Metformin Has Anticancer Activity and Improves Ruxolitinib Treatment in JAK2^{V617F} Cells

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INTRODUCTION: JAK2/STAT signaling pathway is constitutively active in myeloproliferative neoplasms (MPN) and has been target with ruxolitinib, a selective JAK1/2 inhibitor, which improves constitutional symptoms, but does not reverse bone marrow fibrosis, suggesting the need for new therapeutic approaches. Metformin is an activator of AMPK, an intracellular energy sensor that inhibit mTOR activity, with a potential anticancer activity. Studies to assess the effect of combining ruxolitinib and metformin may be interesting in MPN. **OBJECTIVES**: To investigate the cellular and molecular effects of metformin combined or not with ruxolitinib, in MPN cells. **MATERIALS AND METHODS**: HEL and SET2 (JAK2^{V617F}) cell lines treated or not with ruxolitinib (300 nM) and/or metformin (5 and 10 mM) were submitted for cell viability (MTT), clonogenicity (colony formation), cell cycle (flow cytometry), apoptosis (annexin-V/PI and caspase 3/PARP1 cleavage) and PCR array for PI3K/AKT-related genes. Gene and protein expressions were evaluated by qPCR and Western blot. Statistical analyses were performed by ANOVA test or Student's t-test. **DISCUSSION AND RESULTS**: In HEL and SET2 cells, metformin or ruxoltinib treatment significantly reduced cell viability and clonogenicity (p<0.05). Ruxolitinib plus metformin significantly decreased cell viability and induced apoptosis when compared with monotherapy (p<0.05). Metformin and/or ruxolitinib resulted significant reduction in G_2/M phase. PCR-array identified 14, 11 and 17 genes modulated by ruxolitinib, metformin and combined treatment, respectively; both drugs reduced cyclin D1 and upregulated p27. Ruxolitinib treatment reduced STAT3, STAT5, ERK1/2, 4EBP1 and P70S6K phosphorylation. Metformin also reduced activation of same proteins, but combined treatment presented more intense inhibition of ERK1/2 and P70S6K activation compared to metformin or ruxolitinib monotherapy. **CONCLUSION**: Metformin exerts an anticancer activity and downregulated JAK2/STAT and mTOR/P70S6K/4EBP1 signaling in JAK2^{V617F} cells. PCR-array identified cyclin D1 and p27 as target of metformin and ruxolitinib, corroborating cell cycle findings. Combined treatment, ruxolitinib plus metformin, may be an effective approach to reduce cell viability of JAK2^{V617F}cells.

Key words: Myeloproliferative neoplasms; Metformin; Ruxolitinib

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