Insights from tissue “omics” analysis on intestinal remodeling in celiac disease

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Abstract
Celiac disease (CeD) is a prevalent intestinal disorder that only develops in genetically susceptible individuals when they mount a harmful CD4+ T-cell response towards gluten peptides. Intake of gluten leads to inflammation and remodeling of the small intestine with symptoms such as nausea and diarrhea. The only current treatment is a lifelong gluten free diet. The immunological basis for CeD is well characterized but the mechanisms that drive intestinal remodeling are still poorly understood. Transcriptome or proteome analysis of intestinal biopsies gives a global snapshot of all processes that occur in the tissue, including alterations in the epithelial cell layer. This paper will introduce concepts of intestinal remodeling, recapitulate the current understanding of CeD pathogenesis and discuss findings from relevant tissue “omics” studies. On the basis of this review, I give perspectives on what tissue “omics” studies can tell us about disease pathogenesis with a particular focus on the gluten induced intestinal remodeling.

KEYWORDS
celiac disease, inflammation, intestine, omics

1 | INTRODUCTION

Transcriptome or proteome analysis of tissue samples give complex datasets due to lack of cellular and spatial resolution, but also give unbiased insight into biological processes. Such studies can be of great value for conditions with unknown etiology, and for conditions that are well characterized where specific questions remain unanswered. Celiac disease (CeD) falls in the latter category. The immunological basis for CeD is well characterized but the mechanisms that drive intestinal remodeling in patients who eat gluten are still poorly understood. Intestinal remodeling largely reflects changes in the epithelial compartment which is dynamic and highly responsive to external cues. Proteome or transcriptome analysis of intestinal biopsies provides information about both the epithelial cell layer as well as active immune processes. Interpretation of such tissue “omics” data requires a good understanding of tissue composition and must be done in the context of existing knowledge of the disease. The next section will provide an overview of intestinal function, composition and remodeling followed by an introduction to CeD pathogenesis. This will set the basis for a discussion on what we can learn from published tissue “omics” studies in CeD followed by some future perspectives.

2 | THE SMALL INTESTINE

2.1 | Tissue structure and function

The primary function of the small intestine is to absorb nutrients from ingested food. This task is performed by a single layer of epithelial cells, the intestinal villi, which are covered by a thin layer of mucus produced by goblet cells. The intestinal villi are composed of a central core of capillaries and lymphatic vessels, surrounded by a layer of muscularis mucosae. The outer layer of the intestine is composed of smooth muscle tissue, which allows for peristalsis and segmentation movements to aid in the absorption of nutrients. The enterocytes, the absorptive cells, are responsible for the absorption of nutrients such as sugars, amino acids, and lipids. The enterocytes are also responsible for the secretion of bicarbonate ions, which help to maintain the pH balance of the intestinal contents. The enterocytes are supported by a layer of subepithelial connective tissue, which provides structural support and contains blood vessels and nerves. The enterocytes are also in close contact with the lamina propria, which is a layer of loose connective tissue that supports the blood vessels, lymphatic vessels, and immune cells. The lamina propria contains various immune cells, including lymphocytes and macrophages, which are involved in the immune response to pathogens and antigens. The enterocytes are also in close contact with the submucosa, which is the outer layer of the intestinal wall. The submucosa contains blood vessels, lymphatic vessels, and nerves, which support the enterocytes and regulate the intestinal function. The enterocytes are also in close contact with the muscularis mucosae, which is a layer of smooth muscle tissue that provides the peristaltic and segmentation movements. The enterocytes are also in close contact with the serosa, which is the outer layer of the intestinal wall. The serosa contains blood vessels and nerves, which support the enterocytes and regulate the intestinal function.
FIGURE 1 Structure and cellular composition of the human small intestine. (A) The small intestine can be structurally and functionally divided into epithelium, lamina propria and immune cells. (B) Composition of the epithelial cell layer. (C) The lamina propria consists of a mix of mesenchymal cells, extracellular matrix proteins and vasculature. (D) Immune cells are abundant also in the healthy intestine. (EEC; enteroendocrine cell, ISC; intestinal stem cell, LP; lamina propria, TA; transit amplifying cells) (Figure 1A). To maximize the surface area for nutrient uptake, the intestine is folded and contains finger-like projections (villi). At the base of the villi are small invaginations (crypts) that harbor proliferative intestinal stem cells (ISC). The epithelial layer is continuously renewed to preserve tissue function and integrity. Newly generated epithelial cells migrate out of the crypts up the villi until they reach the tip where they are shed into the gut lumen. Each crypt contains ~5–16 ISCs that give rise to ~250 new epithelial cells each day; each villus receives cells from multiple crypts and sheds ~1400 cells per day [1]. Thereby, the entire epithelial cell layer is renewed every 4–6 days. Appropriate regulation of crypt cell proliferation and differentiation is essential to maintain tissue structure and function. The epithelial layer forms a physical barrier of protection for the underlying lamina propria which harbors mesenchymal cells, vasculature, and immune cells (Figure 1A).

