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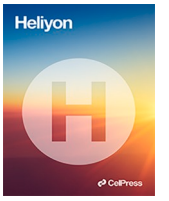

Review article

TWISTed fibroblasts: New drivers of intestinal fibrosis in Crohn's disease

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ABSTRACT

Fibrosis is the pathological consequence of chronic inflammation. In Crohn's disease (CD), fibrostenotic complications occur with 50–70 % frequency as a failure to properly repair the tissue damage. Intestinal stenosis requires surgical intervention and relapses in most patients. Mesenchymal cells encompassed of heterogeneous cell subsets orchestrate this complex process. The lack of a full characterization of the stromal diversity and function in CD has consequently slowed the development of anti-fibrotic targets. Two recent studies align together demonstrating $FAP^{+}TWIST1^{+}$ fibroblasts as the primary mesenchymal population driving intestinal fibrosis in CD. Genetic and pharmacological targeting of Twist1 in mouse models proved the functional role of $Fap^{+}Twist1^{+}$ fibroblasts and indicate the use of the Twist1 inhibitor harmine as a potential therapeutic strategy to revert fibrosis.

1. Fibrostenotic complications in Crohn's disease

Fibrosis consists in the substitution of functional organ parenchyma with scar tissue composed of an exaggerated abundance of extracellular matrix (ECM) proteins, such as collagens. It is the final event of the persistent inflammation and tissue remodeling accompanying repeated cycles of injury and attempted repair. Fibrosis may virtually affect every organ and currently there are no effective anti-fibrotic therapies [1,2]. In the context of intestinal disease, particularly in patients affected by Crohn's disease (CD), fibrosis is responsible for stenotic complications requiring surgical removal in 40 % of patients. Yet surgery is not curative nor preventive as fibrostenotic complications will relapse in nearly half of the patients [3]. Therefore understanding the cellular and molecular basis of fibrosis development and designing adequate therapeutic strategies represent an urgent need in the field.

Intestinal fibrosis represents a maladaptive wound healing process attempting to repair the intestinal wall and therefore arises as a consequence of the interconnected involvement of immune cells, inflammatory mediators, tissue architectural components such as fibroblasts and the extracellular matrix (ECM) [4,5].

Fibroblasts, the principal effectors of fibrosis, are heterogenous cells of mesenchymal origin which fundamentally sustain tissue architecture during homeostatic conditions [6]. Their transient activation into myofibroblasts allows wound closure during physiological tissue repair, however, the persistent maintenance of such activation state triggers pathological fibrosis [7].

Intestinal fibroblasts are of diverse nature and the advent of single-cell sequencing techniques has revealed the multiplicity of

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fibroblast subpopulations present in the intestinal tissue and their differential roles during development, homeostasis and disease [8]. Their identity, as specified by different and sometimes overlapping markers, and functionality are heterogeneous [8]. For this reason, the phenotypic and molecular profiling of fibroblasts by single-transcriptomic techniques needs to be combined with the *in vivo* assessment of their functional role during chronic inflammation in order to identify mechanisms governing their capacity to drive fibrosis.

2. Identification of FAP$^+$ fibroblasts as drivers of CD-associated fibrostenosis

Recently, two back-to-back studies have identified a population of FAP$^+$ TWIST1$^+$ fibroblasts functionally involved in the pathogenesis of intestinal fibrosis in CD [9,10]. In the first study, Ke and Abdurahiman et al. [9] performed single-cell RNA-sequencing (scRNAseq) on patient-matched, full thickness transmural terminal ileum samples from fibro-stenotic CD patients undergoing ileocecal resection. They analyzed ileum samples displaying three distinct disease stages: non-affected, inflamed with ulceration and stenotic. By applying multiple computational tools, they identified FAP$^+$ fibroblasts as a unique population present in inflamed and stenotic ileum, geographically located in the submucosa and deeper layers of CD ileum. These FAP$^+$ fibroblasts display the most abundant ECM-producing capacity and express neutrophils and monocytes chemoattractant chemokines.

