session 9 – micro-RNA in lymphoid malignancies

104 RNA INTERFERENCE-BASED GENETIC SCREENING MEETS CANCER GENOME RESEQUENCING

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Cancers develop addiction to signaling and regulatory pathways that sustain proliferation and survival, and such pathways provide an ''Achilles heel'' that can be exploited therapeutically. We have recently developed high-throughput, comprehensive methods to discover these functionally critical pathways by combining RNA interference-based genetic screens with analysis of somatically acquired aberrations in tumor cell genomes. One recurrent theme is the activation of the NF-kB pathway in lymphoid malignancy owing to constitutive activation of IkB kinase beta (IKKb). In diffuse large B cell lymphoma (DLBCL), we have defined two major subtypes of diffuse large B cell lymphoma (DLBCL), termed activated B cell-like (ABC) DLBCL and germinal center B cell-like (GCB) DLBCL, and have shown that IKKb is constitutively active and required for the survival of ABC DLBCL but not GCB DLBCL cells. Using a RNA interference-based genetic screen, we have identified a variety of signaling molecules that contribute to IKKb activation in ABC DLBCL, most notably CARD11, MALT1 and BCL10, which are normally involved in NF-kB signaling downstream of antigen receptors in lymphocytes. We have also initiated a comprehensive search for somatically acquired translocations, amplifications and mutations in lymphoid malignancies. This effort has uncovered diverse genetic events that activate NF-kB signaling in lymphoma and multiple myeloma, creating an addiction that may be exploited therapeutically with inhibitors of IKKb. Thus, some essential pathways uncovered by our RNA interference genetic screens are directly targeted by oncogenic alterations in cancer. However, other genes identified by these genetic screens are not genetically altered but are nonetheless required for the proliferation and/or survival of one type of lymphoid malignancy but not others, a phenomenon known as ''non-oncogene addiction''. This novel class of essential genes provides new therapeutic targets in cancer.

105 ROLE OF MICRORNAS IN NORMAL B CELL DEVELOPMENT AND LYMPHOMAGENESIS

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The discovery of microRNAs (miRNAs) has added a new dimension to the mechanisms that regulate gene expression in normal cell development and their disruption during tumorigenesis. Nonetheless, the full set of miRNAs is not known for many species including humans, since presently known miRNAs have been identified by virtue of their abundant expression in a few cell types, while tissue-specific miRNAs may remain unknown. In order to understand the role of miRNA in mature B cell function and lymphomagenesis, we generated miRNA libraries from human B cells at different stages of development, including naïve, germinal-center and memory B cells, and from a Burkitt lymphoma cell line. The combination of cloning and computational analysis allowed identifying 220 miRNA expressed in human B cells, including 112 miRNA not previously reported. These results indicate the presence of a sizable fraction of tissue- and stage of differentiation-specific miRNAs of potential relevance for normal physiology as well as for tumorigenesis. Using microarray-based analysis, we then examined the patterns of miRNA expression during normal B cell development/activation and in several major types of B cell malignancies. The results indicate that: 1) miRNA expression is specifically regulated during the germinal-center reaction, with specific miRNAs expressed in naïve, germinal-center and memory B cells; 2) miRNA expression signatures can distinguish B cell malignancies from their normal B cell counterparts, and can also identify diagnostically relevant tumor subtypes; 3) In vitro and in vivo studies suggest the involvement of specific miRNAs in the pathogenesis of distinct B cell malignancies.

106 MICRORNA CONTROL IN LYMPHOCYTE PHYSIOLOGY AND PATHOLOGY

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MiRNAs have recently emerged as important factors in the post-transcriptional control of intracellular protein concentrations in metazoan organisms. These RNA molecules of approximately 22 nucleotides pair with mRNAs transcribed from protein coding genes, and specify their degradation or translational repression. MiRNAs display distinct temporal and spatial expression patterns and regulate a diverse range of physiological processes including hematopoiesis, developmental timing, organogenesis, cell differentiation, cell proliferation, and apoptosis. Expression profiling studies have detected specific miRNA expression signatures in a variety of human cancers, and miRNA genes are frequently located at genomic regions associated with carcinogenesis, suggesting that miRNAs may be involved in this process.

We set out to study miR-17-92 to test the hypothesis that de-regulation of individual miRNA expression could cause cancer. The miR-17-92 miRNA cluster is located at human chromosome 13q31, in a genomic region that is frequently amplified in lymphomas and other cancers, and the mature miRNAs encoded by this locus are expressed in high amounts in cancer cells. Retroviral expression of this miRNA cluster in the hematopoietic system accelerated the onset of cMyc-mediated lymphomagenesis in a transgenic mouse model involving bone marrow reconstitution. To elucidate the mechanism by which the miR-17-92 cluster promotes lymphomagenesis, we generated mice with elevated miR-17-92 expression in lymphocytes. These mice developed lymphoproliferative disease and autoimmunity, and died prematurely. Lymphocytes from these mice showed increased proliferation and reduced activation-induced cell death. miR-17-92 miRNAs suppressed expression of the tumor suppressor Pten and the pro-apoptotic protein Bim. These results suggest that overexpression of miR-17-92 contributes to lymphomagenesis by downregulating the protein levels of multiple components of a gene network that is essential for cell proliferation and survival. This demonstrates that overexpression of a single miRNA cluster could play a role in early phases of lymphomagenesis.

107 CONCOMITANT GENE EXPRESSION PROFILING AND MICRORNA PROFILING REVEAL A ROLE FOR MICRORNAS IN B CELL DEVELOPMENT AND LYMPHOMAS AT EVERY STAGE

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MicroRNAs (miRNA) are negative regulators of gene expression and have been shown to play pivotal roles in cellular differentiation and oncogenesis. We hypothesized that miRNAs play a role in B cell differentiation that distinguish stages in B cell differentiation could have a role in distinguishing different lymphoma types

Tonsillar B cells from young patients were sorted to high purity using flow-cytometry into naive B cells, germinal center (GC) B cells, plasma cells and memory cells. These were profiled for gene expression using Affymetrix U133+ arrays. Over 50% of the genes differentially expressed in B cell differentiation were predicted as targets of miRNA ($P<1E-10$).

We undertook profiling of all known miRNA (miRBASE 9.2) using a highly sensitive multiplex real-time PCR assay. We noted excellent reproducibility across biologic replicates and a 2000X dynamic range. At a false discovery rate $< 1\%$, we found miRNAs that distinguished B cell stages. Target genes of the differentially expressed miRNAs were found to be down-regulated in the appropriate B cell populations, offering strong evidence that the differentially microRNAs exert a major influence on B cell differentiation.

A predictor constructed of miRNAs was 100% accurate in identifying the lineage of tumor samples from patients with chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma and Burkitt lymphoma, demonstrating that B cell stage-specific miRNAs are strongly conserved in malignancies.

Thus, we have identified a strong role for microRNAs in the differentiation of B cells at every stage. The stage-specific microRNAs are conserved in the corresponding lymphoid malignancies. This study could serve as the starting point for the development of miRNA-based diagnostic assays and offers a new window into the biology of normal and malignant B cells.