2.2 The epithelial cell layer

The villi are lined with differentiated epithelial cells of committed lineages that develop from absorptive or secretory progenitors located in the crypts (Figure 1B). About 90% of the differentiated epithelial cells in the human duodenum are enterocytes which are short-lived absorptive cells specialized in uptake, metabolism and transport of nutrients from the gut lumen to the underlying blood vessels or lymph. The second most abundant cell type is goblet cells (4%–10%) that secrete mucus proteins and trefoil factors into the gut lumen, creating a mechanical barrier of protection [2]. The chemosensory Tuft cells and hormone-secreting enteroendocrine cells serve important regulatory functions, but these cells constitute less than 1% of the epithelial cells [3]. Paneth cells are fully differentiated secretory cells located at the crypt bottom where they provide support for the ISCs and secrete antimicrobial peptides into the crypt lumen. Paneth cells live for 1–2 months and are cleared by infiltrating macrophages [4]. The epithelial cells are large in size and epithelial proteins or transcripts will contribute to a large proportion of tissue “omics” datasets.

2.3 The lamina propria

The epithelial cells migrate on the basement membrane which is a sheet of extracellular matrix proteins that forms a physical barrier to the lamina propria (Figure 1C). Basement membrane proteins are deposited by subepithelial mesenchymal cells and epithelial cells, and have a slow turnover rate of several weeks [5]. The basement membrane provides cues that regulate epithelial cell migration, such as laminins, where different isoforms show zonal expression and exert different effects on epithelial cell migration and function along the crypt-villus axis [6,7].
The interstitial matrix of the lamina propria is predominantly composed of glycosylated extracellular matrix proteins that regulate tissue volume and structure via glycan hydration and osmotic pressure. Matrix composition and structure is dynamically modulated by crosslinking or enzymatic cleavage by migrating cells. The subepithelial network of blood capillaries is connected to submucosal arterioles and venules and serves as a transport route for nutrients and for immune cells that migrate to the gut. In the villus center lays the lacteal, a specialized lymphatic vessel that transports cells, chylomicrons and molecules to draining lymphoid tissue and eventually blood. The lamina propria mesenchymal cells (fibroblasts, myofibroblasts, pericytes, and telocytes) are increasingly recognized as contributors to regulation of epithelial function [8]. Comprehensive "omics" analysis of lamina propria mesenchyme and extracellular matrix requires dedicated sample preparation and data analysis pipelines.

2.4 | The immune cell compartment

The intestine is the organ that hosts the largest number of immune cells in the body (Figure 1D). Tissue homeostasis requires a fine balance between immune cell responsiveness to luminal pathogens and tolerance or ignorance towards food and commensal bacteria. The frequency and phenotype of intestinal immune cells can change dramatically upon infection or immune mediated pathologies such as CeD.

The epithelial cell layer is patrolled by intraepithelial lymphocytes (IELs) that migrate in the narrow space between the basement membrane and the epithelial cells. IELs are present from crypt to villus tip with highest density in the mid-villus region. The vast majority of CD3+ IELs in the human duodenum are CD8+ T-cell receptor (TCR) γδ+ while ~10% express TCR αβ. IEL frequency can be transiently or permanently elevated in response to drugs, infections or in autoimmune disease. Compared to epithelial cells, the IELs are small in size and make a limited contribution to the epithelial transcriptome or proteome.

