University of Helsinki

Dissertationes Universitatis Helsingiensis 66/2024

MECHANISMS OF POLYPOSIS IN PEUTZ-JEGHERS SYNDROME MODELS

Eva Domènech Moreno

Helsinki Institute of Life Science (HiLIFE),
Doctoral Program in Integrative Life Sciences,
Faculty of Biological and Environmental Sciences,
University of Helsinki,
Finland

Academic Dissertation

To be publicly discussed with the permission of the Faculty of Biological and Environmental Sciences, University of Helsinki, in Biomedicum 1, lecture hall 2, Haartmaninkatu 8, Helsinki on April 12th 2024, at 13

HELSINKI 2024

SUPERVISORS

Professor **Tomi P. Mäkelä**, MD, PhD
iCAN Digital Precision Cancer Medicine Flagship, University of Helsinki, Helsinki, Finland.

Saara Ollila, PhD
Translational Cancer Medicine Program, University of Helsinki, Helsinki, Finland.

THESIS COMMITTEE

Professor **Satu Mustjoki**, MD, PhD
Director of Translational Immunology Research Program. University of Helsinki, Helsinki, Finland.

Tuomas Tammela, MD, PhD
Associate Member, Cancer Biology and Genetics Program, Sloan Kettering Institute, New York, United States.

FACULTY APPOINTED REVIEWERS

Tapio Lönnberg, PhD
InFLAMES Flagship, Turku Bioscience Centre, Turku, Finland

Juha Väyrynen, PhD
Research Unit for Translational Medicine University of Oulu, Helsinki, Finland

OPPONENT

Associate Professor, **Vasiliki Koliaraki**, PhD
BSRC Alexander Flemming Athens, Greece

CUSTODIAN

Professor **Juha Partanen**, PhD
Molecular and Integrative Biosciences Research Programme University Helsinki, Finland

GRAPHICS

Figures 1 (p. 12-13) and 2 (p. 15) designed by Sara Domènech Moreno

Publisher: Helsingin yliopisto
Series: Dissertationes Universitatis Helsingiensis 66/2024

ISBN 978-951-51-9698-9 (print)
ISBN 978-951-51-9697-2 (online)
ISSN 2954-2898 (print)
ISSN 2954-2952 (online)
PunaMusta, Joensuu 2024

"Les proteïnes són les màquines de la vida
(Proteins are the machines of life)"
–Max, school teacher

"To remember that a category is at best a proxy;
at worst, a shackle."
–Lulu Miller
Why Fish Don't Exist

ABSTRACT

Peutz-Jeghers Syndrome (PJS) is a rare gastrointestinal (GI) polyposis syndrome driven by germline mutations in the tumor suppressor kinase *LKB1* (*STK11*). PJS patients develop multiple hamartomatous GI polyps and have a higher cancer risk. Previous findings indicate a role for stromal cells in PJS polyp development. This study aimed to further clarify the cell types and molecular mechanisms driving PJS polyposis and to find new therapeutic approaches. To achieve this, we generated several mouse models to study the requirement of *Lkb1* loss at specific cell types. Additionally, we conducted extensive transcriptomic and single-cell omics analyses of PJS polyps and established organoid co-cultures to address the effects of *Lkb1*-deficient stroma on epithelial growth. To scale the organoid experiments and ensure unbiased quantification, in this study, we also aimed to generate better tools for gastrointestinal organoid classification by exploring the feasibility of using artificial intelligence.

We found that the specific loss of *Lkb1* in mesenchymal progenitors or a subset of GI fibroblasts is sufficient to recapitulate PJS polyposis in mice. The polyps exhibited an inflammatory transcriptome with an activated JAK-STAT3 signaling, and JAK inhibition significantly reduced polyp burden in the PJS mouse model. Using single-cell sequencing, we also discovered that PJS polyps are enriched for $ST2^+$ (*Il1rl1*) fibroblasts that closely resemble inflammation-associated GI fibroblasts. These fibroblasts present a genetic profile with key molecules involved in tissue regeneration, including *Il11*, *Fgf7*, *Hgf*, *Ptgs2*, *Wnt5a*, and *Nrg1*. Additionally, we found that $ST2^+$ fibroblasts are sensitized to inflammatory stimuli and that inflammation exacerbates PJS polyposis. This $ST2^+$ fibroblast signature was associated with strong autocrine and paracrine IL11 signaling, which was required to develop the transcriptional changes induced upon the loss of *Lkb1*. Furthermore, cell-cell interaction analysis predicted activated signaling from *Lkb1*-deficient stroma to epithelial cells, including factors that promote epithelial growth and regeneration, such as *Nrg1*, *Wnt5a* and *Hgf*. Importantly, inhibition of the IL11 signaling using a blocking antibody successfully reduced polyp initiation and burden in mice.

The results presented in this thesis identify the cell of origin of PJS polyposis and a mechanism that can be targeted therapeutically. In addition to the significant advancements in PJS biology, this study effectively demonstrates the feasibility of object detection algorithms for gastrointestinal organoid classification. We provide the first open-source, user-friendly tool, named Tellu, for automated intestinal organoid classification.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications (I, III) and a manuscript (II), which can be found appended. The articles are referred to in the text by their Roman numerals (I-III). In addition, some unpublished data is presented.

I. Stromal Lkb1 deficiency leads to gastrointestinal tumorigenesis involving the IL11 -JAK/STAT3 pathway.

Saara Ollila, **Eva Domènech-Moreno**, Kaisa Laajanen, Iris P.L. Wong, Sushil Tripathi, Nalle Pentinmikko, Yajing Gao, Yan Yan, Elina H. Niemelä, Timothy C. Wang, Benoit Viollet, Gustavo Leone, Pekka Katajisto, Kari Vaahtomeri & Tomi P. Mäkelä.

