miRNA regulation during glucocorticoid treatment in children with inflammatory bowel disease

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To date, a curative pharmacological therapy for inflammatory bowel diseases (IBD) does not exist and the therapeutic approach is mainly aimed at controlling inflammation, with drugs capable of inducing and maintaining remission. Despite the introduction in therapy of highly effective biological agents, in IBD patients with moderate to severe disease glucocorticoids (GCs) are effective in inducing remission and are still considered the standard for treatment especially for ulcerative colitis. Given the high incidence of suboptimal response, associated with a significant number of side effects, the identification of subjects that are most likely to respond poorly to these agents 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, microRNAs (miRNAs) represent a new and promising field of research.

miRNAs are small (18 – 24 nucleotides) non-coding RNAs, which bind the 3'UTRs and the coding exons of their target genes and inhibit gene expression: a single miRNA can regulate a multitude of mRNAs (approximately 200), and each mRNA can be regulated by multiple miRNAs. There is a lot of interest in identifying the role of miRNAs in the modulation of drug response, but studies about this topic are still very limited, and the possible correlation between miRNAs expression and variability on GC response in IBD patients has not yet been examined.

The aim of this research is the identification of novel pharmacogenomic biomarkers useful for the personalization of GC therapy in paediatric IBD, through the evaluation of the different pattern in miRNA and mRNA expression profiles during GC treatment.

Six IBD paediatric patients (mean age at the enrolment 15.1 years, 5 ulcerative colitis and 1 Crohn's disease, 3 males and 3 females) were enrolled at the Paediatric 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' PBMC at T0 and T4, and used to analyze miRNA and mRNA profiles using next generation sequencing platform Ion Proton System.

Of all miRNAs sequenced, 89 were differentially regulated (p-value less than 0.05; range from 3.36×10^{-7} to 0.006129). After normalization analysis, a total of 45 miRNAs was upregulated after GC treatment, and 44 downregulated. The absolute fold changes ranged from 1.89 to 6.95 for up-regulated miRNAs, and -3.37 to -1.83 for downregulated ones.

Interestingly, among miRNAs upregulated after GC treatment, miR-192-5p contained GC responsive elements (distance from transcription start site 1189-1204 pb), which represent the sites responsible of a direct regulation by the GC receptor. Moreover, considering RNA sequencing results, an increased GILZ expression was observed at T4: GILZ is induced by GCs, and plays a key role in their antiinflammatory and immunosuppressive effects. Bioinformatic analysis revealed that GILZ is a putative target gene of miR-1229-3p and miR-887-3p, that were indeed downregulated by GC treatment.

In conclusion, integration of miRNA and mRNA analyses in a larger number of patients could represent innovative strategies that will allow to better understand GC mechanism of action and resistance.