Identification of cellular models to study the role of the long noncoding RNA GAS5 as modulator of glucocorticoid response

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GAS5 is a long noncoding RNA that interacts with the DNA binding domain of the activated glucocorticoid receptor (GR). This binding prevents the transcription of glucocorticoid (GC) responsive genes, modulating GC response, as demonstrated in a recent work in our laboratory on peripheral blood mononuclear cells (PBMCs) treated with methyl-prednisolone (MP). The aim of this study was to investigate the role of GAS5 as modulator of GR activity in immortalized human cell lines. The inhibitory effect of MP on cell proliferation at different concentrations (20 μg/ml-0.019 ng/ml) for 72 h was determined by [methyl-3H] thymidine incorporation assay on HeLa, CCRF-CEM and LoVo cell lines.

The inhibition of proliferation at 250 ng/mL of MP ($I_{250ng/ml}$) was calculated in HeLa ($I_{250ng/ml}$= 77%), CCRF-CEM ($I_{250ng/ml}$= 15%) and Lovo ($I_{250ng/ml}$= 26%) cells, to establish individual cell lines GCs sensitivity.

Gene expression analysis was performed to evaluate GAS5 levels on these cells after 72 h treatment with MP (250 ng/ml). The results showed a downregulation of GAS5 in GCs sensitive HeLa cells in comparison with untreated cells (Relative Expression (RE)= 0.47) and an upregulation in GCs resistant LoVo cells (RE= 1.43): this different expression profile was already demonstrated in PBMCs good and poor responders for GCs. In contrast a downregulation of GAS5 was detected in resistant CCRF-CEM cells (RE= 0.49).

RNAi experiments were performed to knockdown GAS5 on HeLa cells treated with MP for 72 h. Data showed an increased response to the drug in HeLa (+)SiRNA cells ($I_{250ng/ml}$ and $I_{10\ ng/ml}$ MP = 82% and 53%, respectively) compared to HeLa (-)SiRNA ($I_{250ng/ml}$ and $I_{10\ ng/ml}$ MP = 76% and 32%, respectively) confirming that GAS5 interferes with GC effects.

These results suggest that these cell lines could be an useful model for studying the role of the long noncoding RNA GAS5 as modulator of GCs response.