Effects of lifestyle and environmental factors on intestinal epithelial cell morphogenesis

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Chapter 1

Introduction & scope of the thesis

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The intestinal epithelium is a polarized cell monolayer which is crucial for its function

The intestinal epithelium is a 1-cell-thick layer (a monolayer) separating the lumen of the gut from the rest of the body. The intestinal epithelium functions as the gateway between these compartments; taking up fluids and nutrients from the gut, and preventing these to leak into the lumen, thus performing the main task of the gut.

In order to form a functional tissue, individual cells must organize themselves in context of each other and their surroundings, in a process termed 'morphogenesis'; from the Greek 'beginning of the shape'[1]. To be able to organize as a tissue, (intestinal) epithelial cells adhere to their neighbor cells, utilizing transmembrane proteins. The mature intestinal epithelial cells are, like all epithelial cells, polarized, i.e. display an asymmetric distribution of cell components allowing for different functions on different locations in the cells[2]. IECs have distinct membrane domains, an 'apical' domain facing the intestinal lumen, and a 'basolateral' domain facing away from the lumen, with the lateral part facing and contacting the neighboring IECs, and the basal part facing and contacting the underlying tissue. The lateral surface houses intercellular adhesions, including E-cadherin-based adherens junctions and Claudin-based tight junctions, which ensure monolayer strength and impermeability, respectively[3]. The basal part harbors transmembrane proteins attaching the IEC to the basement membrane, produced by the IECs themselves and local fibroblasts. The apical and basolateral domains are separated by tight junctions (TJs) sealing the intercellular space. TJ localization is part of apical-basal polarity; and prevents diffusion of membrane proteins between the apical and basolateral plasma membrane domains it separates[3-6]. The TJs, by virtue of the pore-forming Claudins, are rate limiting for

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epithelial permeability of electrolytes and water[6-8] and restrict the localization of secreted molecules in either gut lumen or lamina propria[9,10]. Tight junctions also recruit transcription factors and prevent their translocation to the nucleus, and in this way contribute to the regulation of cell proliferation[11]. The apical membrane of intestinal epithelial cells harbors numerous densely and uniformly packed finger-like projections filled with actin filament bundles tethered to the subapical actin web, called microvilli. Microvilli are essential for enterocyte function; enlarging the apical absorptive area 6- to 15- fold while minimally influencing cellular volume[12,13].

Polarity of IECs is initiated and maintained by the intracellular trafficking machinery[14]. This epithelial polarity program (further detailed in the next chapter) consists of three evolutionary conserved polarity complexes; one consisting of Crb3a, Pals1 and PATJ, one consisting of Par3, Par6, atypical (a)PKC and the small GTPase Cdc42, and one consisting of Lgl, Dlg and Scribble[14]. Dysregulation of these polarity complexes has been correlated with impaired intestinal epithelial morphogenesis and homeostasis[15-18]

IECs originate from stem cells that reside in crypts, which are invaginations in the intestinal wall. These stem cells give rise to highly proliferative IECs that differentiate and polarize as they migrate from the crypts to the surface epithelium (in the colon) or to the villi (in the small intestine), were they are eventually shed into the lumen. This morphogenic process is tightly controlled in time (3-4 days) and space to ensure maintenance of monolayer organization and, consequently, an adequate barrier function. In order for the monolayer to remain intact and in contact with the basement membrane, every cell that divides must maintain its polarized configuration and divide in a planar orientation, i.e. perpendicular to the apical-basal axis[19-21].

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Concluding, specific cellular characteristics are crucial for epithelial morphogenesis and therefore function. These characteristics are the capacity to (1) engage in cohesive interactions with neighbor cells, (2) create distinct apical and basolateral membrane domains separated by tight junctions, (3) establish a polarized distribution of cellular components, and (4) to maintain their spatial relationship to the environment, *i.e.*, remain in a monolayer (planar orientation) separating lumen from the underlying tissue[1]Fig 1.

External factors disrupt epithelial barrier function

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), are characterized by a chronic inflammation of the intestine, relapsing diarrhoea, and additional symptoms such as vomiting, abdominal pain, and weight loss. UC is limited to the colon, were it affects the superficial mucosal layer[22]. CD is not limited to any part of the gastro-intestinal tract, but is predominant in the ileocecal region, and is characterized by transmural ulcers[23]. The aetiology of IBD is not fully elucidated. The concordance between monozygotic twins is 20-50% for CD and 16% for UC, implying a significant role for environmental (extrinsic) factors. Extrinsic, *i.e.*, non-epithelium derived factors include smoking tobacco, appendectomy, hygiene as per the 'hygiene hypothesis[24], infectious pathogens and antibiotics, other medications such as aspirins or NSAIDs, a lack of fibers in diet, major life stressors, anxiety and depression[25]. These factors can be classified as 'western lifestyle'. Indeed, the incidence of IBD has increased in the last decades in the western world, as well as in countries adapting to western lifestyle[26,27].

