located in the most frequent chromosomal imbalance were studied by quantitative RT-PCR (qPCR) confirmed by immunohistochemistry and functional studies (MTT assay with specific inhibitors). Relevant genes were sequenced to identify potential oncogenic mutations.
Results: CGH array revealed a significant increase of 9q34 gain in relapsing tumours. Candidate genes over-expressed included Notch 1. The entire Notch pathway was studied by qPCR and more than 80% of the ependymomas had at least 1 ligand (JAG1, DLL1 and DLL3), one receptor (Notch 1 and 2), and target genes (HES1, HEY2 and c-Myc) over-expressed. Immunohistochemical analysis of Hes1 was strongly positive in 14 out of 17 samples. Six samples (8.3%) from 3 different patients exhibited Notch 1 missense mutations: one in the HD-C domain and the two others in the TAD domain. All 3 were patients with posterior fossa tumours and 9q34 gain. No mutations were found in the FBXW7 gene, whose product is involved in Notch 1 ubiquitylation complex, but its expression was lower than control brain in all tumour samples. We therefore studied the effect of 4 different gamma-secretase inhibitors (GSI) on primary cell-cultures. These inhibitors will prevent the third cleavage of Notch and here by the activation of the pathway. All 4 GSI were found to be active at a concentration below 5 μM and completely abrogated cell growth similarly to the positive control DAOY medulloblastoma cell line. GSI at 3 μM were able to suppress neurospheres formation in a limiting dilution assay with ependymoma stem cell.
Conclusion: Our results suggest that Notch pathway is a key-player in tumorigenesis and progression of pediatric ependymomas. Notch 1 is the first oncogene with mutations found in ependymomas. Notch 1 overexpression could be explained in part by the down regulation of its degradation complex (FBXW7). Notch pathway may represent a potential new target for the treatment of ependymoma.

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Specific activation of microRNA 106b targets the ubiquitin ligase ITCH to enable the p73 apoptotic response in chronic lymphocytic leukemia

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Background: Chronic lymphocytic leukemia (CLL) is a disease characterized by enhanced survival of lymphocytes. Mutations in p53 and low levels of p73 offer a survival advantage in CLL. MicroRNAs (miR) are a class of regulatory molecules that are largely downregulated in chronic lymphocytic leukemia (CLL). Mutations as well as epigenetic mechanisms may be responsible for the observed silencing of miRs in CLL. Consequently, epigenetically directed agents, such as histone deacetylase inhibitors (HDACi) may restore the expression of miRs to therapeutic advantage in CLL. The purpose of this investigation was to determine the molecular mechanisms by which miRs influence p73-dependent apoptotic signaling.
Materials and Methods: Primary CLL cells were obtained after informed consent, leukemia cells were isolated and exposed to LBH 589 (Panabinostat) and other HDACi for up to 48 h. ChIP assays, RNA and protein were isolated and utilized for various assays.
Results: Exposure of CLL cells to LBH589 (Panobinostat) and other HDACi resulted in upregulation of miR106b in conjunction with its primary host transcript, Mcm7. Upregulation of miR106b was associated with an increase in the levels of acetylated histone H3, increased recruitment of RNA polymerase II, E2F1 and myc onto the miR106b promoter. Mutation of E2F1 and myc abrogated the transcriptional induction of miR106b in response to LBH589. By analyzing the homology between miRNA106b and the Itch mRNA sequence we found that seven nucleotides from the 5' end of this miRNA were complementary to bases 1382–1388 of the Itch cDNA. Ectopic expression of miR106b in K562 leukemia cells and primary CLL samples demonstrated that miR106b caused a downregulation of Itch protein without any effect on its mRNA. Significantly, upregulation of miR106b in CLL cells in response to LBH589 was associated with a decline in the levels of the E3 ubiquitin ligase Itch. In unstressed cells, the constitutive action of Itch mediates the proteosomal degradation of p73 to maintain low levels. Consequently, the miR106b-mediated decline in the levels of Itch caused an accumulation of p73. Increases in p73 were associated with a consequent induction of the transcript and protein levels of the proapoptotic Bcl-2 family member PUMA, declines in mitochondrial-membrane permeability, and induction of cell death in CLL samples.
Conclusions: Our results define a mechanism by which exposure to LBH589 activates a microRNA-initiated cascade that relieves the suppression of p73 to target malignant cells.

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OXA-01, a novel potent mTORC1/TORC2 kinase inhibitor, demonstrates broad spectrum antitumor activity in preclinical models of human cancer

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Background: The mammalian target of rapamycin (mTOR) protein kinase plays a central role in regulating cell proliferation and cell survival. The PI3K/AKT signaling pathways that activate mTOR are frequently altered in many human cancers. mTOR exists as multi-protein complexes with distinct signaling functions. Complexes containing mTOR and RAPTOR (mTORC1) phosphorylate S6K and 4E-BP1 and are rapamycin sensitive. Whereas, complexes that contain mTOR and RICTOR (mTORC2) phosphorylate AKT and 4E-BP1 and are insensitive to rapamycin treatment. Our goal was to identify a potent dual inhibitor of mTORC1 and mTORC2 with potentially improved therapeutic utility.
Methods and Results: OXA-01 was discovered and optimized by a combination of medicinal chemistry exploration and use of a PI3K-gamma-derived structural homology model. It is a selective small molecule inhibitor of both mTORC1 and mTORC2 kinase activity with IC_{50} values of 29 nM and 7 nM, respectively. In MDA-MB 231 breast carcinoma xenograft model, a single 50 mg/kg oral dose of OXA-01 resulted in sustained inhibition of 4E-BP1 phosphorylation up to 8 h in tumor tissue, corresponding to plasma levels >12 μM. At 16 h following OXA-01 administration, drug concentration decreased to <0.1 μM with concomitant return of p4E-BP1 levels to control. Once daily administration of OXA-01 at 50 mg/kg for 14-days resulted in 50% inhibition of MDA-MB 231 tumor growth (TGI) compared to control vehicle treated animals. In comparison, at a higher dose of 75 mg/kg on a twice daily schedule, significant inhibition of 4E-BP1 phosphorylation was observed up to 24 h. Such extended target suppression was associated with improved efficacy corresponding to 100% TGI in the MDA-MB 231 xenograft model. Consistent with its ability to inhibit mTORC2, OXA-01 administration resulted in sustained inhibition of phosphorylation of AKT (Ser473) in tumors up to 24 h post treatment. In addition to significant efficacy in MDA-MB 231 breast carcinoma model, OXA-01 demonstrated robust anti-tumor activity in several different human xenograft models of various histologies, including carcinomas of colon, non-small cell lung, prostate, ovarian and melanomas.
Conclusions: These data suggest that a dual mTORC1/2 kinase inhibitor, such as OXA-01, may have significant therapeutic utility, warranting further clinical investigation of this novel class of anticancer agents.

Thursday, 23 October 2008 16:30–18:15

PLENARY SESSION 7

Targeting protein translation and protein–protein interaction in cancer

251 INVITED

An approach for targeting protein–protein interactions

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Many important biological processes involve protein–protein interactions and are thus potentially important targets for drug discovery. However, due to the lack of deep pockets and large surface areas involved in these interactions, they may be difficult to target. A strategy for targeting protein–protein interactions will be presented that involves the identification of suitable protein targets from NMR-based screening, the discovery of small lead molecules that bind to the protein using SAR by NMR, and structure-based lead optimization. An example of this approach will be described for the discovery of a Bcl-2 family inhibitor that is currently in clinical trials for the treatment of cancer.