Trajectory analysis revealed GREM$^-$CD34$^+$ fibroblasts as the cell-of-origin of the pathological FAP$^+$ fibroblasts. Along their differentiation path, these cells lose CD34 expression and gain expression of FAP and Collagen I. This transcriptional reprograming was found mediated by the inflammatory cytokines IL1A, IL1B, TNF and TGFB1, identified by NicheNet prediction tool. Cellular source of these molecules was identified in a subset of CD150$^+$ highly inflammatory monocytes located in the deeper muscolaris layer of diseased ileum and closely co-localizing with FAP$^+$ fibroblasts. No elevation of this population was registered in the blood of CD patients, suggesting its tissue resident nature. *In vitro* co-culture and conditional media experiments confirmed that the CD150$^+$ inflammatory monocytes are able to activate fibroblasts to a collagen-producing FAP$^+$ phenotype. Authors detected the CD150$^+$ inflammatory monocytes also in the colon segments from CD but not from UC patients, indicating their unique association with CD.

In the second study, Zhang, Wang and Sun et al. [10]. conducted scRNA-seq investigation on matched fibrotic and non-fibrotic surgical specimens from the ileum of CD patients who underwent intestinal resection for fibrotic stricture. Among the four subsets of fibroblasts cluster the authors identified, FAP$^+$ fibroblasts displayed the highest ECM-producing capacity and resulted highly enriched in fibrotic areas. In this case pseudotime analysis revealed that FAP$^+$ fibroblasts are derived from FGFR2$^+$ fibroblasts, a pre-existing population that is predominantly located in non-fibrotic areas. In search for microenvironmental components possibly inducing such fibroblasts reprogramming, among the clustered immune cells the authors identified a population of CXCL9$^+$ macrophages displaying a high pro-fibrotic score and closely co-localizing with FAP$^+$ fibroblasts in fibrotic regions. These macrophages demonstrated high IL1B and TGFB1 ligand activity, with their respective receptor being expressed by the FAP$^+$ fibroblasts.

Importantly, this study highlighted that the modulation of the cellular components driving intestinal fibrosis is evolutionary conserved. In fact, scRNA-seq performed on colon from healthy and chronic DSS-treated mice revealed a substantial homology between human and murine fibroblasts, with the mouse Cd81$^+$Pi16$^-$ subset displaying a transcriptional profile analog to human FAP$^+$ fibroblasts. Moreover, the abundance and transcriptional similarity of Cxcl9$^+$ macrophage with respect to the human counterpart was confirmed in the mouse setting. This result also indicates that the intestinal fibrogenic response is driven by common cellular pathways independently from its localization either in the ileum (analyzed in the human scRNA-seq) or in the colon (analyzed in the murine scRNA-seq).

To identify regulators of the FAP$^+$ fibroblasts' pathological transcriptional activity, the two studies employed single-cell regulatory network inference and clustering (SCENIC) analysis and identified the transcription factor TWIST1 as the driver of the observed pro-fibrotic phenotype.

3. Functional role of TWIST1 in fibrosis

TWIST1 is a basic helix-loop-helix transcription factor known for being one of the core proteins transcriptionally driving the process of Epithelial-to-Mesenchymal Transition (EMT) [11]. Twist1 exerts its physiological role during embryogenesis, where it is required for mesodermal development and dorsal-ventral specification [12]. Twist1 null Drosophila and mouse embryos resulted in embryonic lethality due to gastrulation defects [13,14] and failure in the cranial neural tube closure [15]. Heterozygous loss-of-function mutations of the human *TWIST1* gene causes Saethre-Chotzen syndrome characterized by clinical manifestations as craniosynostosis and facial asymmetry [16,17]. At molecular level, TWIST1 acts as transcriptional activator or repressor by binding the E-box cassette present in the regulatory elements of many genes essential for various type of organogenesis [12]. Signaling pathways activating TWIST1 include TGFβ, TNFα, Notch and Wnt signaling pathways as well as hypoxia and mechanotransduction [12,18]. TWIST1 is normally not expressed in adult tissues, except for quiescent stem cells located in mesoderm-derived mesenchymal tissues such as muscle, adipose tissue and bone marrow [12]. However, its reactivation in pathological conditions is linked to promoting tissue fibrosis and participating to multiple aspects of cancer progression [12,19–21].