Amongst life style factors especially smoking

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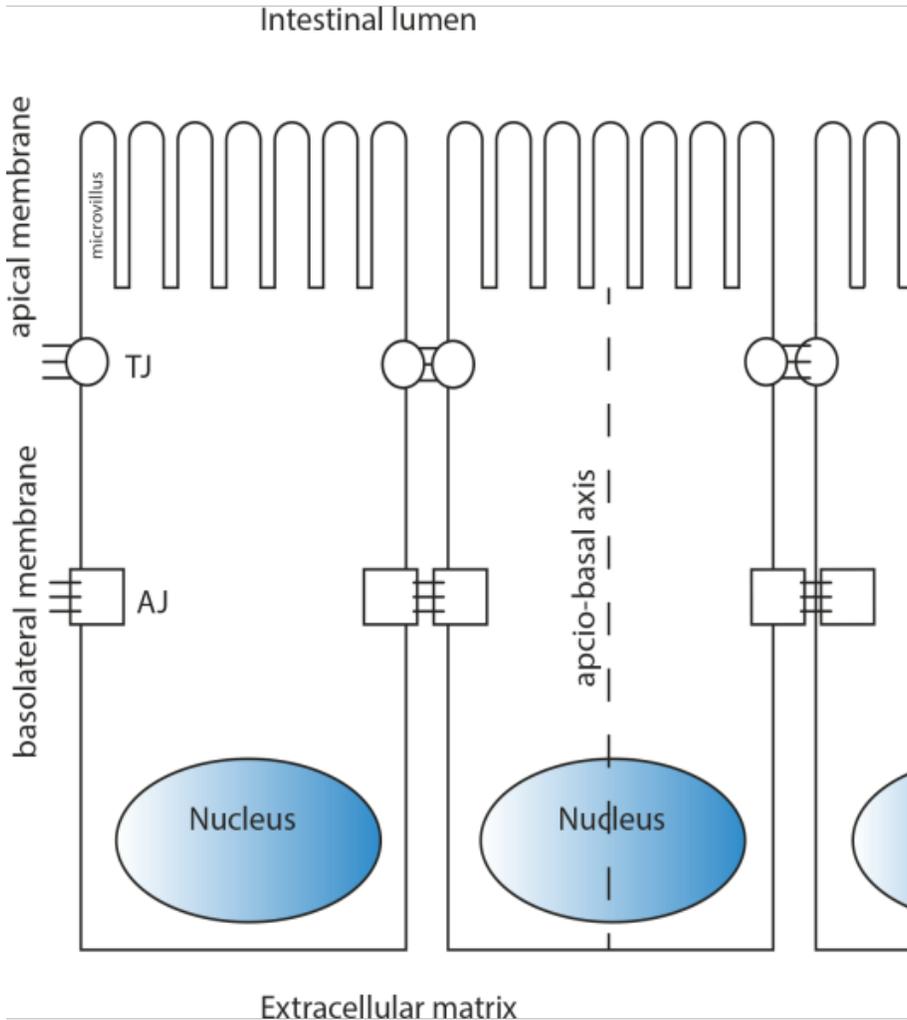
Figure 1 Schematic overview of typical polarized intestinal epithelium showing different membrane domains separated by tight junctions (TJ), the apical domain harboring microvilli and facing the intestinal lumen, and the basolateral membrane facing extracellular matrix and basolateral membranes of neighbor cell, to which it is anchored with adherens junctions (AJ). Also depicted is the theoretic apicobasol axis, perpendicular to which cell division takes place in order to maintain the monolayer.

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stands out; with smokers having a higher change of developing CD and a lower change of developing UC relative to non-smokers[28,29]. While IBD was emerging it seemed a disease disproportionally afflicting people with higher socio-economic status, which is seemingly at odds with their generally lesser exposition to above stated extrinsic factors; however, socio-economic status as an independent predictor has not been uniformly found in recent research[30].

On tissue and cellular level IBD is characterized by mucosal and epithelial injury and increased permeability[31-33], including changes in epithelial tight junctions, mucosal lesions, epithelial restoration failure, and changed functionality of the epithelial cells, which are correlated with immune deregulation[34]. Local inflammatory mediators and lifestyle factors play an important role in this perturbation of epithelial function. Increased expression of pro-inflammatory cytokines (i.e. such as interferon gamma (IFNγ) and Tumor Necrosis Factor (TNF), IL-1β, IL-6, IL-17, IL-22, IL-23) plays a crucial role in the perturbation of epithelial function in IBD[33,35-37]. IFNγ and TNF directly target and negatively affect intestinal epithelial cells. For instance, IFNγ and TNF disrupt a previously intact intestinal epithelial barrier in a synergistic manner by eliciting intracellular signalling pathways that negatively affect tight junctions that control paracellular transport[38-40] and control the release of transcription factors[41,42]. The importance of cytokines in the pathogenesis of IBD is further underscored by the effectiveness of anti-TNF monoclonal antibodies in the treatment of IBD in the clinic[37,43]. The effectiveness of anti-TNF monoclonal antibodies could not be found with recombinant TNF-receptors, possibly because of a less stable bond, or because of a not fully elucidated enhanced pro-inflammatory cytokine production[44-48].
Cigarette smoking as a Life style factor has been

