session 3 – biology and novel rational therapeutic targets in DLBCL

045 RESTORING APOPTOSIS TO LYMPHOMA BY TARGETING BCL-2

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Cancer cells exhibit many violations of normal cellular behavior that would induce apoptosis (programmed cell death) in normal cells. It is therefore likely that all cancer cells require a block in apoptotic signaling in order to survive. We have developed a strategy, which we call BH3 profiling, which can identify the position of the block in the apoptotic pathway in cancer cells.We have applied this strategy to a panel of eighteen diffuse large B-cell lymphoma cell lines.We found that many of these cell lines escape apoptosis via the overexpression of anti-apoptotic proteins like BCL-2. Such cells require BCL-2 expression to sequester pro-death molecules like BIM from signaling death downstream. We refer to cells in which antiapoptotic proteins are required to tonically sequester pro-death proteins as ''primed''. BH3 profiling is able to identify primed cells, and moreover identify the individual antiapoptotic protein responsible for survival in those cells. Cells identified as BCL-2 dependent by this method are found to be sensitive to small molecule BCL-2 antagonists like ABT-737. ''Unprimed'' cells that employ other blocks such as down-regulation of pro-apoptotic proteins are resistant to BCL-2 antagonists. Moreover, we find that conventional chemotherapeutic agents kill primed cells with greater efficiency than unprimed ones. This suggests that response to many conventional agents may often be determined by the arrangement of the apoptotic machinery, an arrangement that can be determined by BH3 profiling. Systematic study of the apoptotic pathways of cancer cells, facilitated by techniques such as BH3 profiling, offers the possibility of tailoring therapy according to the type of block in apoptosis employed by an individual tumor.