In the context of intestinal disease, while the process of EMT has been associated with IBD [22], the specific role exerted by TWIST1 is poorly understood. Few studies reported its activation in the immune compartment during colitis [23–25] but a more extensive functional investigation was so far missing. The studies by Ke&Abdurahiman et al. [9] and Zhang&Wang&Sun et al. [10] demonstrate for the first time the functional involvement of Twist1 in driving a pro-fibrotic phenotype in CD. On the basis of the transcriptional and computational identification of TWIST1 as a driver of the pro-fibrotic activity of the FAP$^+$ fibroblasts, both groups employed an *in vivo* strategy to demonstrate its functional role in fibroblasts. They generated a genetically engineered mouse model of tamoxifen-inducible

Twist1 conditional knock-out (cKO) in Collagen I expressing cells (Col1α2-Cre^{ERT2};Twist1$^{L/L}$ mice) and subjected these mice to chronic Dextran Sodium Sulfate (DSS) treatment to induce colon fibrosis. Twist1 cKO mice display improved disease activity index, which is a multi-factorial score monitoring the clinical progression of colitis, and reduced collagen I, collagen III and fibronectin deposition in the colon. Isolated primary fibroblasts from the mouse colon of WT and Twist1 cKO mice, as well as TWIST1-silenced human CCD18-Co fibroblasts, display reduced Fap and collagen III expression *in vitro*. Pharmacological targeting of Twist1 by treatment with the inhibitor harmine administered to DSS-treated mice – although with different regimens applied by the two studies – confirmed an efficient suppression of intestinal fibroblasts activation and collagen deposition pointing out Twist1 as a therapeutic target for intestinal fibrosis.

Considering the observed fibrosis-promoting role of TWIST1 and the pro-inflammatory nature of the FAP^{+} fibroblasts – a phenotype originally reported in the cancer field, where FAP^{+} fibroblasts are termed inflammatory cancer-associated fibroblasts (iCAFs) – it would be interesting to know whether TWIST1 may directly orchestrate the inflammatory response in intestinal fibrosis and whether this is directly related to its activation in fibroblasts. A significant reduction in the macrophage population was indeed observed in the colon of DSS-treated Twist cKO mice [10], possibly implying its direct role in modulating immune infiltration. Although a more extensive immune and cytokine profiling will be needed, this result is in line with previous works conducted in the context of renal fibrosis which reported a modulation of the immune and secretome profile following Twist1 deletion in either the epithelial or the endothelial compartments [26,27]. Moreover. Twist1 expression in cells of the immune compartment has been

Table 1
Functional roles of Twist1 in fibrosis emerged from *in vivo* mouse models.