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found to negatively affect intestinal epithelial function in animal models. These studies reveal a causal relationship between the breathing in of cigarette smoke and therefore basal exposition to IECs (via blood vessels), and the effects on morphogenesis. In this way, cigarette smoke exposure impaired intestinal barrier function[49,50], increased apoptosis and autophagy in the ileum[51,52], decreased cystic fibrosis transmembrane conductance regulator (CFTR) activity in the intestinal epithelium[53], and increased bacterial translocation, intestinal villi atrophy[49], and altered tight junction protein expression in a small/large intestine-specific fashion[49,54]. In cell models, exposure to cigarette smoke extract has been demonstrated to perturb epithelial barrier function and TJs in pulmonic epithelia cells[55,56] and, likewise, impaired intestinal barrier function has been associated with the smoking-related lung disease chronic obstructive pulmonary disease (COPD)[50,57]

As the intestinal epithelium is the first defense layer against whatever is in the gut, it is prone to damage by mechanical or immune-mediated stressors. If a patch of epithelium is damaged; functionality is lost. Epithelial wound healing consists of three different processes; migration of dedifferentiated epithelium, 'restitution', subsequent proliferation, and the final morphogenetic step of differentiation and thereby of regaining function[58]. The behavior of IECs is tightly controlled trough locally excreted growth factos, cytokines and/or bacterial products. These factors induce signaling cascades leading to activation of master transcription factors such as NF-kB and STAT-3 in IECs. These induce anti-apoptotic and proliferative effects, leading to augmented survival and division of IECs[59].

In IBD repeated intestinal epithelial damage with disruption of barrier function is a key feature, and mucosal healing has recently been established as a key prognostic parameter[59].

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Inherent and acquired polarity dysfunction effects on morphogenesis and function in IECs

Dysregulation of (intestinal) epithelial morphogenesis is precursor to epithelial dysplasia, polyposis and eventually colon cancer[8,60,61]. Cancer arises in the epithelium of the crypt, where the IECs proliferate and differentiate. Uncontrolled proliferation is one of the first steps in cancer progression, and occurs after IECs have obtained the capacity to survive while no longer in a monolayer, no longer attached to the underlying tissue. Hyperproliferation leads via an adenoma stage to a cancerous stage while molecular alterations stack (chromosomal instability, microsatellite instability, hypermethylation, early APC mutation, late p53 mutations)[62,63]. Inflammatory bowel diseases are associated with an enhanced risk of colorectal cancer up to three times the risk in the general population, with a longer disease duration, greater extent and severity of inflammation associated with an increasing risk[62]. An intricate interplay between cytokines, immune cells and intestinal epithelial cells, possibly in predisposing genetic and/or environmental contexts, likely drives the development of IBD associated colorectal cancer[64,65]. Inflammation in IBD leads to increased proliferation and regeneration and thus increased cell turnover with resultant increase in the number of mutations[66]. Consequently, the events leading to eventual cancer happen faster. Histologically, IBD-associated colorectal cancer follows a different pathway, that is, from inflamed, regenerative epithelium, to hyperplastic epithelium, to flat dysplasia, and finally to invasive adenocarcinoma[66]. Epithelial regeneration and dysplasia may be difficult to distinguish histologically. Also, as opposed to sporadic colorectal cancer development, APC mutations occur relatively late, and p53 mutations occur relatively early, and can even be found in non-dysplastic lesions[62]. These findings have led to a theory of 'field cancerization', where a complete

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area of inflammation-affected epithelium is prone to progress to a cancerous phenotype[66]. Thus, loss of polarity and differentiation in IECs to cancer progression can be caused by a combination of intrinsic and extrinsic factors.

Loss of polarity mechanisms leads to loss of epithelial characteristics.
Defective epithelial barrier function frequently leads to diarrhea, and can be caused on the cellular level by defective cell polarity mechanisms. Tight junctions are the rate limiting factor for epithelial permeability in an otherwise intact monolayer. Therefore, disruption of tight junctional protein function or localization might cause impaired barrier function. Tight junctions are dynamically endocytosed and recycled via apical recycling endosomes[67-73]. Microvillus inclusion disease (MVID) is a rare disease clinically characterized by an early-onset severe secretory diarrhea, caused by polarization defects due loss of function of Myosin Vb, a key regulator of recycling endosomes, encoded by the *MYO5B* gene[74,75].
MVID IECs are characterized by microvillus atrophy, the intracellular retention of transmembrane apical brush border proteins, and the name-giving cytoplasmic microvillus inclusions[76-79] including the main brush border anion transporter cystic fibrosis trans-membrane conductance regulator and the NHE-2 and -3 Na+/H+ exchangers[80] and some basolateral proteins[79,81].

