Pharmacoepigenomic markers involved in glucocorticoid response in children with inflammatory bowel disease

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Despite the introduction in therapy of highly effective biological agents, glucocorticoids (GCs) are very effective in inducing remission in inflammatory bowel disease (IBD) patients with moderate to severe disease, and are still widely used. Given the high incidence of suboptimal response, associated with a significant number of side effects, particularly severe in children, the identification of patients that are most likely to respond poorly to GCs is extremely important. However, the mechanisms of this variability are scarcely understood and there is presently no means to predict the response in advance. In this context, the role of epigenomic determinants, in particular microRNAs (miRNAs) and the possible correlation between their expression and variability in GC response is a promising field of research. miRNAs are small non-coding RNAs and usually work as post-transcriptional regulators for gene expression through pairing with the 3'-untranslated region of the mRNA target genes.

The aim of this research is the identification of pharmacoepigenomic markers useful for the personalization of GC therapy in pediatric IBD, through the evaluation of the different patterns in miRNA expression profiles during GC treatment and their association with therapy outcomes.

Nineteen IBD pediatric patients (mean age at enrolment 12.8 years, 14 ulcerative colitis and 5 Crohn's disease, 9 males and 10 females) were enrolled at the Pediatric Clinic of IRCCS Burlo Garofolo in Trieste in a prospective study, and treated with prednisone 1 to 2 mg/kg/day for 30 days. Peripheral blood was obtained from these patients at diagnosis (T0) and after 4 weeks of steroid treatment (T4). RNA was extracted from patients' peripheral blood mononuclear cells at T0 and T4, and used to analyze miRNA and mRNA profiles using next generation sequencing platform Ion Proton System. Selected miRNAs with significant differences in expression were validated by quantitative real time-PCR (qRT-PCR), using TaqMan miRNA assays. Patients were classified on the basis of their clinical response into 2 groups: steroid sensitive (SS; n=15), and steroid resistant (SR; n=4). SS subjects were further stratified into steroid dependent subjects (SD; n=8).

Of all miRNAs sequenced in T0, 20 were differentially expressed (p-value < 0.05) between SS+SD and SR patients, while in T4 this number decreased to 10 miRNAs.

In order to validate these results by qRT-PCR methods, we selected 4 miRNAs (miR-1180-3p, miR-876-5p, miR-1255a and miR-31-3p) that were differentially expressed in T0, based on the fold changes and previous knowledge associating these miRNAs to GC effects. In particular, a good agreement between sequencing and real time data was observed for the miR-1180-3p, upregulated in SR respect to SS+SD patients. It is known that miR-1180-3p directly targets key inhibitors of the nuclear factor (NF)-κB signaling pathway, whose activation is markedly induced in

IBD patients. We hypothesize that, in SR patients, abnormally high levels of miR-1180-3p, through the aberrant activation of NF-κB signaling, contributes to GC-induced resistance.

To identify dysregulated miRNAs with their corresponding predicted target, a preliminary association study between miRNAs and gene expression profiles on 6 patients (3 SS vs 3 SR) was also performed: in T0 58 genes resulted upregulated and 45 downregulated, while in T4, 82 genes were upregulated and 100 downregulated. Among the miRNAs emerged in T0, the downregulated miR-100-5p and miR-618 were negatively associated with the inhibitor of DNA binding 1 (ID1) and C-C motif chemokine receptor 2 (CCR2) expression, respectively. ID1 and CCR2 transcripts, upregulated in SR patients, have already been associated with IBD and proinflammatory damage.

If these results are confirmed in a larger number of patients, miRNAs could be considered novel pharmacoepigenomic markers useful for the personalization of GC therapy in pediatric IBD.