Poster presentations (Wed, 2 Nov)

Basic science

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Characterization of a novel p53-interacting protein

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Mutations in the p53 tumor-suppressor gene occur in more than 50% of human cancers of diverse types and a mouse model with homozygous deletion of p53 shows early onset of multiple tumor types. These studies emphasize the importance of p53 function in tumor development. Research over the last several years has revealed that p53 has a number of biological activities including cell-cycle arrest, apoptosis and DNA repair. However, the exact molecular mechanism by which p53 suppresses tumor formation still remains elusive. A key aspect to understanding p53 function is the identification and analysis of proteins that interact with it.
Using the Sos Recruitment System (SRS) library screen, we have identified a novel p53-interacting protein 1 (pip1). Pip1 is a specific p53-interacting protein in the SRS. The interaction of p53 and pip1 was further confirmed by *in vitro*, *in vivo* binding assays and protein colocalization studies. Pip1 gene is located on human chromosome Xq22. Northern blot analysis showed that the size of its message is approximately 3 kb and that pip1 is preferentially expressed in mouse brain, heart, liver and kidney. The ORF of full-length-pip1 cDNA encodes a protein of 428 amino acids with calculated molecular weight of 46 kDa.
The interaction of p53 and pip1 in mouse tissues can only be detected in the presence of ionizing radiation, suggesting that this interaction might be important in DNA-damage-induced p53-signalling pathway. One of the most interesting findings is that the cellular localization of pip1 is affected by p53 in transiently transfected cells. In the absence or low level of p53, pip1 is exclusively localized in cytosol, whereas pip1 is primarily observed and colocalized with p53 in nucleus when p53 was coexpressed with pip1. This observation not only provides another evidence of the interaction of these two proteins but also renders us some clues for the function of pip1 on p53. On the other hand, we found that pip1 downregulates the transactivation activity of p53 on both p21 and mdm2 promoters. More importantly, depending on the cellular context, pip1 can suppress p53-induced apoptosis and potentiate the G2/M checkpoint initiated by p53, whereas, as controls, pip1 can not rescue cells from the apoptosis which is not induced via p53 signaling pathways, and pip1 has no effect on cell cycle profiles in the absence of p53. Recently we have made a significant discovery of the biochemical consequences of the interaction between p53 and pip1. In several stressed conditions, the stabilization of p53 is considerably attenuated and the kinetics of p53 accumulation and degradation is completely altered when pip1 is overexpressed in cultured cells. In all, our results of both binding and functional studies strongly suggest that pip1 might function as a negative regulator, as mdm2 does, in DNA-damage-induced p53-signaling pathway.

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p53 and chemosensitivity in astrocytic gliomas

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Over 2000 Canadians are diagnosed yearly with glial neoplasms, accounting for two-thirds of primary brain tumors. Sadly, current therapies fall short of providing effective treatment. Our research is directed towards developing therapies based on molecular alterations in these tumors. More specifically, we have been focusing on the relationship between the integrity of the tumor suppressor protein p53 and sensitivity to traditional cytotoxic chemotherapies. Typically p53 is thought to play a protective role in the genome. Under a variety of genotoxic and non-genotoxic stressors, p53 is 'activated', inducing cell cycle arrest or apoptosis. The relationship between response to cytotoxic therapies, survival time and p53 status is largely unknown for astrocytic gliomas. However, dramatic tumor responses to drugs such as 1, 3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and the DNA methylating agent, temozolomide (TMZ), sometimes occur and some patients with astrocytic gliomas have long survival times.
In this study, we test the premise that p53 disruption is a chemosensitizing genetic alteration in astrocytic gliomas. This hypothesis is supported by the observation that fibrillary astrocytomas, evolving slowly and stepwise to higher malignancy stages, typically harbor p53 mutations and have a significantly better prognosis. Here, using the MTT assay, we demonstrate that p53 inactivation sensitizes tumor cells of astrocytic derivation to several cytotoxic chemotherapies commonly used clinically, such as BCNU, TMZ, Cisplatin and CPT-11; sensitization is associated with an inability to induce p21CIP1 expression and failure of cell cycle arrest in G1 or G2. Furthermore, chemosensitization following inactivation of p53 is independent of MGMT, a DNA repair protein silenced in drug sensitive high-grade astrocytic gliomas. The cells used in our experiments contained a methylated MGMT promoter, and as such did not express MGMT.
Our data suggest a correlation between p53 status and response to chemotherapy. These observations are potentially significant in that p53 is mutated in 40% of human gliomas, and thus, like MGMT, could serve as an independent predicative marker. This work has the potential to refine and improve the prescription of chemotherapies for individual patients with astrocytic gliomas.