IECs need a tight control of their metabolic pathways to ensure proper morphogenesis
The two main energy metabolism pathways in mammalian cells are mitochondrial oxidative phosphorylation and (aerobic) glycolysis. During oxidative phosphorylation, adenosine triphosphate (ATP) is formed following the oxidation of nutrients

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in mitochondria. During glycolysis, glucose is converted into pyruvate thereby generating ATP. Glycolysis is relatively inefficient compared to oxidative phosphorylation, generating two ATP molecules per molecule glucose, versus 36 in oxidative phosphorylation. This, however, can be compensated for by the higher rate by which aerobic glycolysis can take place, and aerobic glycolysis allows proliferating cells to use glycolytic intermediates as macromolecular precursors facilitate biomass accumulation[82]. Rapidly proliferating tissue has a relative increase in glycolysis. In the mouse intestine a relative increase in glycolysis is found in the (proliferating) crypt epithelium compared to the (differentiated) villus epithelium. A particular proliferative tissue, the epithelium-derived colon carcinoma, also undergoes this metabolic shift, and recently a mouse model professing an increase in glycolysis has been found to have an increased change of colon carcinoma development[83]. Taken together, intestinal epithelial cells need to control their metabolic pathways in order to maintain homeostasis in their dynamic environment.

The need for a reductionist *in vitro* model mimicking key *in vivo* aspects to study intestinal epithelial integrity and morphogenesis

An intestinal disease phenotype is the product of activities of many cell types, including IECs, communities of commensal or pathogenic microbiota living in the gut and immune cells. While animal models and clinical studies are very useful to assess correlation and causation of interventions to total disease phenotypes, such approaches do not easily allow to determine the specific role of the epithelium. The epithelium, however, is the functionally impaired cell layer. Therefore, a reductionist approach is warranted for studying epithelium specific effects. By isolating the epithelium, we can assess effects of different factors

considered pathogenic or ameliorating, excluding that the epithelial effect perceived is in fact secondary to a contextual change. In this way, deeper knowledge of disease mechanisms may be obtained.

For *in vitro* assessment of epithelial morphogenesis, a model mimicking key aspects of *in vivo* morphogenesis is necessary. Cell lines that are able to obtain morphological and biochemical features of intestinal epithelial cells, such as CaCo-2 cells[84-89] that show contact-inhibition of growth and changes in the expression levels of mRNA[86,87,90] and proteins[84,88] associated with their proliferation and differentiation which are similar to those during enterocyte differentiation in vivo[87], are therefore suited best for morphogenesis studies. The recent advances in culturing primary intestinal stem cells, giving rise to organoids bring the possibility of creating a model system that might be even closer to the in vivo situation. However, cell lines are well characterized and isogenic. Organoids will differ from donor to donor, and indeed even from part to part in the same intestine[91]. Therefore, organoids are per definition not as well characterized, making the distinction between a treatment effect or specific-organoid-intrinsic factor more difficult to make. This makes cell lines perhaps more suitable as a primary tool to decipher mechanistic features of a disease phenotype.

Three-dimensional cultures are a relatively new method in the assessment of epithelial function and morphogenesis. Certain intestinal epithelial cell lines, amongst which is CaCo-2, are capable of forming a three-dimensional structure when given the correct cues and physical surroundings. When plated in the presence of matrigel; a gel resembling extracellular matrix, CaCo-2 cells clonally proliferate and start polarizing after the first division. In a two-cell stage the IECs create an apical membrane at the cell-cell interface, and further division happen perpendicular to the apical-basal axis. As fluid is excreted from

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the apical side, a lumen is created between the cells. Eventually the cell cluster thus resembles a hollow ball lined by a monolayer of IECs whose apical membranes are directed to the lumen with microvilli protruding into the lumen, and basally a basement membrane is recruited, analogous to the *in vivo* situation[92]. Analogous to *in vivo* situation, the 3D-cultured IEC must be capable of (1) interlinking to neighbor cells, otherwise gross organization would be lost, (2) separating membrane domains, otherwise the cell could not organize itself respective to its environment or create a lumen, (3) the IEC must have functional TJ, otherwise the sphere would not be impermeable, (4) distributing cellular components in a polarized fashion, as evidenced by e.g. the microvillus localization, (5) dividing in planar orientation, otherwise a monolayer could not be created or maintained. Thus, to be able to form such a spheroid, the IECs morphogenesis in culture must resemble in vivo morphogenesis in all crucial ways (See figure 2 for a schematic overview of a spheroid). Therefore, this model is an appropriate approach for studying morphogenesis[8,93-96], analogous to *in vivo* epithelial (re)generation.

Scope of this thesis
The aim of this thesis is to investigate the direct effects of different pathogenic factors on intestinal epithelial morphogenesis and function. Previous studies have mainly focused on the assessment of either the complex interplay of all cell types in the gut, or used conventional, two-dimensional cell culture systems lacking essential characteristics of *in vivo* epithelium. In this thesis we developed and employed a reductionist model system that bridges the gap between conventional two-dimensional cell culture and *in vivo* animal models/humans to investigate the direct effects of potentially pathogenic extrinsic factors (including pro-inflammatory cytokines, changes in energy metabolism, and

