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POSTER ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

IGF2BP1 Stabilizes and Inhibits Degradation of Pro-Oncogenic Transcripts in ETV6-RUNX1 Positive B-Cell Acute Lymphoblastic Leukemia

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Insulin like growth factor 2 mRNA binding protein 1(IGF2BP1) belongs to a family of oncofetal proteins expressed in embryonic life followed by re-emergence during malignant transformation. It is an RNA binding protein known to be dysregulated and overexpressed in multiple malignancies. We have demonstrated its specific overexpression in ETV6-RUNX1 translocated B-cell Acute Lymphoblastic Leukemia (B-ALL).

RNA immunoprecipitation using anti-IGF2BP1 antibody, followed by sequencing (RIP-Seq) in Reh cell line (ETV6-RUNX1 positive) identified multiple oncogenic transcripts belonging to the non-canonical NF-κB and PI3K signaling pathways. Overexpression of genes from these pathways *(IL6ST, GADD45A, NFAT5, MDM2, CDK6, FGFR1)* was also observed in our B-ALL patient samples specifically in the ETV6-RUNX1 positive patients (Total n=111; ETV6-RUNX1 positive: 39) in comparison to MACS sorted, peripheral blood CD19 positive B-cells from healthy volunteers as well as in comparison to non ETV6-RUNX1 translocated B-Acute lymphoblastic leukemia patients. This was also corroborated by public B-ALL gene expression data from the TARGET database in the cBioportal.

However, knockout of IGF2BP1 in Reh cell line using CRISPR-Cas9 technology led to significant downregulation of only some of these targets. This implied that the expression of some of the mRNA targets binding to IGF2BP1 was not influenced by its knockout.

To better elucidate the role played by IGF2BP1 in the stability of its binding mRNA targets, we decided to study the rate of degradation of target transcripts. mRNA stability was assessed to quantify the rate of degradation of target mRNA after inhibition of transcription using actinomycin. Since actinomycin causes global transcriptional block, we first quantified the effect of actinomycin on the expression of multiple (n=7) housekeeping genes against each other, and selected 3 (*HGPRT, B2M and PPIA)* which showed least change after actinomycin treatment. These 3 housekeeping genes were used for further quantification of target transcripts. Dual specificity phosphatase 1(*DUSP1*) a transcript known to be rapidly degraded, was used to validate the transcriptional inhibition by actinomycin.

We quantified the rate of degradation of target transcripts in Reh cells using BTYNB, a small molecule inhibitor of IGF2BP1. BTYNB binds to IGF2BP1 and prevents binding to target transcripts which leads to their destabilization and reduced half-life. We used a combination of BTYNB and actinomycin to study rate of degradation after IGF2BP1 inhibition.

Effect of IGF2BP1 on the stability of three target mRNAs involved in non-canonical NF-κB signalling pathway was quantified by comparing their rate of degradation in BTYNB treated cells against the DMSO (vehicle) treated cells. Rate of degradation was measured by actinomycin treatment of both these groups followed by hourly RNA isolation and qPCR over 12 hours.

MYC is a known mRNA binding target of IGF2BP1. BTYNB was also shown to decrease the expression of *MYC* mRNA by accelerating its degradation. Simultaneously there was an increase in the expression of *MYC* pre-mRNA indicating a possible for IGF2BP1 in splicing of *MYC* RNA. In the NF-κB signalling pathway, we had previously identified that NFAT5 expression was not altered significantly after IGF2BP1 knockout even though it binds to IGF2BP1. BTYNB followed by actinomycin treatment demonstrated a dramatic increase in the rate of degradation of *NFAT5* implying that IGF2BP1 prevents the degradation of *NFAT5* transcript and directly influences its stability. Interestingly, the rates of degradation of other targets like *IL6ST* and *GADD45* were not altered.

These findings suggest a heterogeneous influence of IGF2BP1 on the stability of its target transcripts. Some of the transcripts seem to be directly stabilised by IGF2BP1 while others may be influenced indirectly.

Taken together, these findings suggest a role of IGF2BP1 in multiple phases of the RNA synthesis and degradation warranting further studies to elucidate role of IGF2BP1 in each of these steps. IGF2BP1 appears to have a critical role in preventing the degradation and stabilizing *MYC and NFAT5* transcripts.

Disclosures No relevant conflicts of interest to declare.

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