Plasma cells are the most abundant immune cells in the lamina propria. Intestinal plasma cells predominantly secrete J-chain linked IgA and IgM which are transported via the poly Ig receptor across the epithelial cells into the gut lumen. Therefore, both the lamina propria stroma and the epithelial cell layer contains immunoglobulin proteins but not transcripts. Plasma cells are large and express abundant immunoglobulins and protein synthesis machinery components. Tissue resident CX3CR1+ macrophages form an interdigitating network close to blood capillaries throughout the lamina propria, while dendritic cells are highly migratory and continuously traffic to and from secondary lymphoid organs [9]. Changes in myeloid cell abundance and phenotype upon inflammation can be challenging to infer from tissue "omics" data due lack of cell type specific markers and overlapping phenotypes. Granulocytes express high amounts of cell-type specific proteins; neutrophils are present only during inflammation, while eosinophils are frequent also in the healthy intestine [10]. T cells populate the entire lamina propria from the crypt to the villus tip. Many immune cells, in particular T cells, act through secretion of cytokines which can also influence epithelial function.

3 | EPITHELIAL HOMEOSTASIS AND REMODELING

3.1 | Regulation of crypt proliferation and epithelial cell differentiation

Epithelial homeostasis is maintained by the crypt compartment, a highly specialized niche that governs ISC renewal, cell division and differentiation. ISCs are interspersed between Paneth cells at the bottom of the crypts and have near unlimited capacity to divide due to low telomerase activity (Figure 2A) [11]. Preservation of stemness requires Wnt and Notch signaling as well as active inhibition of the BMP signaling pathway [3]. Wnt proteins are secreted by Paneth cells and lamina propria mesenchymal cells which also secrete R-spondins that bind to LGR5 and further amplify Wnt signaling. Wnt proteins form a very local gradient with highest concentration in the crypt bottom [12]. Notch signaling requires cell–cell contact. Paneth cells are the main source of Notch ligand for ISCs. Symmetrical cell division of ISCs results in crowding in the crypt so that some cells eventually lose contact with Paneth cells. This leads to loss of Notch signal which activates the transcription factor ATOH1 that drives commitment to the secretory lineage and also induces expression of Notch ligand. A cell with Notch ligand will provide Notch signal to 6–8 neighboring cells. Progenitor cells that receive Notch signal but lose Wnt signal upon migration away from the crypt bottom become absorptive cells. This mechanism ensures a high ratio of absorptive to secretory progenitor cells. While secretory progenitor cells immediately exit the cell cycle, absorptive progenitor cells divide 2–3 times within the transit amplifying (TA) zone which further skews the ratio in favor of absorptive cells. Progenitors that lose both Notch and Wnt signaling become goblet cells. BMP signaling induces epithelial cell differentiation and BMP proteins are expressed by mesenchymal cells along the entire crypt-villus axis. BMP signaling is therefore actively suppressed in the crypts to maintain stemness (Figure 2A).

3.2 | Epithelial remodeling

Dynamic regulation of the epithelial layer is essential to preserve intestinal function, and continuous epithelial turnover facilitates induction of rapid and transient changes. Adaptation to nutrient availability occurs on the level of gene expression and does not cause changes in tissue morphology but greatly affects tissue function. A high carbohydrate diet was found to induce expression of carbohydrate metabolizing enzymes and transporters in mature enterocytes [13]. This adaptation occurred after 5 days of diet and was caused by changes in the transcriptional program of absorptive progenitors in the TA zone. Surprisingly, these gene expression changes were induced by cytokines secreted by diet-sensing lamina propria T cells.