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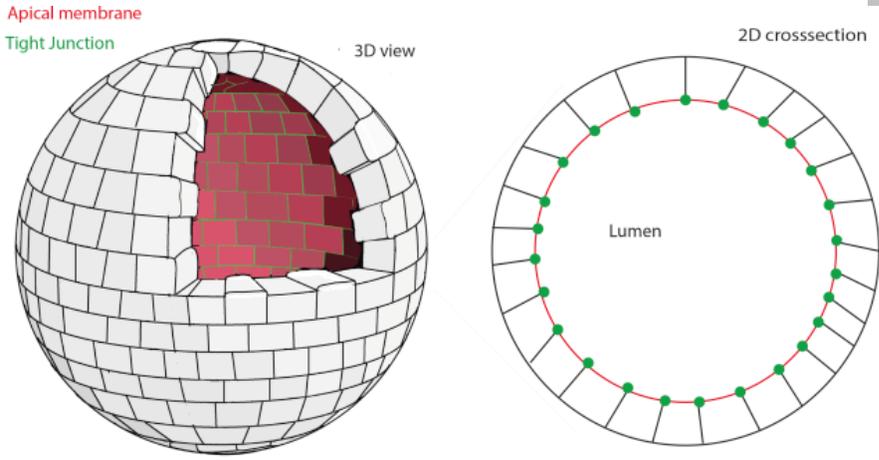cigarette smoke) as well as intrinsic factors on intestinal epithelial morphogenesis and integrity.

Figure 2 Schematic overview of a typical intestinal epithelial spheroid, showing an established lumen, faced by apical membranes delineated by tight junctions. On the left a see-into 3D model is shown, on the right a cross-section of the sphere on the left is shown.

References

1. Morali O, Savagner P, Larue L. Epithelium–Mesenchyme transitions are crucial morphogenetic events occurring during early development. . *In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience; 2000-.*

2. Massey-Harroche D. EpithelialCellPolarityasReflectedinEnterocytes. *Microsc Res Tech. 2000;49(4):353-62.*

3. Giepmans B, van Ijzendoorn S. Epithelial cell-cell junctions and plasma membrane domains. *Biochim. Biophys. Acta*. 2009;1788:820--831.

4. Aijaz S, Balda MS, Matter K. Tight junctions: Molecular architecture and function. *Int. Rev. Cytol. 2006;248:261–298*.

5. Sawada N. Tight junction-related human diseases. *Pathol. Int. 2013;63:1–12.*

6. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell. Mol. Life Sci. CMLS 2013;70:631–659*.

7. Laukoetter MG, Bruewer M, Nusrat A. Regulation of the intestinal epithelial barrier by the apical junctional complex. *Curr. Opin. Gastroenterol. 2006;22:85–89.*

8. Bryant DM MK. From cells to organs: Building polarized tissue. *Nat Rev Mol Cell Biol. 2008 Nov;9(11):887-901.*

9. Marques R BI. Expression and functional importance of innate immune receptors by intestinal epithelial cells. *Cell. Mol. Life Sci. CMLS 2011;68:3661–3673.*

10.   Wells JM, Rossi O, Meijerink M, et al. Epithelial crosstalk at the microbiota-mucosal interface. *Proc. Natl. Acad. Sci. U. S. A. 2011;108 Suppl 1:4607–4614*.

11.   Matter K, Aijaz S, Tsapara A, et al. Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. *Curr. Opin. Cell Biol. 2005;17:453–458*.

12.   Khurana S GS. Regulation of cell structure and function by actin-binding proteins: Villin's perspective. . *FEBS Lett. 2008;582:2128–2139.*

13.   Helander HF FL. Surface area of the digestive tract - revisited. *Scand J Gastroenterol. 2014;49(6):681-9.*

14.   Rodriguez-Boulan E MI. Organization and execution of the epithelial polarity programme. *Nat. Rev. Mol. Cell Biol. 2014;15:225–242.*

15.   Sakamori R, Das S, Yu S, et al. Cdc42 and Rab8a are critical for intestinal stem cell division, survival, and differentiation in mice. *J. Clin. Invest. 2012;122:1052–1065*.

16.   Melendez J, Liu M, Sampson L, et al. Cdc42 coordinates proliferation, polarity, migration, and differentiation of small intestinal epithelial cells in mice. *Gastroenterology 2013;145:808–819.*

17.   Charrier LE, Loie E, Laprise P. Mouse. Crumbs3 sustains epithelial tissue morphogenesis in vivo. *Sci. Rep. 2015;5:17699.*

18.   Whiteman EL, Fan S, Harder JL, et al. Crumbs3 is essential for proper epithelial development and viability. *Mol. Cell. Biol. 2014;34:43–56.*

19.   Fleming ES, Zajac M, Moschenross DM, Montrose DC, Rosenberg DW, Cowan AE, Tirnauer JS. Planar spindle orientation and asymmetric cytokinesis in the mouse small intestine. *J Histochem Cytochem. 2007 Nov;55(11):1173-80.*

20. Quyn AJ, Appleton PL, Carey FA, Steele RJ, Barker N, Clevers H, Ridgway RA, Sansom OJ, Näthke IS. Spindle orientation bias in gut epithelial stem cell compartments is lost in precancerous tissue. *Cell Stem Cell. 2010 Feb 5;6(2):175-8.*

