HIGH-THROUGHPUT MIRNOME AND TRANSCRIPTOME PROFILING IN THALIDOMIDE SENSITIVE PEDIATRIC INFLAMMATORY BOWEL DISEASE PATIENTS

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Background and aim: Thalidomide has anti TNF-alfa, immunomodulatory and anti-angiogenic properties, and plays a pivotal role in the treatment of multiple myeloma. Recent studies have demonstrated the mechanism of action of this drug and have shown that it directly binds cereblon, a substrate receptor of the cullin-4RING E3ubiquitin ligase complex which recognizes specific targets for their ubiquitination and degradation. In the last twenty years, thalidomide has emerged as a powerful immunomodulator in the treatment of pediatric patients with inflammatory bowel disease, in particular Crohn's disease and ulcerative colitis, refractory to standard therapies. However, thalidomide use is often limited by its safety profile. The risk of teratogenicity is nowadays controlled with strict pregnancy prevention programs. Peripheral neuropathy is one of the most frequent adverse events and is a common cause of treatment discontinuation. The molecular mechanisms by which thalidomide regulates inflammation has not been clarified yet. In this context, the aim of this research is the identification of determinants useful for better understanding the molecular mechanisms underpinning thalidomide action in pediatric IBD, by evaluating high-throughput microRNAs (miRNAs) and messenger RNAs (mRNAs) profiles during thalidomide treatment.

Methods: Ten IBD paediatric patients (mean age at enrolment 13.1years, 6Crohn's disease, 6males) refractory to previous pharmacological therapies and responsive to thalidomide were enrolled. Peripheral blood was obtained before (T0) and after twelve weeks (T12) of thalidomide treatment. MiRNA and mRNA profiles were sequenced using next generation sequencing Illumina platform. The differential miRNA and mRNA expression analysis between the samples collected before and after thalidomide treatment was performed using the edgeR package. Differentially expressed miRNAs and mRNAs were identified based on a fold change threshold of |2|, coupled with a false discovery rate-corrected p-value lower than 0.05. In order to detect the potentially altered pathways by differentially expressed miRNAs and mRNAs, the DIANA-miRPath software and KEGG database were used, respectively.

Results: Of all miRNAs sequenced, 10 were differentially expressed from T0 and T12(7upregulated and 3downregulated). Out of these, five upregulated miRNAs could putatively recognize the 3'UTR of several Hox genes, a group of transcription factors that play important roles in the development of structures such as limbs, lungs and nervous system. Interestingly, the pathways related to adherents junction, ECM-receptor interaction and ubiquitin mediated proteolysis were detected by the identified miRNAs using DIANA algorithm which predicts miRNA-target interactions. The RNA-seq analysis identified 84mRNAs deregulated after treatment, 27of which were downregulated. The gene set enrichment analysis highlighted the potentially altered pathways including neuroactive ligand-receptor interaction, cell adhesion molecules and drug metabolism pathways.

Discussion and conclusion: In this first study about the evaluation of miRNome and transcriptome in cells of IBD patients treated with thalidomide, we identified several miRNAs and mRNAs differently expressed which could help to elucidate the mechanism of action of thalidomide in young IBD patients. Moreover, our results may suggest new molecular targets involved in the adverse effects of this drug.

These results could represent a first step towards their translation as pharmacogenomic biomarkers.