustrate the multiple research avenues that will arise from understanding the mechanisms of prolyl isomerization (20, 21).

Tumor necrosis factor (TNF) receptor (TNFR)-associated factor 2 (TRAF-2), a member of the TNFR-associated factors is a RING and zinc finger protein that has E3 ligase activity and that regulates the cellular response to stress and cytokines by controlling the JNKs, p38, and nuclear factor $\kappa$ B (NF$\kappa$B) signaling cascades. It is activated after its direct association with TNFR-2, recruitment to TNFR-1 and interaction with the adapter protein TNFR-associated death domain protein, or after its oligomerization. After its activation, TRAF-2 is ubiquitinated and is degraded in a process that requires Siah2, a protein that also has E3 ligase activity. Dr. Ronai showed that mutation within the RING and a proximal zinc finger domain abolished TRAF-2 ubiquitination and inhibited TNF-$\alpha$-induced activation of JNK, but not of p38 or NF$\kappa$B, suggesting that the RING and therefore TRAF-2 E3 ligase activity are required for selective activation of the JNK signaling cascade. Interestingly, p38, JNK and their transcription factor substrate ATF-2 are implicated in the resistance of melanoma to radiation and chemotherapy. In contrast, an ATF-2 peptide (spanning amino acids 50–100) sensitizes melanoma cells to apoptosis and reduces their tumorigenicity. This peptide abolishes TRAF-2 nuclear localization, thereby inhibiting ATF-2 transcription. The ATF-2 peptide also binds to JNK and affects JNK activation of c-Jun. This suggests that high levels of c-Jun lacking its partner ATF-2 are, in concert with JunD, responsible for sensitization of the resistant melanoma cells to apoptosis. Inhibition of c-Jun or JunD activities abolishes sensitization, further suggesting that ATF-2 is essential to the acquisition of resistance to apoptosis. Interestingly, both TRAF-2 and ATF-2 are up-regulated in human tumors. Hence, TRAF-2 appears as the gatekeeper of stress signaling and changes in its stability and localization are expected to affect the level of ATF-2 activities and, therefore, the sensitization of tumors to treatment (22, 23).

The NF$\kappa$B/Rel transcription factor is involved in coordinating immune and inflammatory responses and in controlling cell survival. Activation of NF$\kappa$B impairs apoptosis by numerous triggers, including ligand engagement of "death receptors" such as TNFR. The anti-apoptotic activity of NF$\kappa$B is also crucial to oncogenesis and chemo- and radio-resistance in cancer. Dr. Franzoso presented evidence showing that cytoprotection by NF$\kappa$B involves the activation of pro-survival genes. Using an unbiased screen for cDNAs capable of blocking apoptosis in NF$\kappa$B/RelA/-/- fibroblasts, he has identified Gadd45$\beta$/Myd118 as a pivotal mediator of the protective activity of NF$\kappa$B against TNF-$\alpha$. gadd45$\beta$ is up-regulated rapidly by TNF-$\alpha$ in 3DO cells and by CD40 in B cells, and the inducing mechanisms
require NFκB. The expression of Gadd45β is essential to antagonize TNF-α- and Fas-induced killing, and it blocks TNF-induced apoptosis in NFκB-null cells. Gadd45β does not affect the formation of the death-inducing signaling complex or caspase 8 activity at the death-inducing signaling complex. The protective activity of Gadd45β against TNF-α-induced cell death depends on the inhibition of the JNK cascade, which is central to the control of apoptosis by NFκB. In particular, Gadd45b inhibits MKK-7 upstream of JNK. Overall, the findings of Dr. Franzoso define a novel protective mechanism that is mediated by NFκB complexes and establish a role for persistent activation of JNK in the apoptotic response to TNF-α. They also raise the relevance of targeting the JNK cascade by NFκB in attempt to modulate several biological processes, including oncogenesis and cancer chemoresistance (24).