21. Casaletto JB, Saotome I, Curto M, et al. Ezrin-mediated apical integrity is required for intestinal homeostasis. *Proc. Natl. Acad. Sci. U. S. A. 2011;108:11924–11929.*

22. Laass MW, Roggenbuck D, Conrad K. Diagnosis and classification of crohn's disease. . *Autoimmun Rev 2014;13:467-471. 2.*

23. Conrad K, Roggenbuck D, Laass MW. Diagnosis and classification of ulcerative colitis. *Autoimmun Rev 2014;13:463-466. 3.*

24. Strachan D.
Hay fever, hygiene, and household size.
BMJ 1989 299, 1259–1260.

25. Ananthakrishnan AN.
Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol. 2015 Apr;12(4):205-17.*

26. Molodecky NA, Soon IS, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology 2012;142:46-54.*

27. Burisch J, Pedersen N, Cukovic-Cavka S, et al. East-west gradient in the incidence of inflammatory bowel disease in europe: The ECCO-EpiCom inception cohort. *Gut 2014;63:588-597.*

28. Heide F van der, Dijkstra A, Weersma RK, et al. Effects of active and passive smoking on disease course of crohn's disease and ulcerative colitis. *In lamm. Bowel Dis. 2009;15:1199–1207.*

29. Heide F van der, Dijkstra A, Albersnagel FA, et al. Active and passive smoking behaviour and cessation plans of patients with crohn's disease and ulcerative colitis. *J Crohns Colitis 2010;4:125–131.*

30. Ng SC BC. Geographical variability and environmental risk factors in inflammatory bowel disease. *Gut 2013;62:630-649.*

31. Salim SY SJ. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *In lamm Bowel Dis. 2011 Jan;17(1):362-81.*

32. Odenwald MA TJ. Intestinal permeability defects: Is it time to

1

treat? *Clin Gastroenterol Hepatol. 2013 Sep;11(9):1075-83*.

33. Atreya R NM. Chemokines in inflammatory bowel diseases. *Dig Dis 2010 28: 386–394*.

34. McGuckin MA, Eri R, Simms LA, Florin TH, Radford-Smith G. Intestinal barrier dysfunction in inflammatory bowel diseases. . *In lamm Bowel Dis 2009 15: 100-113.*

35. Papadakis KA TS. Role of cytokines in the pathogenesis of inflammatory bowel disease. *Annu Rev Med 2000 51: 289-298.*

36. Sands BE KG.
The role of TNFalpha in ulcerative colitis. *J Clin Pharmacol 2007 47: 930-941.*

37. Francescone R, Hou V, Grivennikov SI. Cytokines, IBD, and colitis-associated cancer. *In lamm. Bowel Dis. 2015;21:409–418*.

38. Bruewer M, Utech M, Ivanov AI, et al. Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J. 2005;19:923–933*.

39. Beaurepaire C, Smyth D, McKay DM. Interferon-γ regulation of intestinal epithelial permeability. *J Interferon Cytokine Res. 2009 29: 133-144*.

40. Shen L, Su L, Turner JR. Mechanisms and functional implications of intestinal barrier defects. *Dig Dis 2009 27: 443–449*.

41. Balda MS MK. Tight junctions and the regulation of gene expression. *Biochim Biophys Acta 2009 1788: 761-767*.

42. Hollande F, Shulkes A, Baldwin GS. Signaling the junctions in gut epithelium. *Sci STKE 2005 277: pe13*.

43. Ford AC, Sandborn WJ, Khan KJ, et al. Efficacy of biological therapies in inflammatory bowel disease: Systematic review and meta-analysis. *Am J Gastroenterol. 2011;106:644–659*.

44. Berg L, Lampa J, Rogberg S, van Vollenhoven R, Klareskog,

L. L. berg, J. lampa, S. rogberg, R. van vollenhoven, L. klareskog increased peripheral T cell reactivity to microbial antigens and collagen type II in rheumatoid arthritis after treatment with soluble TNF alpha receptors *Ann Rheum Dis, 60 (2001), pp. 133–139*.

45. Sandborn WJ, Hanauer SB, Katz S, et al. Etanercept for active crohn's disease: A randomized, double-blind, placebo-controlled trial. *Gastroenterology. 2001;121:1088–1094*.

1

46. Zou J, Rudwaleit M, Brandt J, Thiel A, Braun J, Sieper J. J. zou, M. rudwaleit, J. brandt, A. thiel, J. braun, J. sieper up regulation of the production of tumor necrosis factor alpha and interferon gamma by T cells in ankylosing spondylitis during treatment with etanercept *Ann Rheum Dis, 62 (2003), pp. 561–564.*

47. Haraoui B KM. Emergence of Crohn's disease during treatment with the anti-tumor necrosis factor agent etanercept forankylosing spondylitis: Possible mechanisms of action. *Semin Arthritis Rheum. 2009;39(3):176-81.*

48. Dubeau MF, Iacucci M, Beck PL, Moran GW, Kaplan GG, Ghosh S, Panaccione R. Drug-induced inflammatory bowel disease and IBD-like conditions. . *In lamm Bowel Dis. 2013 Feb;19(2):445-56.*

49. Zuo L, Li Y, Wang H, et al. Cigarette smoking is associated with intestinal barrier dysfunction in the small intestine but not in the large intestine of mice. *J Crohns Colitis 2014;8:1710–1722*.

