Noncoding RNA Gas 5 as marker for predicting response to glucocorticoids

M. Lucafò[1], S. De Iudicibus[1], A. Di Silvestre[2], M. Pelin[2], E. Piscianz[1], A. Tommasini[1], G. Decorti[2]

[1] Institute for Maternal and Child Health I.R.C.C.S. 'Burlo Garofolo', Trieste, Italy
[2] Dept. of Life Sciences, University of Trieste, Italy

Glucocorticoids (GCs) are largely used in patients with chronic inflammatory diseases, however a considerable variability in response to these drugs is evident. GCs exert their biological effects through binding to the GC receptor (GR), which translocates from the cytoplasm into the nucleus and binds, through its DNA-binding domain (DBD), to glucocorticoid response elements (GREs) in the regulatory regions of GC responsive genes.
Growth arrest-specific 5 (Gas 5) is a long (~650 bases in humans) noncoding RNA (ncRNA) that was originally isolated during a screening for potential tumor suppressor genes expressed at high levels in growth arrest (1) even though its functions are not yet well known. Recently, it was found that Gas 5 interacts with the DBD of the ligand-activated GR and suppresses GR-induced transcriptional activity of glucocorticoid-responsive genes by inhibiting binding of GRs to target genes GREs (2).
The aim of this study was to evaluate the association between individual variability in the anti-proliferative efficacy of methyl-prednisolone (MP) with GC receptor gene (NR3C1) and Gas 5 expression in lymphocytes obtained from healthy subjects.
A preliminary study was conducted on 10 buffy coats of healthy donors, and peripheral blood mononuclear cells (PBMCs) were purified for proliferation and gene expression analysis. The effect of MP at 72 hours on PBMCs proliferation was determined by [methyl-3H] thymidine incorporation assay. Using nonlinear regression, a sigmoidal dose-response curve was extrapolated for each subject, and the Imax (the percentage of inhibition observed at 250 ng/ml of MP) was calculated. Subjects were divided into two groups to define individual MP response: MP responder had an Imax>40% (8 subjects; median 58, range 43 - 86%) and MP resistant had an Imax<40% (2 subjects; median 8.5, range 3 - 14%). Total RNA was extracted with a dedicated kit from samples treated for 72 hours with and without MP (250 ng/ml); NR3C1 and Gas 5 gene expression was analyzed using TaqMan® technology.
After MP treatment a downregulation of both Gas 5 and NR3C1 genes was observed, compared to untreated controls, in healthy donors PBMCs responsive to MP treatment (Log_2 Fold change values: Gas 5= - 0.7; NR3C1= - 0.4). On the contrary, the two resistant subjects showed an upregulation of the same genes (Log_2 Fold change values: Gas 5= + 2.3; NR3C1= + 1.3; Unpaired t-test, MP responder *vs* MP resistant: Gas 5= *p-value*<0.0001, NR3C1: *p-value*=0.06). We hypothesize that, in resistant PBMCs, as a consequence of Gas 5 interaction, a reduced availability of the activated GR for binding to GREs target genes suppresses GC transcriptional activity.
These preliminary results could suggest, if confirmed in a larger number of samples, that Gas 5 ncRNA evaluation, associated with a lymphocytes proliferation assay, could represent a useful tool to predict the clinical response to steroid treatment in patients with chronic inflammatory diseases.

1. Schneider C, et al. (1988) *Cell.* 54:787–793.
2. Kino T, et al. (2010) *Sci Signal*. 3(107).