Differential proteomic characterization of B cell proliferative states: analysis of tumor-specific and proliferation-specific proteomes in normal and malignant B cells

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Introduction:

The dual bromodomain protein Brd2 is closely related to the basal transcription factor $TAF_{II}250$, which is essential for *cyclin A* transactivation and mammalian cell cycle progression. In transgenic (Tg) mice, constitutive lymphoid expression of Brd2 causes diffuse large B cell lymphoma (DLCL), which represents most diagnosed human non-Hodgkin's lymphoma (NHL) cases, in part through upregulation of the *cyclin A* locus.

In an effort to understand the mechanism underlying Brd2-driven B cell lymphoma and how it differs from proliferating or resting B cells, we undertook 2D-PAGE-based comparative proteomic analyses of Tg lymphoma in relation to proliferating and resting B cells. We hypothesized that, by analogy to our transcriptional profiling results, non-malignant proliferating cells and malignant proliferating cells share the induction of a variety of common proteins; differential proteomic analysis allows the subtraction of these non- malignant proliferative proteins from the malignant proteome to uncover malignancy specific biomarkers. This will elucidate the mechanisms of lymphomagenesis, maintenance, and progression.

Method:

We used 2D-PAGE separation of murine nuclear extracts subjected to size exclusion chromatography, purified from normal resting B cells, normal proliferating B cells, and Tg malignant B cells, to define 2D reference maps. All cells were derived from syngeneic mice, thus eliminating genetic variance. Resolved, stained and quantitated protein spots were excised and digested with trypsin. Peptides were desalted and subjected to MALDI-TOF MS and peptide mass fingerprint analyses or to LC-MS and LC-MS/MS analyses. Mass spectra were analyzed with MoverZ (Genomic Solutions) or MassLynx (Waters) software. PMF analysis was conducted using Mascot (Matrix Science), BUPID (Boston University) and BUDSS (Boston University), and LC-MS/MS analysis was conducted using Mascot and ProteinLynx (Waters). Relative protein quantification was obtained by digital analysis using PDQuest (Bio-Rad).

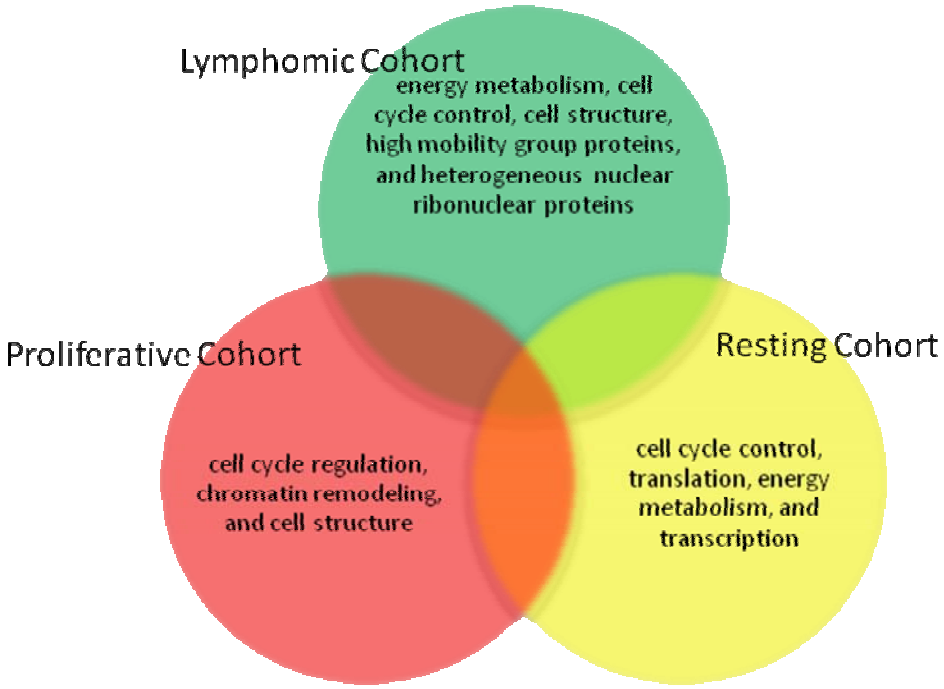
Results:

Differential comparison of the 2D gels of protein nuclear extracts from resting, stimulated and Tg lymphomic cell highlighted the distinct proteomic phenotypes of each cell type. Global differences in patterns supported the hypothesis that there exists a basal B cell proteomic profile, a general proliferative proteomic signature that is present in both the lymphomic and stimulated B cells, and, most importantly, a discrete group of lymphomic cell-specific proteins that are unique to the Tg lymphoma.

Differential analysis allowed the observation of the absolute presence/absence of novel marker proteins and the up- and down-regulation of other implicated proteins. Thus, by subtracting the proliferative proteomic signature from the entire lymphomic-cell proteome we could identify key proteins that may be involved in or markers of lymphomagenesis.

Proteins subjected to uniquely lymphoma-specific over-expression included those known to be involved in cell cycle control, energy metabolism, cell structure, and mRNA transcription and splicing

control. This study thereby represents a paradigm in personalized medicine applied to the diagnosis of lymphoid malignancies, in which a patient's particular lymphoma-specific protein signature may be resolved from the patient's background profile of B cell proliferative proteins.



Most of the differentially expressed proteins fall into three main cohorts, each having specific functions, as shown here. By subtracting the proliferative proteomic signature from the entire lymphomic cell proteome, we were able to identify a unique set of proteins whose expression was specifically modulated in the lymphomic cell group.

Conclusions:

Our results illustrate that lymphomagenesis is more complex than upregulated or dysregulated normal cellular proliferation; it involves additional expression changes in many proteins, several of which were not previously linked to lymphoma. It demonstrates a realistic model of personalized disease diagnosis in which a patient's B cell ex-vivo proliferative signature can be defined for baseline comparison to suspected new or recurrent lymphomas. Moreover, by shedding light on the protein mechanics particular to proliferation and carcinogenesis, our results may lead to identification of novel targets for chemotherapeutic intervention.

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