046 THERAPEUTIC TARGETING OF THE BCL6 ONCOGENE IN DIFFUSE LARGE B-CELL LYMPHOMAS

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Expression of the BCL6 (B-cell lymphoma 6) transcriptional repressor is required for B-cells to form germinal centers (GC) and undergo immunoglobulin affinity maturation. BCL6 contributes to the GC B-cell phenotype of clonal expansion and genetic recombination by repressing target genes involved in DNA damage responses such as ATR, CHEK1, TP53 and CDKN1A. BCL6 can also repress the PRDM1 gene and thus inhibit plasma cell differentiation of GC B-cells. Translocations or mutations of negative regulatory elements that occur as byproducts of class switch recombination or somatic hypermutation can lead to constitutive expression of BCL6. Such events are among the most common genetic lesions found in human diffuse large B-cell lymphomas (DLBCL), and can contribute to malignant transformation. Animals engineered to recapitulate deregulated expression of BCL6 in germinal center B-cells develop DLBCL similar to the human disease. Oncogenic transcription factors like BCL6 are ideal targets for development of therapeutic inhibitors since they exert a profound influence on cellular phenotype. Directly targeting such factors could transcriptionally reprogram tumor cells to either revert to a normal phenotype or escape from aberrant survival programs. One of the main barriers thus far to development of such inhibitors is that most transcription factors mediate their effects through protein-protein interactions, which are often quite complex and may not be suited to inhibition by small molecules. In recent years this limitation has been overcome by the harnessing of protein transduction domains (PTDs) including the nine residue cationic HIV-TAT motif. PTDs allow even full-length proteins to be effectively transduced into virtually all cell types both in vitro and in vivo. The TAT PTD penetrates cells via macropinocytosis, and enters the cytoplasm by leaking through the macropinosome membrane as the pH drops within. Co-administration of a fusogenic motif from the influenza virus hemagglutinin protein can greatly facilitate escape of PTDs from macropinosomes. Since TAT also functions as a nuclear localization signal it is well suited for the delivery of transcription factor inhibitors. BCL6 is a member of the BTB-POZ (bric a brac, tramtrack, broad complex – pox virus zinc finger) family of proteins. Our crystallography studies showed that homo-dimerization of the BCL6 BTB domain forms an extended lateral groove motif along the dimer interface, which is required to recruit the SMRT and N-CoR corepressors. Amino acid side chains protruding into this groove make extensive contact with an 18-residue BCL6 binding domain (BBD) peptide that is conserved between N-CoR and SMRT. The BCL6 lateral groove residues that contact N-CoR and SMRT are unique to BCL6 and are not present in other BTB proteins. A recombinant peptide containing the SMRT BBD along with a cell penetrating TAT domain and other motifs was able to block interaction of BCL6 with SMRT and N-CoR. This BCL6 peptide inhibitor (BPI) could re-activate BCL6 target genes and kill BCL6-expressing DLBCL cell lines in vitro. DLBCL cells thus require the continued presence and function of BCL6 for their survival, suggesting that BCL6 is a bona fide therapeutic target in this disease. In order to create a more drug like inhibitor with superior potency and stability, through a series of rational design steps we generated a short retro-inverso/fusogenic peptidomimetic molecule. This retro-inverso BPI (RI-BPI) inhibitor retained its specificity for BCL6 and could disrupt BCL6 repression complexes in DLBCL cells. RI-BPI was non-toxic and non-immunogenic in animals even when administered for up to 1 year. RI-BPI induced profound growth arrest and cell death in murine DLBCL xenografts and induced expression of BCL6 target genes critical for evasion of key cellular checkpoints such as p53 and ATR. RI-BPI contains a portion of the SMRT BBD that makes multiple important inter-molecular contacts with the BCL6 lateral groove. We used a computational strategy to screen one million small molecules for their ability to potentially dock to the same region. The chemistry of protein contacts in this region led us to predict that small molecules that could dock to this site would have the best chance of destabilizing the BCL6-corepressor complex. Among the top-scoring 100 molecules from this screen, we identified 10 compounds that could specifically inhibit the repressor activity of the BCL6 BTB domain in reporter assays and that displayed direct binding to purified BCL6 BTB domains. Using these leads as molecular scaffolds we generated small libraries of molecules derived from each parental compound. The most active of these families was called the 57 series. Series 57 compounds could all specifically block BCL6 repression in reporter assays, and disrupt corepressor/BCL6 complexes at low micromolar concentrations as shown in fluorescence polarization assays. X-ray crystallography of the most active member of the 57 family (called 57-6) showed that the small molecule docked as predicted in the critical region of the lateral groove. Moreover, 57-6 induced an allosteric conformational change in the entire lateral groove that explains how these small molecules so effectively disrupt the BCL6/corepressor complex. 57-6 was also biologically active, since it could induce expression of BCL6 target genes including p53 and ATR in BCL6-positive DLBCL cells as shown by QPCR. 57-6 had no effect on negative control genes nor in BCL6-negative DLBCL cells. The mechanism of action was confirmed in ChIP assays showed that 57-6 abrogated BCL6 mediated corepressor recruitment to BCL6 target genes but had no effect on negative control genes. Most importantly, 57-6 specifically killed BCL6-positive DLBCL cells but had no effect on BCL6-negative DLBCL cells. A dose escalation experiment in mice revealed no toxic effects. In xenotransplantation experiments, 57-6 potently inhibited the growth of already established human DLBCL tumors in mice, again without toxicity to other organs. In summary, we used a rational approach to design specific and potent peptidomimetic and small molecule inhibitors of BCL6, which could serve as targeted agents for DLBCL in clinical trials. Our data show that transcription factors are druggable targets that can be harnessed to potentially improve cancer therapy.

047 MOLECULAR HETEROGENEITY AND RATIONAL THERAPEUTIC TARGETS IN DLBCL

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Diffuse large B-cell lymphomas (DLBCLs) are the most common lymphoid malignancies in adults. Although more than 60% of patients with DLBCL are cured with empiric combination chemotherapy and rituximab, the remainder relapse and many ultimately die of their disease. The striking clinical, genetic and morphologic heterogeneity in DLBCL and its variants suggests that biologically relevant subtypes remain to be defined. To understand the bases of clinical and molecular heterogeneity in DLBCL, it would be useful to have comprehensive molecular signatures of tumors that share similar features. The combination of transcriptional profiling and additional detailed genetic and functional analyses identified three groups of DLBCLs with significantly different molecular signatures and underlying genetic abnormalities. BCR DLBCLs have increased expression of B-cell transcription factors including BCL6 and more frequent BCL6 translocations; these tumors also have more abundant expression of multiple components of the BCR signaling cascade. Previous functional analyses indicate that BCL6 target genes are differentially regulated in BCR DLBCLs and that these tumors are more sensitive to targeted inhibition of BCL6. Since BCR tumors also have increased expression of multiple components of the BCR signaling cascade, we postulated that these DLBCLs might exhibit increased activity of and reliance upon tonic BCR-mediated survival signals. In recent studies, we found that BCR DLBCL cell lines and primary tumors exhibited tonic and ligand-induced BCR signaling. In addition, these DLBCLs were selectively sensitive to targeted inhibition of the spleen tyrosine kinase (SYK), which initiates downstream events and amplifies the initial BCR signal. Taken together, these data suggest that SYK-dependent tonic BCR signaling is an important and potentially targetable survival pathway in BCR DLBCLs. These studies prompted the clinical evaluation of an oral SYK inhibitor in DLBCL and other B-cell lymphomas. More generally, these studies highlight the potential clinical utility of identifying DLBCL subtypes reliant upon specific survival pathways.