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***In vitro* biological activity of SB-497115, an orally bioavailable, small molecule platelet growth factor**

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SB-497115 is an investigational small molecular weight, orally active, thrombopoietin (Tpo) receptor agonist that requires Tpo receptor (TpoR) expression for activity. The therapeutic target for SB-97115 is a decrease in frequency or elimination of severe thrombocytopenia associated with thrombocytopenic diseases such as cancer chemotherapy or immune thrombocytopenic purpura (ITP). SB-497115 requires TpoR to activate the JAK/STAT signalling pathway and stimulates transcription through the STAT based (IRF-1) and megakaryocyte specific (gpIIb) promoters.
An analysis of the receptor selectivity of SB-497115 was undertaken utilizing a panel of various transfected and non-transfected cell lines in which other cytokines, including G-CSF, Epo, IL-3, Interferon-alpha or Interferon-gamma, were active. SB-497115 was inactive over a three-fold concentration range in proliferation, reporter gene, or STAT activation assays performed on cell lines that did not express TpoR. To characterize the kinetics and specificity of SB-497115 in cells, multiple molecular markers for Tpo activity were measured. Western blot analysis for activation of the STAT and MAPK pathways was performed using phospho-specific antibodies on lysates of UT7-Tpo cells treated with SB-497115. The kinetics and level of induction for pathway phosphorylation events were similar to that seen with Tpo. SB-497115 was shown to be equal to or better than rhTpo in the ability to induce differentiation of normal human CD34+ marrow progenitors into CD41+ cells of the megakaryocyte lineage, with an EC50 of 100 nM. SB-497115 demonstrated specificity for human and chimpanzee TpoR with no responses from TpoR of other species, such as cynomolgus macaques.
Data obtained utilizing chimeric human/cyno receptors suggest a model in which TpoR agonist compounds interact with a residue in the transmembrane domain to change the conformation of TpoR or to induce dimerization, resulting in activation of the signal transduction pathways of TpoR. In summary, SB-497115 is a small molecule TpoR agonist demonstrating activity in human cell lines and *in vitro* bone marrow assays and imparting biologically relevant function.

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Sphingosine kinase as a "sensor" to chemotherapy in prostate cancer

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Ceramide and sphingosine 1-phosphate are interconvertible sphingolipids playing opposed roles in apoptosis. Ceramide functions as a pro-apoptotic molecule while sphingosine 1-phosphate exhibits anti-apoptotic properties, leading to the hypothesis that the balance between ceramide and sphingosine 1-phosphate levels determined by S1P-forming enzyme – sphingosine kinase (SK) might decide the cell fate. SK has also been reported to be oncogenic and tumor-related enzyme.
Here, we examined the involvement of SK in susceptibility to antineoplastic agents of prostate cancer cells. Camptothecin, a known apoptosis inducer in LNCaP prostate cancer cells, is much less effective in PC < 3 cell line. On the contrary docetaxel treatment caused massive loss of cell viability in PC-3, but had much lesser effect in LNCaP cells. Both docetaxel and camptothecin induced inhibition of sphingosine kinase and increase of ceramide/sphingosine 1-phosphate ratio only in cell lines sensitive to the drugs, but not in the more resistant ones. Enforced expression of sphingosine kinase in PC-3 and LNCaP cells restored their resistance to chemotherapy, notably by decreasing ceramide/sphingosine 1-phosphate ratio. On the other hand, in both cell lines, siRNA to SK

