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Introduction: We have previously shown that the two ETS transcription factors ETS1 and FLI1, co-mapped in the 11q24.3 region, are recurrently gained in up to 25% of diffuse large B cell lymphomas (DLBCL), and largely co-regulate a series of genes involved in B cell signaling, differentiation and cell cycle (Bonetti et al., 2013; Priebe et al., 2020; Sartori et al., 2021). ABC-DLBCL is a more aggressive subtype of DLBCL compared to GCB-DLBCL, and it is associated with poor outcomes when treated with a standard therapy. As a result, there is an urgent need to elucidate new therapeutic venues for this dismal malignancy. While FLI1 is expressed at a higher level in DLBCL of the germinal center B-cell (GCB) type than in the activated B-cell-like (ABC) DLBCL, ETS1 is more expressed in the latter subgroup. We and others have reported preclinical anti-tumor activity in lymphomas and other tumors with small molecules blocking the binding of ETS factors and RNA helicases (Erkizan et al., 2009; Spriano et al., 2019). In this study, we identified additional therapeutic targets related to these transcription factors, by investigating the ETS1 interactome in ABC DLBCL.

Methods: Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) was done on proteins obtained with pull down of strep-tagged ETS1. Proteins with a spectral count above 4 were considered candidate interactors, and validated by normal and reverse co-immunoprecipitation experiments. RNASeq and small RNASeq analysis was done after DDX21 siRNAs silencing in 2 ABC (HBL1 and U2932) and in 2 GCB (OCI-Ly1 and VAL) DLBCL cell lines. In addition, we performed ChIPSeq for DDX21 on the same 4 cell line models.

Results: Proteins related to RNA processing, more specifically in spliceosome (NOP56 and ALYREF) and in ribosome biogenesis (SF3B1 and DDX21), were among the identified ETS1 interactors in the ABC DLBCL HBL-1 cell line. We focused on the novel ETS1 interactor DDX21, an RNA helicase also regulated by FLI1 (Sartori et al., 2021). DDX21 appeared more expressed in ABC than GCB DLBCL ($P < 0.001$) in 4 datasets (GSE98588, $n = 117$; phs001444.v2.p1, $n = 432$; GSE95013, $n = 33$; GSE10846, $n = 350$). When we silenced DDX21 with siRNAs, toxicity was seen in ABC (U2932) and not in GCB (OCI-Ly1) cell lines. Our results indicate DDX21 is involved in regulating proteins involved in cell cycle (FDR < 0.001), ribosomes (FDR < 0.001), spliceosome (FDR < 0.001) and small nucleolar RNAs (snRNAs).

Conclusions: In ABC DLBCL, ETS1 interacts with proteins involved in spliceosome and in ribosome biogenesis, including DDX21. Highly expressed in ABC than GCB DLBCL, DDX21 sustains the survival of lymphoma cells by regulating cell cycle and RNA processing. Targeting the interaction between ETS1 and DDX21 represents a novel therapeutic modality against ABC DLBCL.

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Keywords: Aggressive B-cell non-Hodgkin lymphoma, Genomics, Epigenomics, and Other -Omics, Molecular Targeted Therapies

No conflicts of interests pertinent to the abstract.

165 | ALPHA-KETOGLUTARATE SUPPRESSES TUMOR GROWTH OF DIFFUSE LARGE B-CELL LYMPHOMA BY INDUCING FERROPTOSIS

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Background: Metabolic reprogramming is one of the vital characteristics of cancers. Glutamine metabolism is an essential metabolic pathway in tumorigenesis, which provides a ready carbon and nitrogen source to support tumor biosynthesis, energy metabolism, and intracellular homeostasis. As a type of lymphoma with heterogeneity, diffuse large B-cell lymphoma (DLBCL) is characterized by severe metabolic vulnerability represents. Given the importance of glutamine metabolism in human cancer, revealing the characteristics of glutamine metabolism in DLBCL is expected to provide new pathogenesis and effective treatment strategies for patients.

Methods: Peripheral blood serum of 120 DLBCL patients and 60 healthy donors were collected for untargeted metabolomics sequencing, followed by metabolic characteristics analysis. Subsequently, the biological functions and mechanisms of α-ketoglutarate (α-KG) were explored by RNA sequencing. Finally, ROS detection, ATP detection, and lipid peroxidation were used to verify the mechanism of α-KG-induced ferroptosis.

Results: Untargeted metabolomics profiling revealed that the metabolic characteristics of DLBCL patients were significantly different from healthy controls. Among the differentially expressed metabolic pathways, glutamine metabolism accounted for the highest weight, suggesting the importance of glutamine metabolism in the tumorigenesis of DLBCL (Figure 1A). Notably, glutamate, glutamine, and α-KG were the critical metabolites in glutamine metabolism. Clinical data analysis identified that high glutamine concentrations and low decreased α-KG were associated with poor prognosis in DLBCL patients. Subsequently, dimethyl α-ketoglutarate (DM-αKG) was used to reverse glutamine metabolism and increase α-KG concentration. In vitro studies showed that DM-αKG treatment significantly inhibited cell proliferation of DLBCL cells both in vitro and in vivo (Figure 1B). In addition, DM-αKG induced non-apoptotic cell death phenotypes, represented by cell membrane swelling and LDH release. To further explore the functional mechanisms of α-KG in DLBCL, we performed RNA-sequencing in DM-αKG-treated DLBCL cells. As shown in enrichment analysis, DM-αKG treatment showed apparent dysfunction in the hypoxia-inducible factor pathway, oxidative stress response,

and ferroptosis (Figure 1C). In particular, DM-αKG treatment promoted ROS release and lipid peroxidation, followed by impaired ATP production and decreased mitochondrial pathway expression. Moreover, differentially expressed genes analysis identified an increased expression of TP53 in the ferroptosis pathway, indicating the importance of TP53 in α-KG-induced ferroptosis (Figure 1D).

Conclusion: Our present findings were the first to identify the metabolic characteristics of DLBCL patients and elucidate the anti-tumor effects of α-KG in DLBCL.

Keywords: Aggressive B-cell non-Hodgkin lymphoma, Metabolism

No conflicts of interests pertinent to the abstract.

Figure 1