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An EPHA2-centered gene regulatory network associates with hyperbaric oxygen treatment response in perianal fistulizing Crohn's disease patients

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INTRODUCTION

Perianal fistulizing Crohn's disease (PFCD) is a debilitating CD complication characterized by the formation of a tract (fistula) between the anorectal mucosa and other epithelial-lined surfaces. Despite combination of therapeutic agents and surgical treatment, long-term remission rates remain low, often not exceeding 50%[1]. Hyperbaric oxygen therapy (HBOT) is a treatment that delivers 100% oxygen in a pressurized chamber and previously proved clinical efficacy in PFCD[2]. We used HBOT as an adjunct therapy to potentially reduce disease activity and improve amenability to surgical closure in PFCD.

AIM

We aim to investigate the cellular representation and transcriptomic processes in fistula biopsies before and after HBOT through single-cell RNA-sequencing (scRNA-seq) and assess the differences between responders and non-responders.

METHOD

Patients with active PFCD refractory to optimized medical therapy were included and treated with 40 sessions of HBOT (110 minutes with 5-minute air breaks, 100% oxygen at 2.4 atmosphere) (**Fig. 1**).

Biopsies were collected from the internal opening and adjacent rectal mucosa at baseline and 4 weeks into HBOT at which fistula closure surgery was performed. Biopsies were subsequently analyzed with scRNA-seq to find differences between responders and non-responders.

Response was defined by MRI response (MAGNIFI-CD decrease ≥ 2) at 26 weeks follow-up.

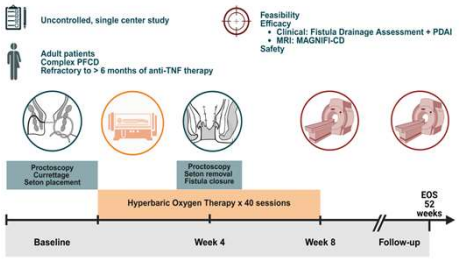

Figure 1: Timeline of the HBOT AMFIBIO trial design.

RESULTS

We included 10 patients (7 responders and 3 non-responders), from which we acquired transcriptomic data of 34,185 cells (**Fig. 2A and 2B**).

At baseline, responder-derived epithelial cells displayed a gene-regulatory network (GRN) in epithelial cells centered around EPHA2 and its downstream ephrin pathway, which disappeared at follow-up (**Fig. 2C**).

Notably, the EPHA2 driven GRN was observable among responders but not non-responders prior to the start of treatment (**Fig. 2D**) whilst EPHA2 expression significantly decreased in responders, it was unaffected in non-responder over time (**Fig. 2E**).

The EPHA2-GRN showed no significant differential expression when comparing inflamed and non-inflamed colonic biopsies from Kong et al.[3], suggesting EPHA2 GRN is not related to an inflammation-dependent process (**Fig. 2F**).

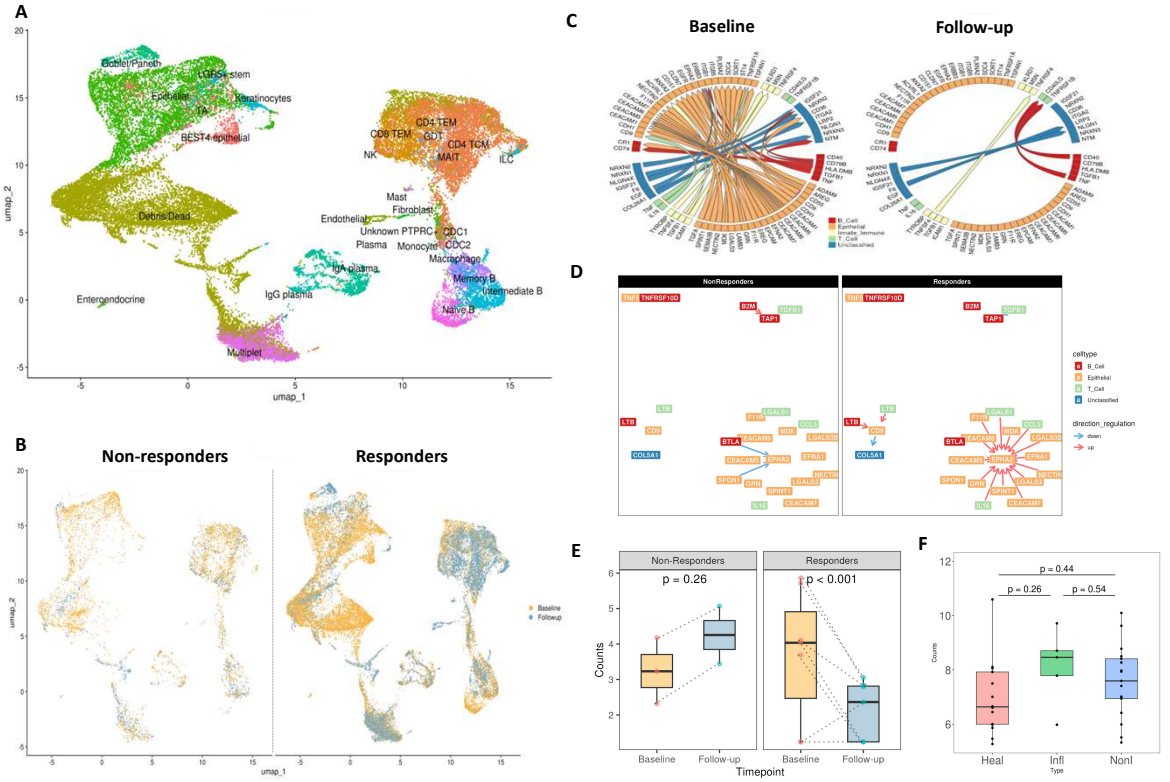

Figure 2: UMAP of 34,185 cells across 10 patients, colored by (**A**) cell type, or (**B**) timepoint and split by response status. **C** – Chord diagram of differential cell-cell interactions of responders at baseline and follow-up. **D** – EPHA2-centered gene regulatory network is upregulated only among responders. **E** – EPHA2 expression indicating significant downregulated across treatment among responders but not among non-responders. **F** – EPHA2 expression as published by Kong et al. grouped by phenotype shows no differential expression between the three conditions.

CONCLUSION

The EPHA2-GRN associates with response to HBOT in PFCD patients *a priori* and presents itself as a potential biomarker for response after fistula closure surgery.

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