Physical damage, infection or inflammation can induce epithelial remodeling that alters tissue morphology. Remodeling can be required to regain an intact barrier after tissue injury. Remodeling can also serve as a host protective mechanism to purge infected epithelial cells or
3.3 Temporal and spatial organization of enterocyte function

In the healthy intestine, epithelial cell age and function is tightly linked to cell position along the crypt villus axis. ISCs have a stable cell cycle time of $\sim 21$ h while absorptive progenitors in the TA zone divide more rapidly with a cell cycle time of $\sim 12$ h (Figure 2B) [11]. Lineage tracing experiments have shown that 3 days is the minimal time window required to generate both goblet cells and enterocytes from the same ISC clone [19]. Epithelial cells migrate with different speed along the crypt villus axis with the highest migration rate in the TA zone [20]. At the crypt-villus junction, absorptive cells exit the cell cycle and start to differentiate but newly differentiated enterocytes at the villus base will still retain high expression of cell division proteins and transcripts [21]. Enterocytes migrate for $\sim 2$–$3$ days until they reach the villus tip where they are shed into the gut lumen. Within this time window, the expression of metabolizing enzymes and nutrient transporters is regulated by a gradient of mesenchymal derived growth factors including BMPs. This contributes to a zonal mRNA expression pattern and spatial organization of enterocyte function along the crypt villus axis (Figure 2B) [22].

The changes in enterocyte function along the crypt villus axis are paralleled by changes in cell metabolism. ISCs display high mitochondrial activity and rely on oxidative phosphorylation in part fuelled by Paneth cell-derived lactate. The rapid division of progenitor cells in the TA zone followed by differentiation requires glycolysis. A gradual metabolic shift occurs towards the villus tip with increased reliance on oxidative phosphorylation and increased mitochondrial frequency (Figure 2B) [1]. Also protein half-life appears to mirror the spatial organization of cellular function. While the median protein half-life of mouse small intestinal epithelial cells was 3.5 days, proteins involved in cellular metabolism and nutrient transport had a shorter half-life ($\sim 2.5$ days), which could reflect the rapid changes in metabolic profile and function along the crypt-villus axis [23].

Epithelial remodeling and ISC stemness is tightly linked to ISC cell metabolism. Fatty-acid $\beta$-oxidation is important to preserve stemness altered expression of ISC markers, induction of a fetal-like transcriptional program as well as increased expression of secretory cell type markers [15]. These changes were induced by IFN-$\gamma$ from CD4+ T cells that infiltrated the granulomas. In a mouse model of graft-versus-host disease, early infiltrating CD4+ T cells were identified as key drivers of intestinal damage [16]. Also here, epithelial remodeling and damage was induced by T-cell secreted IFN-$\gamma$ which affected ISC stemness and homeostasis. Only vasculature close to the crypt base showed a high expression of MAdCAM-1 and this promoted accumulation of gut-homing $\alpha_4\beta_7$ T cells in the pericryptal region [17]. Tight regulation of interferon signaling is also crucial to preserve stemness and restrict secretory-cell differentiation during ISC homeostasis [18]. Cytokine induced changes in the crypt compartment can therefore explain why conditions with very different etiology converge on the level of intestinal remodeling.
while loss of HMGCS2, the rate-limiting enzyme of the ketogenesis pathway, leads to loss of stemness, increased ATOH1 transcription and premature differentiation, resembling the changes observed in response to IFN-γ [24,25]. Changes in ISC metabolism induced by diet, age or microbiome has therefore been linked to changes in the regenerative capacity of the intestine [26].

4 | CELIAC DISEASE

4.1 | Symptoms and diagnosis

CeD affects more than 1% of the western population, and can debut clinically at all ages. Most children present classical symptoms such as diarrhea, malnutrition, and failure to thrive while adults often have vague, extra-intestinal symptoms and can therefore go undiagnosed for many years [27]. Diagnosis has historically required histological evaluation of duodenal biopsies as well as detection of disease specific auto-antibodies in serum. In children, diagnosis can now be set based on autoantibody titers alone whereas biopsies are still required to set the diagnosis in adults. The degree of intestinal damage in CeD is the only clinical factor associated with disease complications such as nutritional deficiencies, osteoporosis, and T-cell malignancies [28].

