131 ACTIVATION OF THE STAT3 SIGNALING PATHWAY PREDICTS POOR SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH R-CHOP

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in the adult population. Addition of rituximab to the standard CHOP chemotherapy regimens results in an improvement of overall survival. Nevertheless, a substantial number of patients still succumb to the disease. We have previously reported aberrant activation of signal pathway, which has begun to change our perspective on how to tailor new therapeutic strategies for the molecular phenotype of different diseases. Dysregulation of the many proteins that govern control of programmed cell death pose the cell to resist traditional chemotherapy regimens, and contribute to their mortality. While many conventional drugs clearly have the potential to induce ‘cell death’, relatively few directly modulate the balance of pro- and anti-apoptotic forces, which collectively set the threshold for programmed cell death. One of the first drugs to selectively target these pathways was oblimersen, an antisense molecule that directly targeted and degraded Bcl-2, an anti-apoptotic protein. Since then, a host of small molecules functioning as BH3 only mimetics have entered the clinic, including AX-011, a stereosomer isolated from gossypol, obatoclax and ABT-263/737. These molecules, which have completed phase I testing as single agents, have produced responses in various disease settings, including CLL and follicular lymphoma, and are now being studied in combination with other antiapoptotic agents. Beyond targeting the regulation of proteins that govern mitochondrial membrane depolarization leading to cytochrome C release, new molecules targeting the extrinsic pathway, including CD95 and other Fas signaling pathways have also completed early phase clinical testing. While these agents have not produced substantial single agent signals to date, the idea of combining them with other agents to aid in lowering the threshold required to induce apoptosis is the most viable strategy for their development moving forward. Similarly, new molecules targeting survivin, and other IAP (inhibitors of apoptosis) family members, including YM 155, have been studied in relatively chemotherapy resistant lymphomas, including refractory diffuse large B-cell lymphoma. Again, while limited single agent signals have been reported, increasing emphasis is being placed on the study of these agents in combination. While many of these targeted Bcl-2 directed agents have been designed to directly modulate a very discrete aspect of the apoptotic pathway, it is also clear that several other classes of drugs, including the proteasome inhibitors, the histone deacetylase inhibitors, also have the potential to modulate these pathways. There is considerable enthusiasm that the integration of these agents into our present treatment regimens will afford new opportunities to overcome the survival instincts of many cancers, that will hopefully lead to improved outcomes.

Patients and Methods: One-Hundred-eighty-eight patients with DLBCL were identified and included 89 patients treated by CHOP/CNOP (CHOP) and 99 patients treated by CHOP/CNOP plus rituximab (R-CHOP). We evaluated STAT3 activation by quantitating the levels of phosphorylated p70S6K (PY-STAT3) in tumor cells using immunohistochemistry. The result was correlated with the pathological characteristics and survival. We also constructed a gene expression-based PY-STAT3 signature derived from STAT3 siRNA treated DLBCL cell lines. We then applied this signature to a cohort of 233 DLBCL cases treated with the R-CHOP regimen for which both gene expression profiles and clinical data are available. Results: The non-GCB subgroup had significantly higher number of PY-STAT3 positive cases compared to the GCB subgroup (59.5% vs 29.8%; P<0.001). PY-STAT3 positivity predicts poor overall survival in the entire R-CHOP cohort (P = 0.041) and in the non-GCB subgroup (P = 0.016), suggesting that the combination of PY-STAT3 with non-GCB phenotype identifies a subset of patients who were at high risk when treated with R-CHOP. Multivariate analysis was performed and showed that the hazard ratios of overall and event free survival for the PY-STAT3 positive cases were significantly higher than the PET-STAT3 negative cases in the non-GCB-DLBCL cohort. When tested in a published cohort of 233 DLBCL cases treated with R-CHOP, our 33-gene PY-STAT3 signature stratified this cohort into 4 subgroups with different immunophenotypes and survival outcomes.

Conclusions: STAT3 activation in DLBCL is a significant prognostic factor, especially in the non-GCB patients treated with the R-CHOP regimen. Targeting STAT3 pathway may therefore provide a novel therapeutic approach for patients with DLBCL.

132 METABOLIC TARGETING IN LYMPHOMA THERAPY

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Introduction: Chemoresistance is the most important predictor of poor survival in lymphoma patients but underlying mechanisms remain poorly understood. Cellular senescence, a DNA damage-initiated terminal cell-cycle block, may be an important component of drug action, but no genetic model exists to assess the specific contribution of therapy-inducible senescence (TIS). We previously established the Eμ-myc transgenic mouse as an excellent model to explore the role of candidate genes and candidate programs (such as apoptosis) in response to chemotherapy because oncogene-induced senescence is characterized by trimethylated histone H3 lysine 9 marks (H3K9me3), we aimed to address the effect of TIS on long-term outcome in Eμ-myc transgenic mice lacking the H3K9 histone methyltransferase Suv39h1.

Methods: Suv39h1-deficient lymphomas were generated; proliferation, apoptosis, cellular senescence, and metabolic parameters were analyzed after various types of DNA-damaging and metabolically targeting therapies. Therapy responses were monitored by whole-body fluorescence, luciferase imaging and 18F-fluoro-deoxyglucose (FDG) and 18F-fluoro-deoxyxymethylin (FLT) positron emission tomography (PET).

Results: Control lymphoma-bearing mice entered TIS and achieved a much better long-term treatment outcome (90% survival) compared to Suv39h1-deficient lymphomas, which did not differ in their apoptotic and proliferative capacity, but lacked a TIS response and rapidly progressed to a terminal disease condition. TIS lymphomas mice exhibited a sharp decline in FLT-PET activity, and CD11c enhanced FDG-PET signals. In vitro, we also observed decreased glucose uptake, a higher glycolytic rate and higher ATP levels. They were more sensitive to glucose deprivation or inhibition of glycolysis when compared to equally treated, but senescence- incapable Suv39h1-deficient lymphoma cells. Importantly, the simultaneous treatment of TIS-capable, Bcl2-overexpressing lymphoma-bearing mice with chemotherapy followed by a glycolysis inhibitor, but not in the reverse order, produced lymphoma regression by TUNEL-positive cell death in vivo.

Conclusions: TIS significantly improves the long-term outcome to anticancer therapy in vivo. However, rare senescence cells may eventually resume proliferation and, thus, give rise to a relapse. The – unexpected – hypermetabolic nature of TIS imposes a therapeutically exploitable cancer cell identity that can be further exploited by metabolic targeting strategies that selectively eliminate senescent tumor cells.