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The "Janus role "of ZBTB46 RNA in Anaplastic Large Cell Lymphoma treatment resistance

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ALK(+) Anaplastic large cell lymphoma (ALCL) is a rare pediatric lymphoma affecting mostly CD4 T cells. It is mainly associated with the t(2;5) chromosomal leading to the formation of a chimeric protein, NPM-ALK, which is always absent in normal cells and participates in oncogenesis through the STAT3 pathway. In ALCL the first-line treatment is a polychimiotherapy. ALK(+) ALCL is relatively sensitive to chemotherapy with high response rate. Nonetheless, event-free survival is still between 65-75% and about 30% of patients relapse early with a very poor prognosis. In case of resistance, treatments targeting ALK can be used, such as crizotinib. Unfortunately, resistance mechanisms to ALK inhibitors have also been observed. It is therefore necessary to better understand these mechanisms in order to find new therapeutic targets and to identify predictive biomarkers. Recent studies have shown the importance of a new class of noncoding RNAs, circular RNAs (circRNAs), in solid cancers by linking these RNAs to tumor stage and treatment resistance. The circRNAs are organized in a closed loop, which increases their stability. Of note that some circRNA can derive from translocations such as NPM-ALK *(Babin et al, iScience, 2018)*. They are therefore robust and can be detected in plasma, which makes them very good biomarkers, especially for diagnosis *(Babin et al, JCI Investigation, 2021)*.

Using RNAseq analysis of a cohort of 48 ALK(+) primary biopsies, we identified a circular RNA (circZBTB46) that was highly overexpressed in patient lymph nodes compared to healthy lymph nodes. Its host gene, ZBTB46, whose expression is normally restricted to dendritic progenitors, was also overexpressed in ALK(+) patients. We are currently evaluating the role of circZBTB46 and its parent gene in the oncogenesis of ALK(+) ALCL and in particular in therapy resistance.

In order to distinguish the role of circZBTB46 from the one of ZBTB46 mRNA (and protein), we used different strategies to modulate the expression of each transcript. We first invalidated each one using CRISPR/Cas9 and siRNA strategy. We performed *in vitro* experiment in order to analyze proliferation, survival and resistance to therapy. These experiments allowed us to identified an opposite role of linear versus circular transcript in resistance

to ALK inhibitor Crizotinib: circZBTB46 seems to increase resistance to crizotinib whereas ZBTB46 mRNA seems to increase sensitivity. These results were confirmed *in vivo* by xenografted in NSG mice.

In a second part, we tried to determine *ZBTB46* regulation in ALK(+) ALCL cells. As ALK/STAT3 pathway is the main oncogenic driver in ALCL, we analyzed the link between this pathway and ZBTB46 expression. By chromatin immunoprecipitation experiments, we showed that STAT3 transcription factor is able to bind *ZBTB46* promoter. Moreover, downregulation of ALK or STAT3 by siRNA strategy leads to inhibition of both circZBTB46 and ZBTB46 mRNA. These results allowed us to identified ALK/STAT3 pathway as a regulator of ZBTB46 expression in ALCL cells.

We are now trying to identified downstream pathways regulated by ZBTB46. To do so, we will perform RNAseq on CRISPR/Cas9 clones depleted for ZBTB46 mRNA or circZBTB46. Moreover, as circular RNA can act as RNA sponge or protein platform, we will next try to identify circZBTB46 partners using RNApulldown technic. In the longer term, we plan to detect these two RNAs in patients' blood samples. If we can easily detect them, they could be used as biomarkers to identify patients with high/low risk of relapse. These transcripts may also be used as therapeutical targets in case of resistance to classical therapies.

Babin et al, Chromosomal Translocation Formation Is Sufficient to Produce Fusion Circular RNAs Specific to Patient Tumor Cells, iScience, 2018, doi: 10.1016/j.isci.2018.06.007

Babin et al, From circRNAs to fusion circRNAs in hematological malignancies, JCI Insight, 2021, doi: 10.1172/jci.insight.151513