4.2 | The gluten induced adaptive immune response

The strongest genetic association for CeD comes from the MHC class II molecules HLA-DQ2.5, HLA-DQ2.2, and -DQ8 that can bind and present deamidated gluten peptides to CD4+ T cells (Figure 3A) [29]. Only patients with CeD have DQ-restricted CD4+ T cells that recognize deamidated gluten peptides in the blood and in the intestine [30,31]. Deamidation is a posttranslational modification catalyzed by the enzyme transglutaminase 2 (TG2). CeD patients develop antibodies towards deamidated gluten peptides as well as disease-specific autoantibodies towards TG2. Autoantibody production also depends on dietary gluten, a finding which supports a hapten-carrier model where TG2-specific B cells internalize TG2-gluten complexes and present gluten to gluten-specific CD4+ T cells [29]. Only patients with CeD have DQ-restricted CD4+ T cells that can bind and present deamidated gluten peptides to CD4+ T cells [30]. The amount of gluten peptides is highest. The lesion is characteristic for but not specific to CeD. However, measurement of intestinal damage is an important clinical parameter to evaluate disease severity and recovery upon treatment. Intestinal damage is classified according to the categorical Marsh score that considers changes in IEL frequency and villous height to crypt depth (Vh:Cd) ratio [46]. Marsh 0 is similar to healthy intestine with <25 IELs per 100 epithelial cells and Vh:Cd > 2 (Figure 3B). Marsh 1 represents an infiltrative state with an IEL frequency of >25 IELs per 100 epithelial cells but normal Vh:Cd ratio. Marsh 2, 3A, 3B, and 3C represent increased IEL frequency in conjunction with decreasing Vh:Cd ratio where the villi length decreases and the crypts expand but the mucosal height remains stable. In Marsh 3C, hyperplastic crypts constitute a large fraction of the epithelial layer. The surface area available for nutrient absorption from the gut lumen is therefore severely diminished.

Tissue damage in CeD occurs primarily in the upper small intestine where the amount of gluten peptides is highest. The lesion is characteristic for but not specific to CeD. However, measurement of intestinal damage is an important clinical parameter to evaluate disease severity and recovery upon treatment. Intestinal damage is classified according to the categorical Marsh score that considers changes in IEL frequency and villous height to crypt depth (Vh:Cd) ratio [46]. Marsh 0 is similar to healthy intestine with <25 IELs per 100 epithelial cells and Vh:Cd > 2 (Figure 3B). Marsh 1 represents an infiltrative state with an IEL frequency of >25 IELs per 100 epithelial cells but normal Vh:Cd ratio. Marsh 2, 3A, 3B, and 3C represent increased IEL frequency in conjunction with decreasing Vh:Cd ratio where the villi length decreases and the crypts expand but the mucosal height remains stable. In Marsh 3C, hyperplastic crypts constitute a large fraction of the epithelial layer. The surface area available for nutrient absorption from the gut lumen is therefore severely diminished.

The untreated CeD lesion is also characterized by immune cell infiltration (Figure 3B). IEL infiltration (Marsh 1) occurs prior to visible epithelial remodeling while immune cell infiltration in the lamina propria correlates with Marsh score. Plasma cells are massively increased and populate the entire lamina propria where ∼10% of the IgA+ cells are specific for TG2 [47]. T cells, inflammatory myeloid cells as well as eosinophils are also increased, while neutrophils can be present to varying degrees. Despite their important contribution to pathogenesis, gluten specific CD4+ T cells represent only ∼1.5% of all lamina propria CD4+ T cells even in untreated CeD [48,49]. Due to the severe inflammation, Marsh 3 lesions may also have visible changes in the lamina propria interstitial matrix structure as well as altered blood vessel organization [50].
4.4 Dynamics of gluten induced intestinal remodeling

Removal of gluten from the diet curbs the adaptive immune response and the intestine recovers, but the kinetics and degree of tissue architecture normalization varies between patients [51]. Reintroduction of gluten, such as by gluten challenge, leads to relapse of disease. Again, the kinetics of intestinal remodeling varies between patients and likely depends on baseline treatment status, gluten dose and length of gluten challenge. A 3-day gluten challenge was found sufficient to unani-
mously activate gluten specific CD4+ T cells, but only patients that were poorly treated at baseline developed visible changes in intestinal architecture [52]. A 2-week gluten challenge induced intestinal remodeling in many but not in all patients [40,53]. Gluten dose was recently
proved to be a crucial factor, as consumption of 10 g gluten per day for 2 weeks caused intestinal remodeling in most patients while 3 g gluten per day did not [54]. Gluten challenge regimes used in clinical practice to confirm CeD diagnosis have therefore typically lasted for ~10 weeks to ensure sufficient exposure to gluten.