Organ fibrosis	Cell type	Functional approach	Twist1 function	Refs.
Skin wound healing	Fibroblasts	Conditional deletion:FSP1^{Cre};Twist1$^{L/L}$	Fibrotic:- Permanent fibroblasts activation	[19]
Skin	Fibroblasts	Conditional deletion:Col1a2^{CreERT2};Twist1$^{L/L}$ UBC^{CreERT2};Twist1$^{L/L}$	Fibrotic:- Myofibroblasts differentiation- Collagen deposition	[29]
Intestinal	Fibroblasts	Conditional deletion:Col1a2^{CreERT2};Twist1$^{L/L}$Harmine treatment	Fibrotic:- FAP^{+} fibroblasts pro-fibrotic phenotype- Collagen deposition	[9,10]
Pulmonary	Fibroblasts	Overexpression:Col1a2^{CreERT2};Twist1-LUC	Fibrotic:- Increased collagen synthesis- Enhanced pro-fibrotic gene expression	[30]
Pulmonary	Fibroblasts	Conditional deletion:Col1a2^{CreERT2};Twist1$^{L/L}$	Protective:- Decreased fibrosis- Decreased T cells accumulation- Lower inflammatory mediators- Limited bone-marrow derived matrix- producing cells	[31]
Pulmonary	Endothelial cells	Conditional deletion:Tie2^{Cre};Twist1$^{L/L}$	Fibrotic:- Vascular leakage- Vascular remodeling- Myofibroblasts accumulation	[33,34]
Atherosclerosis	Endothelial cells	Conditional deletion:Tie2^{Cre};Twist1$^{L/L}$	Fibrotic:- Endothelial cells proliferation- Vascular leakage- Enhanced inflammation	[35]
Renal	Endothelial cells	Conditional deletion:Cdh5^{CreERT2};Twist1$^{L/L}$ Cdh5^{Cre};Twist1$^{L/L}$ Tie 1^{Cre};Twist1$^{L/L}$	Fibrotic:- Partial EndMT- Vascular leakage- Cytotoxic T cells infiltration- Promotion of inflammation- Reprograming of epithelial metabolism	[27]
Renal	Tubular epithelial cells	Conditional deletion:γGT^{Cre};Twist1$^{L/L}$ Ksp 1.3^{Cre};Twist1$^{L/L}$	Fibrotic:- Partial EMT- G2/M cell cycle arrest- Impaired epithelial functionality- Inflammatory secretome- Macrophages recruitment and activation	[26]
Renal	Podocytes	Conditional deletion:Nphs2^{Cre};Twist1$^{L/L}$	Protective:- Protection from injury- Reduced macrophage infiltration	[32]
Renal	Macrophages	Conditional deletion:LySM^{Cre};Twist1$^{L/L}$	Fibrotic:- Macrophage infiltration- M2 polarization- Secretion of pro-fibrotic factors	[36]
Renal	Macrophages	Conditional deletion:LySM^{Cre};Twist1$^{L/L}$ Cx3cr1^{Cre};Twist1$^{L/L}$	Protective:- Function in inflammatory (LySM) but not resident (Cxcr1) macrophages- MMP13 expression- Collagen degradation	[37]

reported in intestinal disease, although with opposite functions. In the context of ulcerative colitis (UC), neutrophil-expressed Twist1 is associated with corticosteroid resistance due to its physical interaction with the glucocorticoid receptor [24]. While Twist1 expression in CD-associated macrophages was shown to suppress the activation of inflammatory pathways [25]. In the context of asthma, a lung pro-fibrotic condition, epithelial-derived Twist1 has been functionally proved to induce T lymphocytes polarization toward a pro-inflammatory profile [28]. Therefore all these studies indicate the capacity of Twist1 to directly modulate the immune compartment.

The two back-to-back studies by Matteoli and Su groups highlighting the pro-fibrotic role of TWIST1 in fibroblasts confirmed earlier studies conducted on skin [19,29] and lung [30] fibrosis. All these studies consistently identified a prominent pro-fibrotic role for Twist in sustaining fibroblasts activation and enhancing the expression of pro-fibrotic genes among which collagen, the major component of the fibrotic extracellular matrix. However, one previous study pointed to a protective role of fibroblast-expressed Twist1 in lung fibrosis, with loss of Twist1 associated with increased fibrosis, accumulation of T lymphocytes and bone marrow-derived matrix producing cells [31].

Interestingly, in kidney and lung fibrosis, and in atherosclerosis, Twist1 was found expressed in a plethora of other cell types such as epithelial [26,32] and endothelial cells [27,33–35], and macrophages [36,37]. Twist1-induced EMT in renal epithelial cells has been identified as an injury-induced mechanisms impairing epithelial functionality and regeneration, and fueling tissue inflammation [26]. On the contrary, Twist1 expression in specialized glomerular epithelial cells called podocytes exerts an injury protective function by reducing macrophages infiltration [32]. In the endothelial compartment, Twist1 activation is associated with Endothelial-to-Mesenchymal Transition (EndMT), vascular leakage and modulation of the inflammatory response [27,33–35]. In macrophages, the role of Twist1 is less clear as both pro-fibrotic and protective roles for macrophage Twist1 were reported [36,37]. Therefore, besides few exceptions, the studies employing genetically engineered mouse models to perturb *Twist1* expression have generally demonstrated a conserved fibrosis promoting role for Twist1 across different cell types and organs (Table 1).