or pharmacological inhibition of this enzyme induced profound apoptosis, which coupled with significant elevation of intracellular ceramide and loss of sphingosine 1-phosphate. *In vivo*, both docetaxel and camptothecin treatment of fluorescent tumors consistent of PC-3/GFP cells o.t. implanted in nude mice induced primary tumor and lymph nodes' volume regression, markedly docetaxel being twice more potent than camptothecin. These events were coupled to sphingosine kinase inhibition and elevation of ceramide/sphingosine 1-phosphate ratio both in primary tumors and in lymph nodes. Markedly, docetaxel treatment abrogated migration of cancer cells and formation of micrometastases.
Collectively our results show that apoptosis induction by chemotherapy in prostate cancer cells is correlated with sphingosine kinase inhibition. Ability of sphingosine kinase to determine the resistance of cancer cells to chemotherapy might propose its role as a responsive element in proapoptotic signaling. Thus modulation of SK activity and thus of ceramide/S1P balance might find an application in cancer treatment.

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Different expression of tight junction proteins in HCC and metastatic liver tumours

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Background: Tight junction (TJ) proteins have already been found implicated in carcinogenesis. A group of integral membrane proteins – occludin, claudins and junctional adhesion molecules – interact with cytoplasmatic tight junction proteins to integrate diverse processes (e.g. tumour suppression, gene transcription, cell polarity).
Material and methods: Expression of claudins, occludin, junctional adhesion molecule (JAM)-1, -2, -3 and zonula occludens (ZO)-1, -2, -3 was analysed in 15 human hepatocellular carcinoma (HCC) and 15 colorectal metastasis in liver to study TJ in liver malignancies. Gene expression levels were measured by real-time PCR, protein expression was determined by immunohistochemistry and Western blot comparing tumours to surrounding parenchyma and to normal liver samples (7).
Results: ZO-1, -2, -3, JAM-1, -2, -3 and occludin mRNAs were significantly downregulated in HCC compared to normal liver (4.6×; 15.3×; 18.2×; 12.9×; 5.9×; 3.3× and 8.2×) and ZO-2, -3, JAM-2 and occludin mRNAs were also significantly downregulated compared to surrounding tissues (3.4×; 5×; 3.2× and 2.2×). In metastasis claudin-4 was significantly upregulated (12.7×), while ZO-1, -2, JAM-1, -2 and occludin were downregulated (6.4×; 9.6×; 9.4×; 18.6× and 12.1×) with respect to normal liver. Immunohistochemistry basically supported RNA expression data. Claudin-3, -4 and -7 staining were very strong in metastasis, while only scattered weak in HCC. TJ proteins were generally weakly expressed on hepatocytes, while strongly on bile canaliculi and arterioles in normal liver.
Conclusions: HCC and metastasis show different pattern of expression of TJ components. Differences in ZO-3, claudin-3 and -4 could be used for differentiation of the primary and secondary tumour. The origin of metastatic tumour could influence TJ protein expression, especially different organs can be characterized by their claudin expression.
This project was supported by grants: NKFP-1/0023/2002, NKFP-1A/002/2004, OTKA T-049559

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Characterization of genes with increased expression in glioblastomas

