Thursday 30 September 16:30–18:15

PLENARY SESSION 7

Chromatin modifying agents

250 INVITED

Modification of the histone code: effects of DNA methylation

S. Clark. *Garvan Institue of Medical Research, Epigenetics and Cancer, Sydney, NSW, Australia*

DNA methylation in mammals involves the addition of a methyl group to the cytosine residue of a CpG dinucleotide. The DNA methylation pattern of a cell is complex and is exquisitely controlled during early development. In fact a loss of DNA methylation results in embryonic death. Methylation of the promoter of a gene is associated with silencing, but the precise mechanism by which DNA methylation confers silencing is still not clear, but the involvement in histone remodeling is critical. Hypermethylation is associated with heterochromatin and binding of deacetylated and methylated histones, whereas hypomethylated DNA is associated with eurchromatin and binding of acetylated and unmethylated histones.
Improper methylation patterns are associated with many disease states, in particular with cancer. Some of the earliest changes in a cancer cell involve reversal of the embryonic methylation pattern, with repeated sequences becoming demethylated and the normally unmethylated regions of house-keeping genes becoming hypermethylated. CpG island associated genes, in particular tumour suppressor, are often hypermethylated in cancer and it is this hypermethylation that results in silencing of these important genes. DNA methylation changes in cancer and therefore promotes changes to the chromatin structure. Therefore both DNA methylation and chromatin modification provide novel cancer markers as well as a novel therapeutic targets for cancer treatment.

251 INVITED

Histone deacetylase inhibition – a promising anticancer therapeutic strategy

P. Atadja[1], M. Hsu[1], P. Kwon[1], R. Pili[2], K. Bhalla[3], A. Wood[1]. *[1]Novartis Institutes for Biomedical Research, Oncology Research, Cambridge, MA, USA; [2]Johns Hopkins University, Sidney Kimmel Cancer Center, Baltimore, MD, USA; [3]University of South Florida, Moffit Cancer Center, Tampa, FL, USA*

Histone deacetylases (HDACs) play key roles in maintaining chromatin structure and regulation of gene expression. Recent studies indicate that abnormal histone deacetylase activity may lead to aberrant expression of oncogenes and/or tumor suppressor genes resulting in cancer. In leukemias, HDACs have been linked with translocated oncoproteins to suppress the expression of genes necessary for normal differentiation. In addition, overexpression of HDACs has been reported in solid tumors such as colon, prostate, gastric and lung. HDACs are therefore a promising target class for the development of anti-cancer drugs. Through iterative design and combinatorial chemistry, we have obtained the HDAC inhibitors LAQ824 and LBH589 which are currently in clinical development. The compounds are cinnamic hydroxamic acids which inhibit HDAC activity in low nanomolar concentrations, selectively induced apoptosis in tumor cells but not in normal fibroblasts or epithelial cells. When administered to athymic nude mice bearing a variety of tumor xenografts, LAQ824 and LBH589 produce tumor stasis or regression in tolerable doses. Analysis of histone-H3 and H4, in drug treated cells and tumor xenografts, showed increased histone acetylation thus correlating inhibition of histone deacetylases with the anti-tumor activity. In studies to understand the mechanism of the antiproliferative effect of LAQ824 and LBH589, we have found that the compounds activate the expression of the cdk inhibitors p21 and p27 and caused hypophosphorylation of Rb. Surprisingly, these cell cycle inhibitory effects did not result in the expected arrest of the cells in the G1 phase. These results suggest the absence or inactivation of normal cell cycle checkpoints leading to apoptosis. Moreover, treatment of tumor cells with LAQ824 leads to increased acetylation and loss of chaperone function in HSP90 with resultant degradation of protein substrates Bcr-Abl c-RAF, pAKT, Her-2-neu, phospho-AKT and other known oncogenic and tumor cell survival proteins. LAQ824 also activates the expression of mediators of pro-apoptotic proteins such as TRAIL, DR4, DR5 and inhibits the expression of anti-apoptotic proteins such as FLIP, Bcl-$_{XL}$, survivin and XIAP. The compounds also inhibit the expression of angiogenenic factors such as VEGF, HIF1-α and TIE-2. Taken together, the HDAC inhibitors produce anti-tumor activity through multiple pathways including cell growth inhibition, apoptosis and anti-angiogenesis. LAQ824 and LBH589 are undergoing clinical investigation in solid and hematological malignancies.