4.5 | Proposed models of intestinal remodeling in CeD

Activation of gluten specific CD4+ T cells and IEL infiltration occurs prior to visible changes in tissue morphology. It is broadly accepted that the intestinal remodeling in CeD must be immune mediated and two not mutually exclusive models have been proposed. The prevailing model is that transformed, cytotoxic CD8+ TCR αβ IELs mediate excessive killing of mature enterocytes which leads to compensatory crypt proliferation, hyperplasia and eventually intestinal remodeling [42,55]. Alternatively, T-cell derived cytokines can cause excessive crypt proliferation and intestinal remodeling [56,57]. This model was proposed more than 30 years ago, based on the morphological resemblance between the CeD lesion and remodeling observed in human intestinal explants following activation of tissue resident T cells. As in CeD, mucosal height was conserved and the decrease in villi height was compensated by increased crypt depth.

5 | TISSUE "OMICS" IN CELIAC DISEASE

5.1 | Transcriptomics versus proteomics

Global transcriptome or proteome analysis can be performed both on fresh frozen and formalin fixed intestinal tissue. Use of frozen tissue requires collection of dedicated biopsies while use of formalin fixed tissue facilitates retrospective studies from diagnostic biobanks and allows for histological assessment of the same biopsies. Proteins and mRNA can be extracted from entire biopsies or from tissue sections. The use of sections also allows for isolation of tissue subcompartments by laser capture microdissection (LMD) followed by protein or mRNA extraction and analysis. Fresh biopsies can also be disrupted into single cell suspensions for transcriptome analysis of single cells or cell populations isolated by flow cytometry.

The first tissue "omics" studies in CeD were performed more than 15 years ago by microarray based transcriptome analysis at a depth of ~5000 and ~10,000 genes, respectively (Table 1) [58–72]. By contrast, the first tissue proteomics study from 2010 relied on comparison of protein expression by 2-dimensional gel electrophoresis followed by mass spectrometry based protein identification. This approach yielded some hundred differentially expressed proteins of which only ~50 were identified. Now, shot-gun proteome analysis of small amounts of formalin fixed tissue can quantify ~4000 proteins, while transcriptome analysis by high-throughput sequencing can reach a depth of ~10,000 genes. Proteomics analysis is naturally biased toward detection of medium to high abundance proteins due to the lack of an amplification step. Small, low abundant proteins such as cytokines or lipophilic transmembrane proteins are also often not detected. Assessment of cytokine activity therefore relies on detection of downstream signaling pathways. Transcriptome analysis will detect cytokines, transmembrane proteins and transcription factors, but always suffers from the discordance between transcript and protein.

Analysis of tissue "omics" data rely heavily on pathway enrichment analysis approaches that consider coordinated changes in many genes. This greatly facilitates the interpretation of very large datasets. However, due to pathway redundancy, the same genes may give apparent regulation of many different pathways. Interpretation of pathway names also requires some caution: for example, enrichment of pathways involved in bacterial or virus responses need not reflect infection, simply regulated expression of the same genes.

5.2 | Tissue "omics" recapitulates known inflammatory features of the CeD lesion

Many canonical features of the CeD lesion can be inferred from "omics" analysis of intestinal biopsies from both pediatric and adult patients (Figure 4A) [58–72]. Increased expression of IFNγ transcript and IFN-γ regulated genes such as WARS, STAT1, TAP, CD74 has been unambiguously reported by all studies as well as increased expression of chemokines and adhesion molecules indicative of immune cell migration and infiltration (CXCL10, CXCL11, and ICAM1) (Figure 4A). The massive infiltration of plasma cells is readily inferred from increased expression of immunoglobulins which is particularly prominent in proteomics studies [62,64]. Presence of disease specific anti-TG2 plasma cells may be inferred from increased protein abundance of the immunoglobulin gene IGVH5-51 that CeD patients have a convergent and stereotypic usage of [64]. Gluten specific CD4+ T cells are not possible to detect directly from tissue "omics" data although increased expression of CTLA4, a marker of activated T cells, is seen in untreated CeD.