Dr. Dong summarized his work concerning the role of the MAPK pathways in cell transformation and skin carcinogenesis. He showed that incubation of JB6 C1 41 cells with TNF-α led to cell transformation and activation of JNKs. Introduction of a dominant negative mutant JNK-1 into these cells specifically inhibited TNF-α-induced activation of JNKs, but not of ERKs or p38. Expressing dominant negative mutant JNK-1 inhibited TNF-α-induced cell transformation but not 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced cell transformation. In Jnk2−/− mice, the number of papillomas induced by TPA was lower than that in wild-type mice. Knockout of Jnk1 did not inhibit skin tumor promotion by TPA. Hence, JNK-1 activation is required for TNF-α- but not TPA-induced cell transformation. In contrast, JNK-2, but not JNK-1, is critical in tumor promotion by TPA and UVB. ERKs and p38 are required for TPA- or EGF-induced cell transformation. Dr. Dong also presented convincing data showing that natural products such as epigallocatechin and theaflavin are potent anticancer agents; the action of which results from inhibiting the phosphorylation of c-Jun by TPA. Overall, the findings of Dr. Dong indicate that tumor promotion may be mediated by different signal transduction pathways and that these latter represent targets for chemotheraphy and chemoprevention (25).

**Stress Signaling in Apoptosis**

Apoptosis is an intrinsic death program that plays a crucial role in the regulation of tissue homeostasis. An imbalance between cell death and proliferation may result in tumor formation. Also, killing of tumor cells by chemical and physical means is predominantly mediated through apoptosis. Therefore, the ability of tumor cells to evade engagement of apoptosis can play a significant role in their resistance to treatments. Understanding the signaling events that regulate apoptosis and how cancer cells evade apoptotic events may be instrumental in developing effective treatments against cancer. In the third session of the meeting, the speakers addressed the underlying mechanisms of apoptosis and the ways by which tumor cells modulate these processes to promote their survival. They discussed how to exploit cell signaling pathways to selectively induce apoptosis in tumor cells.

Talks were given by Drs. Christopher W. Pugh (University of Oxford, United Kingdom), José N. Lavoie (Université Laval, Québec, Canada), Chang-Yan Chen (Boston University, Boston, MA), Normand Marceau (Université Laval, Québec, Canada), Jorge Filmus (Sunnybrook and Women’s College Health Science Center, Toronto, Canada), Heidenori Ichijo (Tokyo University, Japan), and Jacques Landry (Université Laval, Québec, Canada).

As mentioned by Dr. Pouysségur, HIF-1 is a master regulator of the transcriptional response to hypoxia. This transcription factor controls processes such as angiogenesis and erythropoietin production, and it influences cell proliferation and survival. HIF-1 is a heterodimer consisting of αβ isoforms. These isoforms have overlapping, but distinct, effects on gene expression. In his talk, Dr. Pugh showed that HIF activity is dominantly regulated via its α isoform. In normoxia, HIFα is targeted for proteasomal breakdown as a result of iron and oxoglutarate-dependent dioxygenase-induced hydroxylation of particular prolyl residues. Under normal oxygen conditions, asparaginyl hydroxylation by FIH further contributes to negatively regulate the transcriptional activity of HIF-1 by blocking the recruitment of coactivators such as p300. Interestingly, HIF activity is not only increased by hypoxia but also by genetic alterations. The most striking genetic cause of HIF activation in cancer is found in the VHL syndrome. The VHL protein is a tumor suppressor that plays a part in processes that inactivate HIF in normoxia. It acts as the recognition component of the E3-ubiquitin ligase complex involved in HIF-α destabilization and interacts with FIH. In VHL patients, the VHL protein is mutated and nonfunctional, which leads to constitutive HIF activation and the development of tissue-restricted tumors, such as renal cell carcinoma.

Dr. Pugh also mentioned the paradoxical finding that HIF can activate the expression of pro-apoptotic genes. This led him to elaborate on his coselection hypothesis. According to this hypothesis, it is inevitable that strong cell-autonomous selection of one component of a physiological regulatory pathway will be associated with the coselection of many properties that are linked to the pathway. The hypothesis can explain the selection of multiple pro-angiogenic effects that benefit the tumor as a whole. This concept has wide implications for the control of complex physiological regulatory pathways in cancer, as well as for the design of therapeutic targets (26).