50. Li H, Wu Q, Xu L, et al. Increased oxidative stress and disrupted small intestinal tight junctions in cigarette smoke-exposed rats. *Mol Med Rep 2015;11:4639–4644.*

51. Verschuere S, Bracke KR, Demoor T, et al. Cigarette smoking alters epithelial apoptosis and immune composition in murine GALT. *Lab. Invest. 2011;91:1056–1067.*

52. Verschuere S, Allais L, Bracke KR, et al. Cigarette smoke and the terminal ileum: Increased autophagy in murine follicle-associated epithelium and peyer's patches. *Histochem. Cell Biol. 2012;137:293–301.*

53. Raju SV, Jackson PL, Courville CA, et al. Cigarette smoke induces systemic defects in cystic fibrosis transmembrane conductance regulator function. *Am. J. Respir. Crit. Care Med. 2013;188:1321–1330.*

54. Wang X, Tully O, Ngo B, et al. Epithelial tight junctional changes in colorectal cancer tissues. *Scienti icWorldJournal 2011;11:826–841*.

55. Serikov VB, Leutenegger C, Krutilina R, Kropotov A, Pleskach N, Suh JH, Tomilin NV. Cigarette smoke extract inhibits expression of peroxiredoxin V and increases airway epithelial permeability. *Inhal Toxicol. 2006 Jan;18(1):79-92*.

56. Schamberger AC, Mise N, Jia J, Genoyer E, Yildirim AÖ, Meiners S, Eickelberg O. Cigarette smoke-induced disruption of bronchial epithelial tight junctions is prevented by transforming growth factor-β. *Am J Respir Cell Mol Biol. 2014*

Jun;50(6):1040-52.

57. Rutten EPA, Lenaerts K, Buurman WA, et al. Disturbed intestinal integrity in patients with COPD: Effects of activities of daily living. *Chest 2014;145:245–252*.

58. Sturm A DA. Epithelial restitution and wound healing in inflammatory bowel disease. world J gastroenterol. *2008 Jan 21;14(3):348-53.*

59. Neurath MF TS. Mucosal healing in inflammatory bowel diseases: A systematic review. . *Gut. 2012 Nov;61(11):1619-35*.

60. Mimori-Kiyosue Y, Matsui C, Sasaki H, et al. Adenomatous polyposis coli (APC) protein regulates epithelial cell migration and morphogenesis via PDZ domain-based interactions with plasma membranes. *Genes Cells 2007;12:219–233.*

61. Wiebecke B, Brandts A, Eder M. Epithelial proliferation and morphogenesis of hyperplastic adenomatous and villous polyps of the human colon. *Virchows Arch A Pathol Anat Histol 1974;364:35–49.*

62. Dyson JK RM. Colorectal cancer in inflammatory bowel disease: What is the real magnitude of the risk? *World J Gastroenterol. 2012 Aug 7; 18(29): 3839–3848.*

63. Sandouk F, Al Jerf F, Bassel Al-Halabi MHD. Precancerous lesions in colorectal cancer. *Gastroenterol Res Pract. 2013; Published online 2013 May 14doi: 10.1155/2013/457901*.

64. Rhodes JM CB. Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. *2002 Trends Mol Med 8 :10-16*.

65. Saleh M TG. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat Rev Immunol 2011 11:   9-20.*

66. Matkowskyj KA, Chen ZE, Rao MS, Yang GY. Dysplastic lesions in inflammatory bowel disease: Molecular pathogenesis to morphology. *Arch Pathol Lab Med. 2013 Mar;137(3):338-50.*

67. Yu D TJ. Stimulus-induced reorganization of tight junction structure: The role of membrane traffic. *Biochim. Biophys. Acta 2008;1778:709*.

68. Chalmers AD WP. Continuous endocytic recycling of tight junction proteins: How and why? *Essays Biochem. 2012;53:41–54*.

69. Dukes JD, Fish L, Richardson JD, et al. Functional ESCRT machinery is required for constitutive recycling of claudin-1 and

1

maintenance of polarity in vertebrate epithelial cells. *Mol. Biol. Cell 2011;22:3192–3205*.

70. Lock JG SJ. Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol. Biol. Cell 2005;16:1744–1755.*

71. Langevin J, Morgan MJ, Sibarita J-B, et al. Drosophila exocyst components Sec5, Sec6, and Sec15 regulate DE-cadherin trafficking from recycling endosomes to the plasma membrane. . *Dev. Cell 2005;9:365–376*.

72. Desclozeaux M, Venturato J, Wylie FG, et al. Active Rab11 and functional recycling endosome are required for E-cadherin trafficking and lumen formation during epithelial morphogenesis. *Am. J. Physiol. Cell Physiol. 2008;295:C545–556*.