However, not all assumptions made from tissue analysis will be correct. ER stress and unfolded protein response are integral processes to preserve epithelial homeostasis, but many of the same pathways are constitutively active in plasma cells. For example, high expression of PRDX4 in untreated CeD was interpreted to represent epithelial cell processes, but plasma cells represent an equally likely source [62]. Increased expression of CD19 in untreated CeD Marsh 3 was interpreted to reflect an influx of B cells in the lesion [60]. However, B-cells are rare in the lamina propria also in CeD. Increased CD19 expression therefore probably reflects the increased frequency of CD19+ short-lived plasma cells in CeD [73].

Only one study has compared the tissue gene expression profile of untreated CeD to that of other human intestinal inflammatory conditions [68]. Perhaps not surprisingly, many of the changes detected in CeD were also observed in inflamed ileum from Crohn’s disease.
TABLE 1  Tissue "omics" studies of CeD intestinal biopsies

<table>
<thead>
<tr>
<th>Year</th>
<th>First author [reference]</th>
<th>Tissue</th>
<th>Data</th>
<th>Method</th>
<th>Age</th>
<th>Number of patients</th>
<th>Gene coverage</th>
</tr>
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<td>Fresh frozen biopsies</td>
<td>mRNA</td>
<td>microarray, RT-PCR</td>
<td>A P</td>
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<td>4</td>
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<td>4</td>
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<td>RNA-Seq</td>
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<td>A</td>
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Abbreviations: A, adult; CTR, non-CeD control; EP, epithelium; FFPE, formalin fixed paraffin embedded; LMD, laser capture microdissection; P, pediatric; PGC, post gluten challenge; RCD 1, refractory CeD type I; TCeD, treated CeD; UCeD, untreated CeD.

<sup>a</sup>54 of 234 proteins with altered expression identified by MS.

<sup>b</sup>Meta-gene expression values from 47,000 transcripts.

patients. However, a strong IFN-γ signature as well as cell proliferation markers were specific to CeD. Such comparisons are crucial for studies that aim to define disease specific tissue processes.

5.3  Gene expression profile as proxy for a tissue remodeling state

Epithelial remodeling can readily be inferred from tissue "omics" data due to the high abundance of epithelial cell type specific proteins. In untreated CeD, the majority of genes with reduced expression reflect mature enterocyte function while genes reflecting cell proliferation show increased expression (Figure 4A). Diverging expression of APOA1A1, APOA4, APOC3, CYP3A4 versus MKI67 was found to correlate with Marsh score in untreated CeD, while the APOA4 to MKI67 expression ratio correlated with Vh: Cd ratio in response to 10-week gluten challenge [63,66]. The differential expression of APOA4 and MKI67 was confirmed by tissue staining to reflect mature enterocytes and proliferative crypts respectively.

5.4  Gluten-induced immune processes are detected in tissue prior to visible remodeling

Marsh score reflects gradual changes in intestinal morphology, but give no information about ongoing immune processes. Diosdado et al. aimed to address whether distinct immune processes distinguished between Marsh 2, 3A, 3B, and 3C lesions in untreated CeD [60]. Comparing tissue transcriptome expression to that of treated CeD (Marsh 0) they found that genes with differential expression in the presence of gluten changed along a continuum that correlated with Marsh score. Targeted analysis of gene expression in biopsies from pediatric CeD patients collected at the time of diagnosis revealed high expression of CXCL11, IFNG, and CTLA4 both in Marsh 2 to Marsh 3C lesions. In contrast, IL17A, which is not involved in the pathogenesis of CeD, was highly expressed only in the Marsh 3C lesion [63]. This suggests that truly disease specific immune processes may be difficult to infer from a heavily inflamed Marsh 3C lesion. In a follow up study, Bragde et al. aimed to identify tissue biomarkers of early disease, that is, processes that can be detected in the intestine prior to visible changes in tissue
5.5 Inferral of immune mediated gluten induced epithelial remodeling