Defects in regulators of conventional apoptosis pathways are pre-requisites, together with activation of oncogenic functions, for cellular transformation. As a consequence of oncogenic activation, cells have to sustain a higher threshold of apoptotic stimuli and become sensitized to a number of them, which directly interferes with oncogenic signaling. Evidence indicates that the adenovirus death protein E4orf4 triggers the selective killing of transformed cells (27). Dr. Lavoie found that Ad2 E4orf4 induces a unconventional caspase-independent death pathway associated with dramatic changes in cell morphology and apoptosis-like nuclear and chromatin condensation in human cancer cells. She reported that part of this killing activity involved direct binding of E4orf4 to the kinase domain of Src, leading to Src activation, E4orf4 phosphorylation on specific tyrosine residues, and recruitment of specific Src substrates, including p62Dok and cortactin. Notably, the kinase domain of Src alone was sufficient to sustain the in vivo phosphorylation of E4orf4 and cell killing. Conversely, all mutations affecting Src binding strongly inhibited E4orf4-mediated apoptotic changes and cell death. Dr. Lavoie also reported preliminary data suggesting that E4orf4 does not significantly affect major Src-regulated survival pathways, namely the ERKs and protein kinase B pathways. The Rho GTPases rather appear to play a critical role in transduction of the cytoplastic death signal triggered by Ad2 E4orf4. These findings are important because unraveling the mechanisms by which E4orf4 usurps oncogenic signaling (Src) to trigger cell death may uncover novel ways for manipulating up-regulated functions in tumor cells to promote their selective killing (28, 29).

Dr. Chen focused on the relationship between Ras and p53 in regulating apoptosis. She reported that mouse fibroblasts that express wild-type p53 are more susceptible to apoptosis elicited by PKC inhibition if Ras is transiently expressed or up-regulated, as opposed to stably expressed. In the latter case, p53 is frequently mutated. Transiently increased Ras activity induces Bax, and PKC inhibition enhances this induction. In contrast, Bax is not induced in stable Ras transfectants, regardless of PKC inhibition. She also demonstrated that p53 is accumulated by suppression of endogenous PKC activity in a form incapable of inducing transcription. Moreover, a transient increase of Ras activity reactivates p53 function, which is accompa-
nied with the phosphorylation on Ser37. Suppression of JNK abolishes this phosphorylation, and partially blocks the apoptotic process mediated by a transient increase of Ras activity. She concluded that different mechanisms are used by short- and long-term activation of Ras to initiate apoptosis and that the status of p53 may contribute to such differences (30).

Dr. Marceau emphasized the abnormalities in cytoskeletal elements that can lead to apoptosis de-regulation. He spoke about cytokeratins K8/K18, the pair of intermediate filaments (IF) that constitute a hallmark for all simple epithelial cells and that are frequently re-expressed in various types of nonepithelial tumors. The expression of K8/K18 confers resistance features to several stresses in carcinoma cell lines. Interestingly, the IFs of mouse hepatocytes are made solely of K8/K18. Thus, the loss of K8 or K18 in mice via a targeted null mutation in the germ line results in hepatocytes lacking IFs. Hepatocytes respond massively to Fas stimulation, and K8-null mouse hepatocytes are more susceptible to Fas-mediated apoptosis. This implies that K8/K18 provide resistance to Fas-mediated apoptosis. Dr. Marceau showed that K8/K18 regulate Fas-mediated apoptosis at two different levels, namely by modulating Fas density at the cell surface via a change in receptor trafficking, and by affecting the anti-apoptotic ERK-1/2 signaling pathway (31).

Endothelial cells and most epithelial cells undergo apoptosis if they are detached from the extra-cellular matrix. This form of cell death is termed “anoikis.” Dr. Filus has shown that anoikis in intestinal epithelial cells involves the overexpression of Fas ligand, an event that requires detachment-induced activation of p38. One of the initial steps in the development of tumors derived from the intestinal mucosa is the invasion of the surrounding stroma. This invasive process requires the detachment of the transformed intestinal epithelial cells from the basal membrane, which is usually destroyed by proteases secreted by such cells. It is evident, therefore, that intestinal tumor cells have to become resistant to anoikis to invade and metastasize. Oncogenes, such as ras and src, which are activated in a large proportion of colorectal cancers, are strong inhibitors of anoikis. In particular, activated Ras induced a down-regulation of Bak activity, which may contribute to Ras-induced protection against anoikis. TGF-α, a growth factor secreted by many colorectal tumors, can also inhibit anoikis by preventing detachment-induced inhibition of c-Src kinase activity and Bcl-XL down-regulation. Because the restoration of anoikis sensitivity suppresses tumor growth, the work of Dr. Filus may facilitate the design of successful “pro-anoikis” therapy for colorectal cancer (32).