4. Translational significance of FAP^{+}TWIST1^{+} fibroblasts identification

Taken together, the two studies revealing FAP^{+}TWIST1^{+} fibroblasts as the drivers of CD-associated fibrosis [9,10] significantly advance our understanding of the cellular and molecular mechanisms underlying the development of fibrostenotic complications. In fact they identified the major effector cell type responsible for the collagen deposition in the intestine, and highlighted the existence of a close mesenchymal-immune crosstalk orchestrating the fibrogenic response (Fig. 1).

The Fibroblast Activation Protein (FAP) is a transmembrane prolyl serine protease which, similar to TWIST1, is undetectable in

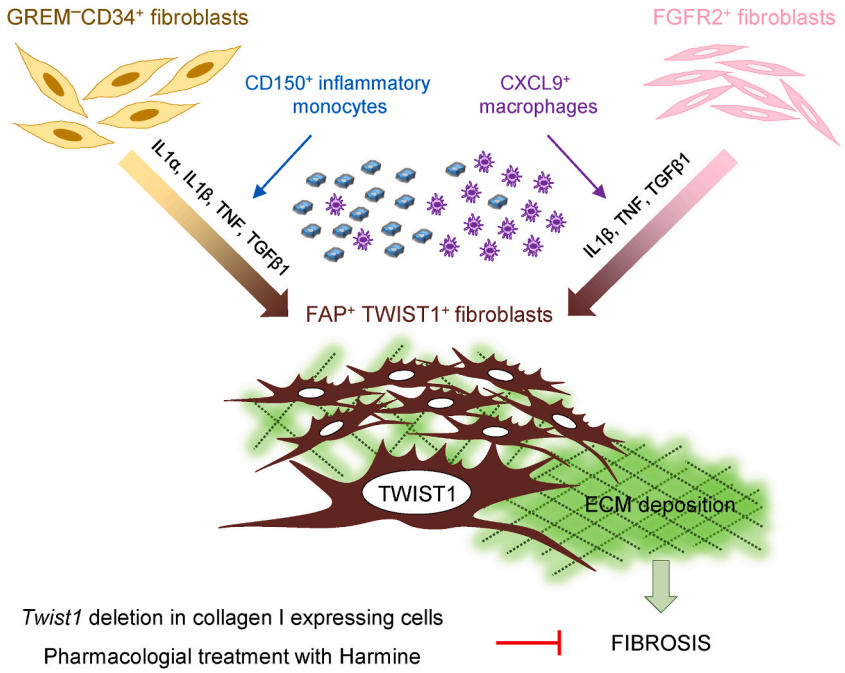

Fig. 1. FAP^{+} Twist1^{+} fibroblasts drive transmural fibrosis in Crohn's disease. The recent studies by Ke et al. and Zhang et al. have identified by scRNAseq FAP $^{+}$ Twist1^{+} fibroblasts as the major effector population responsible for extracellular matrix (ECM) deposition leading to the development of fibrostenotic complications in patients with Crohn's disease. The two works identified two distinct precursor cells: GREM– CD34^{+} fibroblasts which differentiate by the effect of cytokines secreted by CD150^{+} inflammatory monocytes, and FGFR2^{+} fibroblasts, whose reprograming is orchestrated by CXCL9^{+} macrophages. Genetic and pharmacological inhibition of the transcription factor Twist1 was proved efficient in suppressing intestinal fibrosis in mice.

