INVESTIGATIONS ON THE MOLECULAR MECHANISM OF PACSIN2IN THE REGULATION OF TPMT ACTIVITY AND AUTOPHAGY

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Introduction: Thiopurines, in particular mercaptopurine, are commonly used in the treatment of acute lymphoblastic leukemia (ALL) and inflammatory bowel disease (IBD). Thiopurines are inactivated by the enzyme thiopurine-methyltransferase (TPMT). Patients positive for genetic polymorphisms of TPMT present low enzyme activity and an increased risk of developing side effects, such as myelosuppression, that can be harmful and also lead to discontinue the drug treatment. PACSIN2is a member of the protein kinase C and casein kinase substrate in neurons family of proteins and seems to modulate TPMT activity with a molecular mechanism that remains unclear. PACSIN proteins localize on membranes and are involved in cellular processes such endocytosis and caveolae formation. A previous study demonstrates that TPMT activity is reduced after PACSIN2knockdown in a human B-lineage lymphoblastic leukemia cell line (NALM6) and an agnostic gene-expression analysis identified autophagy as one of the pathways significantly affected by the reduction of PACSIN2. This study investigates the role of PACSIN2knockdown and TPMT over-expression in a cellular model in presence or absence of autophagy flux inhibitors in order to understand the impact on autophagy and on thiopurine response.

Material and methods: NALM6cells were engineered in order to obtain a cell line with stable PACSIN2knockdown (KD), one with TPMT*1(*1) overexpression, one with both modifications (KD*1) and one with the stable transfection of an empty vector (MOCK), as control. TPMT gene expression was evaluated through TaqMan Real Time PCR using 18S rRNA as normalizer. TPMT and PACSIN2protein levels were quantified by Western Blot and densitometry using actin as normalizer: the effect of PACSIN2knockdown on the protein level of the autophagy marker LC3-II was evaluated. In order to evaluate drug sensitivity, MTT assay was performed with different concentrations of mercaptopurine (MP). Drug sensitivity was expressed by the half inhibitory concentration (IC_{50}). Experiments were performed in triplicate.

Results: As expected, *1and KD*1present higher TPMT expression at both gene (fold change 58.83and 18.72respectively) and protein levels (fold change 1.76and 1.12respectively) compared to MOCK. Western blot confirmed that KD and KD*1cells presented lower band intensity for PACSIN2compared to MOCK (fold change 0.28and 0.53respectively). Interestingly PACSIN2KD in KD*1lowered TPMT protein level compared to *1(fold change 0.64± 0.15). Under basal conditions, LC3-II protein increased both KD (fold change 2.14± 0.17) and KD*1cells (fold change 3.29± 0.8), while *1presented similar expression (fold change 0.92± 0.4), compared to MOCK. MTT assay showed that *1was the most sensitive cell line to MP (IC_{50} MOCK: $1.1x\ 10^{-6}M$; KD: $1.4x\ 10^{-6}M$; *1: $4.3x\ 10^{-7}M$; KD*1: $8.25x\ 10^{-7}M$). A comparison among IC_{50} showed that *1are two times more sensible to MP than KD*1(fold change 2.1± 0.15, p value 0.0020, t-test).

Discussion and conclusion: PACSIN2KD is related to an increased LC3protein basal level, suggesting that PACSIN2could prevent autophagy induction. MTT assay results suggest that PACSIN2KD decreases mercaptopurine sensitivity in vitro in cell lines overexpressing TPMT, suggesting a role of PACSIN2on TPMT activity. Experiments with autophagy flux inhibitors (chloroquine and quinacrine) are in progress.