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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Integrating Proteomics and Functional Genomics to Identify Targets for Improving Glucocorticoid-Based Treatment of B-Lymphoblastic Leukemia

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Glucocorticoids are a critical component of successful combination chemotherapy regimens for B-lymphoblastic leukemia (B-ALL). Despite advances in immunotherapies, glucocorticoid-based chemotherapy continues to be the essential frontline treatment of patients with B-ALL. This approach has led to high cure rates but at the risk of both acute, potentially life-threatening complications and chronic toxicities. Additionally, glucocorticoids remain ineffective for some patients with B-ALL, which predicts poor outcomes. To achieve better outcomes and reduce the toxicity of treatment, we take a functional genomics approach to understand how glucocorticoids induce cell death and identify strategies to make them more effective. Glucocorticoids work through the glucocorticoid receptor (GR), a ligand-activated transcription factor, to induce cell death in B-ALL. By integrating gene expression and RNA interference screening, we have identified both effector genes (genes whose regulation by glucocorticoids contributes to B-ALL cell death) and sources of resistance that can be targeted to improve treatment (Kruth *et al* Blood 2017). This identified the network of BCL-2 family proteins (e.g., BCL2, BCL2L11, BMF), whose regulation contributes to apoptosis in B-ALL. Surprisingly, it also showed that suppression of B-cell development genes (e.g., EBF1, PAX5, IL7R) contributes to B-ALL cell death. However, only a fraction of the genes that affect the glucocorticoid sensitivity of B-ALL are effector genes. We therefore sought to identify functional interaction partners of GR, defined as proteins that physically interact with GR and influence glucocorticoid sensitivity.

To identify proteins that physically interact with GR, we used proximity labeling. We fused an ascorbate peroxidase domain (APEX2) to the N-terminus of GR and expressed it REH cells, a B-ALL cell line that does not express GR protein. After verifying that APEX2-GR restored glucocorticoid sensitivity in the REH cells, we performed proximity labeling in the presence and absence of dexamethasone. After optimizing these reactions, cells were lysed and labeled proteins were isolated using magnetic streptavidin beads and identified by mass spectrometry. We were able to detect more than 4,000 proteins that interact with GR, including hundreds that are enriched upon addition of dexamethasone.

To identify functional interaction, we intersected the proteins enriched upon addition of dexamethasone with proteins shown by us to modulate glucocorticoid sensitivity in B-ALL (Kruth *et al* Blood 2017). Supporting our working model, we found functional interaction partners that are involved in B-cell development and apoptosis. We found that GR interacts with EBF1 and MEF2C, B-cell development transcription factors that restrain glucocorticoid-induced cell death. This indicates that GR activity and glucocorticoid sensitivity is directly blunted by B-cell development, and further supports the model that glucocorticoids restrain B-cell development. The mechanism of how these interactions influence glucocorticoid sensitivity will guide rational targeting to improve treatment of B-ALL both in general and in different B-ALL subtypes. Additionally, the mitochondrial voltage dependent anion channel 2 (VDAC2) also interacts with GR in a dexamethasone-dependent manner and is integral to glucocorticoid-induced cell death. VDAC2 is important for nucleating and perhaps triggering pore formation through BAK and BAX. How GR influences this activity is not known but suggests a non-genomic role of GR in glucocorticoid-induced cell death.

In summary, integration of functional genomics and proteomics provides rich data sets that will help understand the mechanism of glucocorticoid-induced B-ALL cell death. Targeting of non-canonical (B-cell development) and non-genomic (VDAC2) functions of GR will allow for optimization of glucocorticoid-based chemotherapy regimens.

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