most normal tissue but highly abundant in pathological diseases including fibrosis, arthritis and cancer where it localizes at sites of tissue remodeling [38,39]. In CD surgically resected specimens, FAP expression was detected in fibroblasts localized in the submucosa and subserosa in fibrostenotic areas, while no positive staining was detected in areas of inflammation without fibrosis [40,41]. In the lamina propria, FAP^+ cells were detected in the granulation tissue beneath erosions. A similar histological pattern was observed in UC samples where FAP positivity was identified mainly in fibrotic submucosal areas [41]. Although in the study by the Matteoli group FAP^+ fibroblasts and $CD150^+$ inflammatory monocytes were not consistently detected in transmural colonic samples of UC patients, a previous scRNA-seq based study reported the expansion of an inflammatory fibroblasts population expressing fibrotic genes including *FAP* and *TWIST1* in the colon of UC patients [42]. Moreover, pediatric UC subjects were also shown to exclusively harbor a subset of inflammatory fibroblasts expressing *TWIST1*, *IL1B* and *IL6* [43], a molecular signature similar to the one identified in adult CD-fibrostenotic lesions [9]. Of note, the capacity to produce collagen is an embryonic-derived essential property of FAP^+ fibroblasts. In fact, conditional deletion of *Col1a1* gene in Fap^+ cells results in impaired skeletal development and late embryonic lethality in mice [44].

These observations collectively point to FAP as a universal marker of fibrosis-associated fibroblasts [45]. Targeting FAP holds therapeutic promises for multiple types of fibrotic diseases [46]. In fact previous investigations showed anti-fibrotic outcomes of the use of FAP-specific engineered CAR T cells in experimental models of cardiac injury [47]. Anti-FAP antibody *ex vivo* treatment of CD strictures markedly decreased Collagen I and TIMP-1 production [48]. In the context of liver fibrosis, the protease activity of FAP has been exploited to liberate a liposome-conjugated peptide displaying local anti-fibrotic activity [49]. Lung fibrosis again presents controversies as the use of a small molecule inhibitor of FAP displays anti fibro-proliferative effects [50] while the use of FAP CAR T cells exacerbates fibrosis [51].

A second therapeutic perspective offered by the identification of $FAP^+TWIST1^+$ fibroblasts is represented by TWIST1 itself. In fact, the studies by the Matteoli [9] and Su [10] groups demonstrated that targeting TWIST1, the critical molecular checkpoint driving FAP^+ fibroblasts pathological activity – from which depends their capacity to deposit ECM – could be utilized as a valid strategy to decrease fibrosis. The employed inhibitor harmine, which promotes Twist1 degradation, has been assessed for safety and toxicity in four recently completed phase I clinical trials, three of which related to cognitive functions and one to pancreatic β-cell regeneration. While harmine is a plant-derived molecule acting as a tyrosine phosphorylation-regulated kinase (DYRK) inhibitor and does not specifically target only Twist1, its activity in inducing Twist1 protein degradation has been demonstrated in lung and breast cancer [52,53]. Therefore, it is the first compound with demonstrated activity in modulating Twist1 functions. Considering the role of Twist1 in other fibrotic diseases such as renal and pulmonary fibrosis, the obtained pre-clinical findings on the use of harmine have the potential to be applied also to other organs.

5. Technical and conceptual considerations

These studies underscore the importance and clinical implication of the single cell transcriptomic-based investigation as platform for therapeutic target discovery. Another recent single-cell transcriptomic-based study conducted on full-thickness surgical specimens of non-involved, inflamed and strictured CD intestinal segments identified Cadherin 11 (CDH11)-expressing fibroblasts as major drivers of fibrosis [54]. This $CDH11^+$ subset present the highest score of ECM production and is located in the mucosa and submucosa layers. Genetic targeting and antibody-mediated Cdh11 blockade in DSS-induced experimental colitis was proven to reduce intestinal fibrosis [54].

