The IGF1R/IRS Inhibitor NT157 Induces Apoptosis and STAT3 Inhibition in JAK2^{V617F} Cells

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INTRODUCTION: JAK2/STAT signaling has been well described in myeloproliferative neoplasms (MPN). IGF1R signaling has been recently demonstrated as an important pathway involved in the pathogenesis of myeloproliferative neoplasms, and our group identified that IRS2 cooperates to malignant transformation induced by JAK2^{V617F}. NT157 was designed as IGF1R/IRS inhibitor and is able to induce anti-neoplastic effects in solid tumors. **OBJECTIVES:** To characterize the molecular and cellular effects of NT157 treatment in the MPN cell model HEL JAK2^{V617F}. **MATERIALS AND METHODS:** HEL cells were treated or not with NT157 in different concentrations (0.2, 0.4, 0.8, 1.6 and 3.2 µM) for 24, 48 and 72 hours and submitted for cell viability (MTT), clonogenicity (colony formation), cell cycle (flow cytometry), apoptosis (annexin-V/PI and caspases cleavage) and PCR array for oncogenes and tumor suppressor genes. Protein expression and activation were evaluated by Western blot. Statistical analyses were performed by ANOVA test. **DISCUSSION AND RESULTS:** NT157 treatment decreased cell viability and clonal growth in a dose and time-dependent manner (all $p < 0.05$). NT157 also significantly induced apoptosis as verified by flow cytometry analysis of Annexin-V/PI and confirmed by increased caspases 3, 8 and 9 cleavage. Cell cycle analysis revealed a cell cycle arrest in G2/M associated with a decrease in G0/G1 population (all $p \leq 0.01$) upon NT157 treatment. PCR array identified 23 modulated genes by NT57, including CCND1, CDKN1A and MYB that are involved in cell cycle progression. NT157 inhibited ERK1/2 and STAT3 phosphorylation; doses equal or higher than 0.8 µM were sufficient to completely abolish phospho-STAT3 signal. **CONCLUSION:** NT157 treatment displayed remarkable anti-cancer effects in a hematologic lineage harboring JAK2^{V617F} mutation. The CDKN1A upregulation and downregulation of CCND1 and MYB, identified by PCR array, provide new insights on NT157 action. Therefore, NT157 represents a potential anti-cancer agent by a dual mechanisms, targeting IRS1/2 and STAT3 in myeloproliferative neoplasms.

Key words: Myeloproliferative neoplasms; NT157; IGF1R/IRS

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