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