Cross-analysis of all these scRNA-seq dataset would now be important to assess the degree of similarity between the $CDH11^+$ and the FAP^+ fibroblasts. It is likely to speculate that a certain degree of heterogeneity may exists and that multiple distinct subtypes of mesenchymal cells could contribute to the final fibrotic outcome. Administered therapies, multiplicity of flares and relapses and individual composition of the inflammatory milieu may all be determinant factors triggering the preferential activation of one specific fibroblast population. These works also emphasize the importance of performing clinically meaningful investigations on the whole extension of the fibrotic lesions across the intestinal wall. Data mining on earlier single cell investigations in fact revealed only a moderate similarity of the newly identified FAP^+ fibroblasts across the published datasets [9]. This most likely is due to the different sampling method employed and the consequent lack of the full representation of the cellular components involved in the transmural progression of CD-associated fibrosis. This technical consideration together with the fact that the mesenchymal populations driving fibrosis have been identified only when investigating the lesions in their full thickness, highlight fundamental histological and functional aspects of the involved tissue. In the skin, a paradigm organ for studying wound healing and fibrosis processes, distinct fibroblasts lineages control tissue architecture and are differentially involved during injury and repair. In fact, the lineage present in the lower dermis mediates the initial part of damage repair and the upper lineage recruited during the re-epithelialization [55]. In this context, FAP^+ fibroblasts only exert a minor contribution to wound closure while $aSMA^+$ myofibroblasts are the major actor in driving skin wound repair [56].

With regards to having a layered tissue structure, the bowel wall can be compared to the skin and therefore the regional localization of the various mesenchymal cells determines how they differentially participate to tissue architecture, repair and chronic remodeling. In light of this concept, it is predictable to obtain a substantial heterogeneity in the single cell transcriptomic profiling of the intestinal mesenchymal populations depending on the full-depth versus superficial sample collection. Considering that FAP^+ fibroblasts have been histologically detected in both superficial (submucosal) and deeper (subserosal) layers of the CD fibrostenotic wall [40,41], it would be interesting to investigate whether a differential functionality accompanies these two spatially defined FAP^+ populations. To this purpose, integration of scRNA-seq, spatial transcriptomic and spatial phenotyping platforms may provide further phenotypic, functional and geographical insights into these fibrosis-associated fibroblasts.

Overall, the application of scRNA-seq to analyze IBD patient samples has substantially deepen our understanding of the tissue complexity and heterogeneity at single cell resolution [57]. This has translated into three fundamental aspects: 1) identification of unknown cell types involved in the inflammatory and fibrotic processes, 2) molecular profiling of such populations and 3) discovery of novel therapeutic targets. The subsequent *in vivo* validation in mice by genetic and pharmacological approaches further validate these targets in the context of research investigations carrying intrinsic clinical significance as they were initiated from the analysis of human samples. In this perspective, coordination and close collaboration between researchers and clinicians are fundamental requisites for designing meaningful translational projects.

Although the still high cost of the single-cell sequencing technologies, in the future a patient-specific single-cell profiling may be envisioned to identify the most appropriate therapeutic course and predict the response to therapy in a personalized manner.

Currently there is no therapy capable of preventing or reversing intestinal fibrosis. Pre-clinical studies conducted on mouse models, *in vitro* cell culture and *ex vivo* human samples have indicated multiple cellular and molecular mediators which are under investigation for future therapeutic application. This includes pathways involved in fibroblasts activation, ECM remodeling, soluble mediators of inflammation and the microbiome [58]. The current standard of therapy for CD patients mainly relies on targeting inflammatory pathways but controlling inflammation is not necessarily efficacious in blocking the onset and progression of fibrosis, due to the fact that fibrosis can self-sustain itself independently from inflammation [59,60]. Therefore future effective therapeutic strategies should envision the simultaneous targeting of both inflammatory circuits and fibroblasts, the main effector cells of fibrosis. Although further investigation is certainly needed, these newest advances in profiling intestinal stromal cells and identifying the TWISTed FAP^+ fibroblast population [9,10,61] hold therapeutic promises and represent a step forward the targeting of the intestinal fibrogenic process in Crohn's disease.

CRediT authorship contribution statement

Sara Lovisa: Writing – review & editing, Writing – original draft, Conceptualization. **Stefania Vetrano:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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