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Background: In the present study, we have used the gene expression data available in the SAGE database in an attempt to identify *glioblastoma molecular* markers.
Material and methods: Nine SAGE libraries of human glioblastoma (GB), six SAGE libraries of GB cell lines, and five SAGE libraries of normal human brain (NB) were analyzed to compare gene expression in GB with that of NB by accessing SAGE NCBI web site http://www.ncbi.nlm.nih.gov/SAGE and using the search tool cDNA Digital Gene Expression Displayer (DGED) provided by the SAGE Genie database. Northern blot analysis was performed for confirmation of enhanced expression of activated genes in glioblastoma.
Results: Of 129 genes with more than 5-fold difference ($P \leqslant 0.05$) found by comparison of nine *glioblastoma* vs. five normal brain SAGE libraries, 44 increased their expression in *glioblastoma*s. High expression of 21 genes in *glioblastoma*s as well as in *glioblastoma* cell lines suggested that expression in the bulk tumors was from transformed cells. Increasing of expression of 23 other genes only in *glioblastoma*s but not in *glioblastoma* cell lines suggested that expression in the bulk tumors was from macrophages/microglial cells. Many of the latter genes are among of the top transcripts in activated macrophages and are involved in the immune response and angiogenesis.
Conclusion: Since constituent parts of tumor, *primary tumor tissue and microglia*, both participate in the tumor growth and development, all genes with highest levels of expression in glioblastomas can be used as molecular markers in the analysis of malignant progression of astrocytic tumors. Moreover, several of genes overexpressed in glioblastomas, produce extracellular proteins, thereby providing opportunities for clinical application. Further characterization of these genes will allow them to be exploited in molecular classification of glial tumors, diagnosis, prognosis, and anticancer therapy.

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Pramanicin induces apoptosis in Jurkat leukemia cells: a role for JNK, p38 and caspase activation

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The improvement in our understanding of the regulation of the molecular machinery of apoptosis reveals that the suppression of apoptosis in the presence of a proliferative stimulus is critical for tumour development. It has been clarified that new therapeutic approaches based on drug targets in apoptotic pathways will improve the response of patients to "target specific" therapeutic approaches. Pramanicin is a novel anti-fungal drug with a wide range of potential application against human diseases. In the present study, we showed that pramanicin induced apoptosis in Jurkat T leukemia cells in a dose- and time-dependent manner.
Our data reveal that pramanicin induced the release of cytochrome *c* and caspase-9 and caspase-3 activation, as evidenced by detection of active caspase fragments and fluorometric caspase assays. Pramanicin also activated c-jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinases (ERK 1/2) with different time and dose kinetics. Treatment of cells with specific MAP kinase and caspase inhibitors further confirmed the mechanistic involvement of these signalling cascades in pramanicin-induced apoptosis. JNK and p38 pathways acted as pro-apoptotic signalling pathways in pramanicin-induced apoptosis, in which they regulated release of cytochrome *c* and caspase activation. In contrast the ERK 1/2 pathway exerted a protective effect through inhibition of cytochrome *c* release from mitochondria and consequent caspase activation, which were only observed when lower concentrations of pramanicin were used as apoptosis-inducing agent.
These results suggest pramanicin as a potential apoptosis-inducing small molecule, which acts through a well-defined JNK- and p38-dependent apoptosis signalling pathway in Jurkat T leukemia cells. Studies focusing on different cancer cell lines and/or experimental animal models will further extend our understanding of mechanisms involved in apoptotic response to pramanicin and will allow us to better evaluate the anti-cancer potential of this molecule.

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MDGA1, a novel human protein with a functional role related to cellular adhesion

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Background: We have reported the characterization of the novel human protein MDGA1 (MAM Domain containing Glycosylphosphatidylinositol Anchor-1 protein). The deduced polypeptide exhibits structural features found in different types of Cell Adhesion Molecules (CAMs), such as the presence of both immunoglobulin domains and a MAM domain or the capacity to anchor to the cell membrane by a GPI (GlycosylPhosphatidylInositol) motif. *MDGA*1 encodes a 955 aminoacids protein containing an N-terminal signal peptide followed by six immunoglobulin-like (Ig) domains, one single fibronectin type III (FnIII) domain, a MAM (meprin, A5 protein, receptor protein-tyrosine phosphatase μ) domain and a C-terminal containing a cleavage site for GPI (GlycosylPhosphatidylInositol) anchoring to the cell membrane. The presence of multiple Cell Adhesion Molecule-like domains in MDGA1, lead us to hypothesize a functional role related to cellular adhesion for this protein.