73. Utech M, Mennigen R, Bruewer M. Endocytosis and recycling of tight junction proteins in inflammation. *J. Biomed. Biotechnol. 2010;2010:484987.*

74. Lapierre LA, Kumar R, Hales CM, et al. Myosin vb is associated with plasma membrane recycling systems. *Mol. Biol. Cell 2001;12:1843–1857*.

75. Terrin G, Tomaiuolo R, Passariello A, Elce A, Amato F, Di Costanzo M, Castaldo G, Canani RB. Congenital diarrheal disorders: An updated diagnostic approach. *Int J Mol Sci. 2012;13(4):4168-85.*

76. Davidson GP, Cutz E, Hamilton JR, et al. Familial enteropathy: A syndrome of protracted diarrhea from birth, failure to thrive, and hypoplastic villus atrophy. *Gastroenterology 1978;75:783–790*.

77. Phillips AD, Jenkins P, Raafat F, et al. Congenital microvillous atrophy: Specific diagnostic features. *Arch. Dis. Child. 1985;60:135–140.*

78. Cutz E, Rhoads JM, Drumm B, et al. Microvillus inclusion disease: An inherited defect of brush-border assembly and differentiation. *N. Engl. J. Med. 1989;320:646–651.*

79. Müller T, Hess MW, Schiefermeier N, et al. MYO5B mutations cause microvillus inclusion disease and disrupt epithelial cell polarity. *Nat. Genet. 2008;40:1163–1165.*

80. Ameen N AG. Defective CFTR apical endocytosis and enterocyte brush border in myosin VI-deficient mice. *Traffic Cph. Den. 2007;8:998–1006.*

81. Thoeni CE, Vogel GF, Tancevski I, et al. Microvillus inclusion disease: Loss of myosin vb disrupts intracellular traffic and cell polarity. *Traffic Cph. Den. 2014 15(1):22-42.*

1

82. Lunt SY, Vander Heiden MG. Aerobic glycolysis: Meeting the metabolic requirements of cell proliferation . *Annu Rev Cell Dev Biol. 2011;27:441-64.*

83. Ou J, Miao H, Ma Y, et al. Loss of abhd5 promotes colorectal tumor development and progression by inducing aerobic glycolysis and epithelial-mesenchymal transition. *Cell Rep 2014;9:1798–1811*.

84. Stierum R, Gaspari M, Dommels Y, et al. Proteome analysis reveals novel proteins associated with proliferation and differentiation of the colorectal cancer cell line caco-2. *Biochim. Biophys. Acta 2003;1650:73–91*.

85. Luca AC, Mersch S, Deenen R, et al. Impact of the 3D microenvironment on phenotype, gene expression, and EGFR inhibition of colorectal cancer cell lines. *PLoS One 2013;8. Published online 2013 March 26. doi: 10.1371/journal. pone.0059689*.

86. Halbleib JM, Sääf AM, Brown PO, et al. Transcriptional modulation of genes encoding structural characteristics of differentiating enterocytes during development of a polarized epithelium in vitro. *Mol. Biol. Cell 2007;18:4261–4278*.

87. Sääf AM, Halbleib JM, Chen X, et al. Parallels between global transcriptional programs of polarizing caco-2 intestinal epithelial cells in vitro and gene expression programs in normal colon and colon cancer. *Mol. Biol. Cell 2007;18:4245–4260*.

88. Buhrke T, Lengler I, Lampen A. Analysis of proteomic changes induced upon cellular differentiation of the human intestinal cell line caco-2. *Dev. Growth Differ. 2011;53:411–426.*

89. Natoli M, Leoni BD, D'Agnano I, et al. Good caco-2 cell culture practices. *Toxicol In Vitro 2012;26:1243–1246*.

90. Tremblay E, Auclair J, Delvin E, et al. Gene expression profiles of normal proliferating and differentiating human intestinal epithelial cells: A comparison with the caco-2 cell model. *J. Cell. Biochem. 2006;99:1175–1186*.

91. Middendorp S, Schneeberger K, Wiegerinck CL, Mokry M, Akkerman RD, van Wijngaarden S, Clevers H, Nieuwenhuis EE. Adult stem cells in the small intestine are intrinsically programmed with their location-specific function. . *Stem Cells 2014 32(5), 1083-91.*

92. Jaffe AB, Kaji N, Durgan J, Hall A. Cdc42 controls spindle orientation to position the apical surface during epithelial morphogenesis. *J Cell Biol 2008 183: 625-633.*

1

93. Roignot J, Peng X, Mostov K. Polarity in mammalian epithelial morphogenesis. *Cold Spring Harb Perspect Biol. 2013 Feb 1;5(2).*

94. Shamir ER EA. Three-dimensional organotypic culture: Experimental models of mammalian biology and disease. *Nat Rev Mol Cell Biol. 2014 Oct;15(10):647-64.*

95. Zegers MM. 3D in vitro cell culture models of tube formation. *Semin Cell Dev Biol. 2014 Jul;31:132-40*.

96. Antoni D, Burckel H, Josset E, Noel G. Three-dimensional cell culture: A breakthrough in vivo. . *Int J Mol Sci. 2015 Mar 11;16(3):5517-27*.