252 INVITED

Promising combinations of HDAC inhibitors and other agents

K.N. Bhalla. *H. Lee Moffitt Cancer Centre, Interdisciplinary Oncology, Biochemistry, Molecular, Tampa, USA*

The acetylation status of lysine residues in the histone tails in the chromatin, specific transcription factors, as well as in other proteins including α tubulin and the heat shock protein 90 (hsp90), is dictated by the balance between the activities of histone acetyl transferases (HATs) and histone deacetylases (HDACs). This creates the possibility that modulation of the function of specific HDACs, may not only affect gene transcription but also modify the function of other proteins involved in important biologic functions in the cell. Aberrant activity of HATs and HDACs resulting in aberrant gene transcription is a hallmark of many cancers, including many hematologic malignancies. Several chromosomal translocations in leukemia that produce chimeric fusion oncoprotein that dominantly repress transcription of genes involved in growth inhibition, differentiation and apoptosis by recruiting HDACs to their promoters. These chimeric dominant repressors of gene transcription often collaborate with another set of gene mutations, which result in constitutively active receptor or cytosolic protein kinases, e.g., Bcr-Abl, FLT-3, c-Kit, and PDGFR β. Deregulated activities of these protein kinases signal for increased growth and survival of the leukemia cells. Thus HDACs and deregulated protein kinases are legitimate targets for therapy in leukemia. HDAC inhibitors (HDIs) of the hydroxamic acid analogues (HAA) category, e.g., SAHA, LAQ824 and LBH589, have been shown to induce p21 (transcriptionally) and p27, and promote cell cycle G1 arrest, differentiation and apoptosis of acute leukemia and cancer cells. Recent studies from our laboratory have demonstrated that HAA-HDIs, e.g., LAQ824, also induce acetylation of hsp90 and inhibit its ATP binding and chaperone function, thereby directing its client proteins including Bcr-Abl, FLT-3, c-Raf, and AKT, to polyubiquitylation and proteasomal degradation. HAA-HDIs mediated attenuation of the levels of Bcr-Abl and FLT-3 occurs regardless of the mutational status of these protein kinases, resulting in growth arrest and apoptosis of imatinib mesylate (IM)-refractory Bcr-Abl in CML or AML with mutant FLT-3. Further studies from our laboratory have shown that HAA-HDIs, e.g., LBH589 synergize with the hsp90 inhibitor 17-AAG in exerting cytotoxic effects against those leukemia that are dependent for their growth and survival on hsp90 client proteins. Additional studies show that combination of HAA-HDIs with IM in CML, or with FLT-3 kinase inhibitor PKC412 in AML with mutant FLT-3, exert synergistic antileukemia effects. Thus, the combinations of HAA-HDIs with Bcr-Abl or FLT-3 kinase inhibitors represent a novel non-chemotherapy treatment strategy against leukemia, which may induce targeted disruption of the activity of the dominant, oncoprotein transcriptional repressors, as well as of the deregulated constitutively active protein kinases which are the hallmarks of many sub-types of acute leukemia. Additionally, studies from our laboratory demonstrate that HAA-HDIs recruit three separate mechanisms that may collaborate to a variable extent to induce apoptosis of acute leukemia cells. These mechanisms include a) increase in mRNA and protein expression of the pro-apoptosis molecules, e.g., Bak, Bax, Bid and Bim; b) down regulation in mRNA and protein expression of the levels of antiapoptotic proteins, e.g., Bcl-2, Bcl-x$_L$ and XIAP; c) acetylation and inhibition of hsp90, directing polyubiquitylation and proteasomal degradation of its client pro-survival oncoproteins, and d) direct mitochondria toxic effects that result in increased generation of reactive oxygen species (ROS). Collectively, these effects decrease the threshold for apoptosis and sensitize leukemia cells to conventional antileukemia agents, as well as to novel agents, e.g., the death ligand Apo-2L/TRAIL. Recent studies from our laboratory have also demonstrated that treatment with HAA-HDIs increase the levels of the death receptors, DR4 and DR5, down regulate c-FLIP and enhance the assembly and activity of Apo-2L/TRAIL-induced death inducing signaling complex (DISC), caspase-8 processing and activity and apoptosis of acute leukemia cells. This indicates a potentially promising combination of HAA-HDIs with Apo-2L/TRAIL for the treatment of acute leukemia. Taken together, these studies highlight the biologic rationale and potential activity of several novel HAA-HDI-based combinations that need to be further investigated in vivo as targeted therapies for human leukemia.

253 INVITED

Clinical development of histone deacetylase inhibitors

V. Richon.

Abstract not received.