Inflammatory bowel disease patient-derived organoids for personalized medicine

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BACKGROUND: Inflammatory bowel disease (IBD) includes Crohn’s disease (CD) and ulcerative colitis (UC). Although the cause of IBD is still unknown, accumulating evidence suggests that the intestinal epithelium plays an important role in its pathogenesis. Actually, a curative therapy for IBD does not exist, but many drugs are currently employed. The effects of all these drugs are characterized by a high interpatient variability, associated with a significant number of side effects. We hypothesize that the therapeutic effects of immuno-suppressants used to treat IBD involve also a direct action on epithelial cells, that can be recapitulated in vitro using innovative 3D models named organoids which are capable of simulating the architecture and functionality of the native organ.

AIM: The project aims to establish an in vitro IBD model using human colonic organoids starting from biopsic intestinal samples of pediatric patients with IBD to investigate the effects of drugs used for treating these patients. The model will be validated considering molecular and cellular features, in particular cytotoxicity, expression of the molecular targets of the drugs tested and anti-inflammation actions observed.

METHODS: We have enrolled eighteen patients with IBD (mean age at enrolment 13.78 years, 9 Crohn’s disease, 11 males) and nine non-IBD patients (subjects undergoing colonoscopy, for suspected IBD, used as control, mean age at enrolment 8.26 years, 5 males), and successfully generated organoids from 16 patients (13 patients with IBD and 7 control subjects). Intestinal biopsies of patients were used to isolate crypts and generate organoids. Crypts embedded in Matrigel were cultured and passaged as described by Jung et al. (Nat Med. 2011). The cytotoxicity of the various drugs was evaluated by CellTiter-Glo 3D assay. Gene expression was evaluated using TaqMan® Gene Expression assays.

RESULT 1: EVALUATION OF MUC2 EXPRESSION.
A statistically significant increase in mucin2 (MUC2) expression in IBD patients (RE= 0.01) compared to the control (CTRL) subjects (RE= 0.002) was highlighted (Fig. 1). MUC2 is the primary component of the mucin barrier that separates the intestinal microbiota and the intestinal epithelium and hyperproduction of mucins and abnormal glycosylation is typical for CD patients (PMID: 3994447).

RESULT 2: EVALUATION OF THE CYTOTOXICITY OF DRUGS USED IN THE TREATMENT OF IBD.
Preliminary results were obtained on IBD-derived intestinal organoids and control organoids exposed for 72 hours to increasing concentrations of the drugs used in clinical practice, including glucocorticoids (methylprednisolone), thiopurines (azathioprine and mercaptopurine), thalidomide and infliximab. Data on the cytotoxic effects of the drugs tested on intestinal organoids (both IBD and control) showed that the cells were more sensitive to thiopurines compared to the other immunosuppressant agents (on which a lack of cytotoxicity is observed) and that there is a dose dependent cytotoxicity. Furthermore, the organoids generated from IBD-patients seem to be more sensitive to thiopurines than controls. (Fig. 2A, 2B)

Considering the specific effect of thiopurines on each IBD patient-derived organoids, an inter-individual variability has been observed highlighting how this cellular model could be able to reflect a possible different sensitivity of the patient to the treatment. In particular, a higher sensitivity of ORG12 is observed with the two highest concentrations of azathioprine and the two lowest of mercaptopurine. (Fig. 3A, 3B)

RESULT 3: GENE EXPRESSION ANALYSIS.
The IBD-derived organoids were also used for gene expression analysis of the main targets of the drugs used. In particular, the expression levels of hypoxanthine-guanine phosphoribosyltransferase (HPRT), thiopeure S-methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) as markers for thiopurine drugs were quantified. Globally, TPMT (RE=0.001) appears to be more expressed than ITPA (RE=0.007) in a statistically significant manner, suggesting a higher contribution of the biotransformation by this enzyme in these organoids. (Fig. 4) Furthermore, it has been observed that the patient-derived organoid ORG12, which was found to be more sensitive to the action of thiopurines, has a statistically significant increase in the expression of TPMT compared to the other organoids under examination (** p<0.01; ** p<0.001).

The expression levels of the nuclear receptor subfamily 3 group C member 1 (NR3C1), TSC22 domain family member 3 (GILZ) and the long non-coding RNA GASS as markers for glucocorticoids were also quantified. NR3C1 (RE=0.005) and GILZ (RE=0.002) genes, involved in glucocorticoid activity, were similarly expressed in comparison to the thiopurine markers even though the cytotoxic effect of glucocorticoids was completely absent. A hypothesis is related to the higher expression of GASS (RE= 0.07), a repressor of the glucocorticoid receptor, which could interfere with the activity of the drug. Further experiments will investigate this mechanism. (Fig. 5)

CONCLUSIONS: these preliminary results showed that patient-derived organoids could be a valid models for the evaluation of the possible harmful effect that drugs currently used in the treatment of IBD can exert on the intestinal epithelium. Moreover, intestinal organoids are particularly sensitive to thiopurines and an inter-individual variability in response to this drugs can be highlighted indicating that organoids could be an innovative tool useful for therapy personalization.