Dr. Ichijo reviewed his recent work on the role of the apoptosis signal-regulating kinase ASK-1 in apoptosis. The deletion of the Ask1 gene in mice indicates that ASK-1 plays essential roles in oxidative stress- and endoplasmic reticulum (ER) stress-induced apoptosis. In the case of oxidative stress or after reactive oxygen species production by TNF-α, ASK-1 activation results from its release from an inactive complex with thioredoxin. This triggers activation of ASK-1 by inducing oligomerization and phosphorylation of critical threonine residues in the activation loop of ASK-1. After TNF-α, ASK-1 binds TRAF-2, which helps to further enhance and stabilize the oligomeric activated form of ASK-1. Then, activation of ASK-1 triggers the activation of the JNK and p38 pathways, which leads to apoptosis. Note, oxidative stress and ER stress are closely linked to various physiological phenomena in the control of cell fate, and the resultant apoptosis is implicated in the pathophysiology of a broad range of human diseases. Dr. Ichijo also presented evidence that the ASK-1-p38 pathway played essential roles in innate immune responses. Overall, ASK-1 appears as a multifunctional stress-sensing kinase that controls cell fate in response to various stresses (33, 34).

Oncogenic transformation increases the sensitivity of cancer cells to apoptosis, which may explain why chemotherapy is mostly effective during the early stage of cancer. Unfortunately, transformation is accompanied by a pressure of selection which leads to accumulation of defects in the apoptotic pathways and to resistance to chemotherapy. Dr. Landry investigated the mechanisms of resistance to apoptosis in cancer cells using Rat1 cells with deregulated expression of c-Myc. In these transformed cells, in contrast to parental cells, he showed that cisplatin increases the activation of ASK-1, but not of protein kinase B. This results in the selective activation of the apoptotic pathways involving p38 and JNK in the transformed cells. Dr. Landry showed that p38α is essential for the activation of the mitochondrial apoptotic machinery, upstream of BAX/BAK activation and for HSP-27-mediated membrane blebbing. Inhibiting p38α protects from apoptosis induced by cisplatin and other DNA-damaging agents and it prevents cell death induced by deprivation of serum. The results provide a potential mechanism for explaining the oncogenic action of the p38 phosphatase Wip1, as discussed by Dr. Appella (35, 36).

Acknowledgments

We thank the participants who kindly agreed to review the write-up that summarizes their presentation. We are especially grateful to Drs. Etore Appella, François Houle, and Julie Lefèrivère for critically reading the manuscript.

The organizers thank Bruno Bégin and his team from Hospitalité Québec and all of the sponsors who made the meeting possible: International Union of Biochemistry and Molecular Biology, Canadian Institutes for Health and Research-Institute of Cancer Research, Finances, Économie et Recherche Québec, the Centre de recherche de l’Hôpital-Dieu de Québec, the Faculté de médecine de l’Université Laval, AstraZeneca, Roche, Medicorp Inc., and Bio-Rad. Chantale Morin holds a studentship from La Fondation Dr. Georges Pénix.

References


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RECENT ADVANCES IN STRESS SIGNALING IN CANCER


Corrections

In the article by C. Morin and J. Huot, titled “Recent Advances in Stress Signaling in Cancer,” which appeared in the March 1, 2004 issue of Cancer Research (pp. 1893–1898), the expression “post-heparin plasma diamine oxidase 2” was erroneously substituted for “HIF prolyl hydroxylase 2” in the “Nutritional Stress and Cancer” section, which followed the “Introduction.” The corrected sentence is as follows: Small interfering RNA-mediated ablation of both HIF prolyl hydroxylase 2 and FIH (singly or simultaneously) was sufficient to activate hypoxia-inducible genes during normoxia.
Recent Advances in Stress Signaling in Cancer
Chantale I. Morin and Jacques Huot


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