CHARACTERIZATION OF THE NOTCH AND MTOR PATHWAY IN CONTEXT OF THEIR CROSSTALK IN HODGKIN LYMPHOMAS

Noémi Nagy
Ágnes Márk[1], Melinda Hajdu[1], Mónika Tóth[1], Titanilla Dankó[1,] Péter Attila Király[1], Csaba Bödör[1], Péter Hollósi[1], László Kopper[1], Anna Sebestyén[1,2]

1: 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
2: Tumor Progression Research Group of the Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

Introduction: Notch is a highly conserved, transmembrane receptor that regulates cell differentiation and apoptosis, and plays a critical role in development. Another important element of the signalling network is the mammalian target of rapamycin (mTOR) pathway, which controls cellular proliferation and survival.
Growing evidence demonstrates that Notch1 regulates PI3K–AKT–mTOR1 signalling in T-ALL, but this aspect is poorly characterized in Hodgkin lymphomas (HL).
We characterized mTOR activity and Notch1 expression in HL cell lines and human tissues, and investigated the interaction of the two pathways by inhibitor and activator treatments and mutational analysis.

Methods: We examined the activity and expression of mTOR and Notch signals in human HL cell lines (KMH2, L1236, DEV) and in human HL biopsies by immunocytochemistry, immunohistochemistry (IHC) and Western-blotting (mTOR, p-mTOR, p-S6, p-4EBP1, Notch1, cleaved-Notch1). Cells were treated with rapamycin (mTORC1 inhibitor), DAPT (γ-secretase inhibitor) and Jagged1 (Notch1 ligand) in vitro. The biological effects (proliferation, apoptosis) were investigated by AlamarBlue® assay and flow cytometry analysis. Target gene expression (Hes1, c-myc) was examined by qPCR. Exons 5, 6, 9, 10 and 11 of FBXW7 ubiquitin ligase (one point of interaction between these signals) and exon 9 and 20 of PI3KCa were sequenced in cell lines.

Results: Increased mTOR and Notch1 activity was observed in HL cell lines and samples of patients. Notch1 receptor was present in all HL cell lines, but no biological effects and no changes in target gene expression were detected after DAPT and Jagged1 treatments. Apoptosis induction and the inhibition of cell proliferation was detected after long-term rapamycin treatment and combined inhibitor treatment in vitro and in vivo. Interestingly, cleaved, constitutively activated Notch1 was found in all samples, even after rapamycin and DAPT treatment. Sequencing of FBXW7 and PI3KCa revealed no mutations in our cases.

Conclusion: The constitutively activated form of Notch1 draws the attention to the importance of Notch1 signals in the context of mTOR activity in HL.
Due to the lack of mutations in the FBXW7 ubiquitin ligase and PI3KCa, the background of increased Notch1 and mTOR activity remains unexplained in HL, and needs more exploring. According to these results, targeted therapy of mTOR could be successful in cases with activated Notch signals as well, however, additional Notch inhibition could give more benefit during therapy.

Supported by OTKA81624 and OTKA84262

POSZTER, ELMÉLETI