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Myelodysplastic Syndromes

TGF-B PATHWAY DEREGULATION IN SF3B1 MUTATED LOW RISK MYELODYSPLASTIC SYNDROME PATIENTS

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Introduction: Myelodysplastic syndromes (MDS) associated with mutations in genes belonging to the splicing machinery, such as SF3B1, SRSF2, U2AF1 and ZRSR2, are characterized by inefficient hematopoiesis and variable risk of evolving into AML. Mutations affecting SF3B1 are among the most common alterations, correlating with low-risk phenotype (LR-MDS) and response to Luspatercept treatment. We were interested in the characterization of the expression profiles and the spliceosome of LR-MDS mutated for splicing factors (SF).

Methods: We selected a study cohort of 50 LR-MDS (30 M and 20 F, med. age 75 yrs, range 40-91) characterized by mutations in SF genes (SF3B1=15, SRSF2=9, U2AF1=11 and SF-wt=15).

Results: RNA-Seq on 50 LR-MDS vs 64 non-haematologic pts revealed 4221 differentially expressed genes (DEGs), 3921 down- and 300 up-regulated. When subgrouping patients according to specific SF mutations, SF3B1-mutated MDS (n=15) showed the highest number of DEGs (622: 10 down- and 612 up-regulated) when compared to SF-wt pts (n=7). Gene Ontology analysis identified 37 deregulated pathways in SF3B1-mut vs SF-wt pts. We found 6 DEGs in TGF-β pathway (CHRD, DCN, SMAD9, FST, ID4, PITX2) and 10 in TGF-β modulators (BDNF, BMPER, COL1A1, COL1A2, FN1, GLI2, IGF1, MMP2, NFIB and PLAU). DEGs were confirmed using Q-rt-PCR assays: DCN (p=0,004), FST (p=0,017), SMAD9 (p=0,030), FN1 (p=0,004), IGF1 (p=0,030) and MMP2 (p= 0,004). We then investigated the presence of alternative splicing events (ASE) within the TGF-β pathway, identifying an intron 15 retention on the USP15 gene, a ubiquitin specific protease. This yields a truncated protein isoform (USP15-TR), which was preferentially expressed in SF3B1-K700E vs wt MDS, without changes in total mRNA. USP15-TR was overexpressed in SF3B1-K700E mutated K562 cells ($p<0.02$) as compared to USP15 full length (USP15-FL), without changes in total mRNA. The stability of USP15-TR was confirmed in K562 SF3B1-K700E cells by non-sense mediated decay assays using cycloheximide. Western blot (WB) analysis confirmed a reduction of USP15-FL protein, with a relative increase of USP15-TR ($p<0.05$). Looking for alterations in protein expression and phosphorylation of TGF-β pathway players, bioinformatic analysis in MDS samples showed reduction of SMAD3 and SMAD7 mRNA expression, confirmed at protein level (WB) in the K562 SF3B1-K700E cell model ($p<0.01$ and $p<0.05$). SMAD2 protein levels were slightly reduced, while phosphorylation was clearly increased, highlighting hyperactivation of the TGF-β pathway in SF3B1-mut cells. This was also confirmed by the ectopic expression of either USP15 isoforms in HEK293T cell, showing an increased SMAD2 phosphorylation in USP15-TR vs USP15-FL engineered cells.

Conclusions: Our preliminary studies highlight the deregulation of TGF-β pathway at mRNA, ASE and protein levels in SF3B1-mut pts and engineered cells, suggesting the potential role of USP15 as biomarker and/or actional target in clinical practice.