Untreated CeD represents a state of chronic inflammation. To identify drivers of remodeling, tissue changes that occur early in response to gluten must be studied (Figure 4B). Two recent studies have compared tissue gene expression in response to a gluten challenge [70,71]. Proteomics analysis of biopsies collected before and after 2 weeks of gluten challenge showed that only a subset of the patients developed epithelial changes and visible tissue remodeling and that these patients had low-level ongoing tissue inflammation already at baseline despite normal intestinal architecture [71]. This suggests that some patients have low level active anti-gluten immunity despite a regular gluten free diet, and that such small differences can contribute to differential mucosal response kinetics between patients. In contrast, a 10-week gluten challenge induced visible intestinal remodeling in all patients [70].

Common to both studies was that gluten exposure resulted in decreased expression of mature enterocyte proteins, increased expression proliferation markers as well as increased expression of epithelial secretory cell type proteins [70,71]. These features therefore reflect events that occur early upon gluten induced remodeling in CeD. Loss of differentiated enterocytes was proposed to result from hyperactive Wnt signaling [70,74]. However, this epithelial phenotype of remodeling also resembles changes that occur after IFN-γ induced disruption of ISC homeostasis [15–17]. How T-cell derived cytokines such as IFN-γ affect Wnt and Notch signaling remains to be fully elucidated [18,75]. Increased expression of granzyme B expression was reported after gluten challenge which agrees with the observed IEL infiltration, but no gene expression signature indicative of massive
enterocyte apoptosis was observed. This suggests that IEL-mediated cytotoxic killing of enterocytes is not an early driver of gluten induced intestinal remodeling.

5.6 | Inferral of enterocyte function to assess treatment status

Because epithelial phenotype changes rapidly in response to stimuli, inferral of epithelial function from tissue "omics" data may represent an attractive approach to evaluate effect of treatment and intestinal recovery in CeD. Expression of proteins that transport lipids or nutrients such as sugars, amino acids and vitamins is reduced in untreated CeD and decreased in some patients as early as 4 days after gluten challenge (Figure 4C) [64]. Exposure to gluten also caused metabolic changes in the epithelial cell layer with reduced expression of fatty acid oxidation pathways as well as mitochondrial proteins [71]. In the Marsh 3 lesion, this expression pattern may reflect an increased fraction of TA progenitor cells compared to differentiated enterocytes. However, also treated CeD patients with normal tissue architecture can have altered expression of enterocyte function proteins [70,71]. Potentially, an increased epithelial turnover rate may result in too little time for enterocyte differentiation and maturation. Cytokines from transformed IELs may also affect the epithelium in treated CeD. Further studies are required to establish whether epithelial phenotype and metabolism can serve as measures of treatment status in CeD.

6 | SUMMARY AND FUTURE PERSPECTIVES

CeD is a unique immune-mediated enteropathy in that we know the disease driving antigen which can be removed or introduced at will, and we can readily collect biopsies from the disease lesion. This allows us to monitor tissue changes in response to disease-driving antigen. As shown in this review, "omics" analyses of intestinal biopsies provide information about ongoing immune processes as well as the epithelial remodeling. However, the lack of cellular and spatial resolution sets a limit to what we can infer from such data. Single cell transcriptome analysis preserves cellular resolution and holds great potential to shed light on tissue pathology [76–78]. When combined with spatially resolved techniques such as multiplexed single molecular fluorescent in situ hybridization, detailed maps of pathological tissue processes can be generated [77]. In CeD, both global and selected tissue immune cell populations have been studied by single cell transcriptome analysis but studies of stromal compartments or the epithelial cell layer have not yet been reported [73,79,80]. We have recently demonstrated that proteome analysis of LMD isolated epithelial cell layer from the CeD lesion successfully captures gluten induced epithelial changes [71]. Deep proteome coverage of tissue regions approaching single-cell resolution can now be achieved thanks to increasingly streamlined sample preparation pipelines and sensitive MS instruments [81,82]. Foreseeably, both single cell and spatially resolved "omics" analysis of biopsies from well characterized patient cohorts can further improve our understanding of the gluten induced intestinal remodeling in CeD.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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