048 DEREGULATION OF THE NFKB PATHWAY BY ONCOGENIC LESIONS IN ABC-DLBCL

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease comprising biologically and clinically distinct subgroups. A key feature of the activated B cell (ABC)-type is the constitutive activation of the NFkB transcription complex. However, the underlying mechanisms remain to be elucidated. The aim of this study was to investigate whether genetic lesions in NFkB pathway components are responsible for deregulated NFkB expression in this disease.

Material and Methods: NFkB activity was first assessed in 85 DLBCL (32 ABC, 38 GCB, 15 unclassified) by immunohistochemical / immunofluorescence staining of p50 and p52, WB analysis of p100/p52, and gene set enrichment analysis (GSEA) for NFkB target genes. The complete coding sequence of 30 NFkB pathway components was analyzed by direct sequencing in 15 ABC-DLBCL (6 cell lines and 9 biopsies); genes found mutated were further analyzed in additional 10 ABC-DLBCL and in a panel of 15 GCB-DLBCL. Copy number changes were detected by FISH.

Results: Nuclear localization of p50 and/or p52, indicative of constitutive NFkB activity, was observed in 20/32 (63%) ABC- and 10/38 (26%) GCB-DLBCL. The more sensitive GSEA showed significant enrichment for NFkB target genes in 95% ABC-DLBCL. Mutations were found in 17/25 cases (68%) and were distributed in a total of 10 genes, including A20, CARD11, TRAF1,2 and 5, RANK, TAK1, BAFF, CD40 and NFKB1B. Of these, A20, which encodes for a negative regulator of NFkB responses, was the most frequently altered, with 5/25 samples carrying premature nonsense mutations or frameshift deletions leading to truncated proteins that lack functionally relevant domains. One additional case had a missense mutation. In cases where FISH analysis was performed, both alleles were inactivated by mutations and/or deletions. A20 mutations were absent in GCB-DLBCL.

Conclusions: Multiple genetic lesions in this key signaling pathway, including inactivating mutations of A20, may contribute to ABC-DLBCL pathogenesis by causing abnormally prolonged NFkB responses. These findings may provide new targets for therapeutic intervention.

049 SUBSETS OF NHL POPULATIONS MEET CRITERIA FOR LYMPHOMA STEM CELLS

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Introduction: Emerging studies suggest that cancer stem cells appear to play an important role in human leukemias, multiple myeloma, and cancers of the brain, breast and colon. No such tumor-initiating population has been identified in non-Hodgkin lymphoma (NHL). Cancer stem cells are defined phenotypically and functionally as relatively rare cells with self-renewal, differentiative, and tumorigenic capacity. Using these criteria, we looked for putative LySC in cultured and primary human NHL populations.

Materials and Methods: Two experimental sources of NHL cells were used: a) primary human DLBCL or Burkitt lymphoma cells derived from malignant effusions obtained after therapeutic procedures, and, b) continuously cultured DLBCL lines. Standard techniques for multiparameter FACS analysis, cell sorting, methylcellulose culture for CFU/progenitor cell enumeration, serial in vitro passage, and a NOD/SCID murine xenograft model were used to identify candidate LySC subpopulations.

Results: In both primary tumor populations and cell lines derived from aggressive lymphoma, we readily identified subsets with differential surface expression of CD22, CD27, CD45R/B220, CD184, and CD38. Enriched CFU potential segregated with phenotypically distinct subpopulations, and a purified subpopulation of lymphoma cells, when expanded in culture, could recapitulate the phenotypic heterogeneity found in the original, unfractionated population. In-vivo tumor-initiating cells were confined to a subset of all specimens tested. These data are consistent with the phenotypic and functional heterogeneity expected in a stem cell based hierarchy of malignant populations.

Conclusions: Our studies provide evidence that NHL tumor-initiating cells may comprise a unique population amenable to prospective immunophenotypic and/or functional identification. Implicit in the concept of all cancer stem cells such as the putative LySC is that the control, and ultimately the eradication of these unique populations is critical for both clinical remission induction and for cure.