The Journal of Clinical Investigation, 2018 Jan 2;128(1):402-414

II. Fibroblast-derived IL11 is a driver and therapeutic target in Peutz-Jeghers syndrome polyposis.

Eva Domènech-Moreno, Wei-Wen Lim, Anders Brandt, Toni T. Lemmetyinen, Emma Viitala, Tomi P. Mäkelä, Stuart A. Cook & Saara Ollila.

Preprint in bioRxiv

III. Tellu: an object detector algorithm for automatic classification of intestinal organoids.

Eva Domènech-Moreno, Anders Brandt, Toni T. Lemmetyinen, Linnea Wartiovaara, Tomi P. Mäkelä & Saara Ollila.

Disease Models & Mechanisms, 2023 Mar 1;16(3):dmm049756.

ABBREVIATIONS

AMPK	AMP-activated Protein Kinase
ANXA1	Annexin A1
AOM	Azoxymethane
APC	Adenomatous polyposis coli
AREG	Amphiregulin
aSMA	Alpha Smooth Muscle Actin
ASPN	Asporin
BMP	Bone morphogenetic protein
BMPR1A	Bone Morphogenetic Protein Receptor 1A
BRG1	Brahma-related Gene 1
BRSK1-2	Grain-specific kinases 1 and 2
C3	Complement Component 3
CAC	Colitis-associated Cancer
CAF	Cancer-associated fibroblast
CBC	Crypt Base Columnar Cells
CBF	Crypt Base Fibroblast
CD34	Cluster of Differentiation 34
CDK1A	Cyclin-dependent Kinase 1A
CLDN4	Claudin-4
CLU	Clusterin
COL6A1	Collagen Type VI Alpha 1 Chain
COL8A1	Collagen Type VIII Alpha 1 Chain
CRC	Colorectal Cancer
CTF	Crypt Top Fibroblast
CTGF	Connective Tissue Growth Factor
CXCL5	Chemokine (C-X-C motif) Ligand 5
DCA	Deoxycholic acid
DLL1	Delta-like 1
DLL4	Delta-like 4
DSS	Dextran Sulfate Sodium
EBF1	Early B-cell Factor 1
ECM	Extracellular matrix
EGF	Epidermal Growth Factor
EHB2	Ehel Cup BHLH transcription factor 2
EHB3	Ehel Cup BHLH transcription factor 3
EP4	Prostaglandin E Receptor 4
EREG	Epiregulin
FAK	Focal adhesion kinase
FAP	Familial Adenomatous Polyposis

FGF7	Fibroblast Growth Factor 7
FGFR2	Fibroblast Growth Factor Receptor 2
FRZ	Frizzled receptor
GFI1	Growth Factor Independent 1
GI	Gastrointestinal
GLI1	GLI Family Zinc Finger 1
GP130	Glycoprotein 130
GREM1	Gremlin 1
GREM2	Gremlin 2
HGF	Hepatocyte Growth Factor
IAF	Inflammatory Associated Fibroblast
IBD	Inflammatory Bowel Disease
IGFBP5	Insulin-like Growth Factor Binding Protein 5
IL11	Interleukin 11
IL11RA1	Interleukin 11 Receptor Subunit Alpha 1
IL13RA2	Interleukin 13 Receptor Subunit Alpha 2
IL1RL1	Interleukin 1 Receptor-Like 1 (ST2)
IL33	Interleukin 33
ISC	Intestinal Stem Cell
JAK	Janus Kinase
JPS	Juvenile Polyposis Syndrome
LEPR	Leptin Receptor
LGR	Leucine-rich repeat-containing G protein-coupled receptor
LIP	Lkb1 interacting protein
LKB1	Liver Kinase B1
LOH	Loss of Heterozygosity
LRP6	Low-density lipoprotein receptor-related protein 6
LY6A	Lymphocyte antigen 6 complex, locus A
M025	Mouse protein-25
MAPK1-4	Mitogen-Activated Protein Kinase 1-4
MLPA	Multiplex Ligation-dependent Probe Amplification
NKX3.2	NK3 Homeobox 2
NOTCH	Neurogenic locus notch homolog protein
NRG1	Neuregulin 1
NSCLC	Non-Small Cell Lung Cancer
NUAK1-2	Novel Kinase Kinase 1 and 2
PDGFRA	Platelet-derived growth factor receptor alpha
PDPN	Podoplanin
PECAM1	Platelet/Endothelial Cell Adhesion Molecule 1
PGE2	Prostaglandin E2
PHTS	PTEN Hamartoma Tumor Syndrome

Pi16	Peptidase Inhibitor 16
PLAU	Plasminogen Activator Urokinase
PROCR	Protein C Receptor
PTEN	Phosphatase and Tensin Homolog
PTGS2	Prostaglandin-endoperoxide synthase 2 (Cox2)
PTPRC	Protein Tyrosine Phosphatase Receptor Type C
REG3B	Regenerating islet-derived protein 3 beta
REG3G	Regenerating islet-derived protein 3 gamma
RSPO	R-spondin
SCA1	Stem cell antigen 1
SEMF	Subepithelial myofibroblast
SIK 1-3	Salt-inducible Kinase 1-3
SMAD4	Mothers against decapentaplegic homolog 4
SMC	Smooth muscle cell
SNF1	Sucrose Non-Fermenting 1
SOX6	SRY-Box Transcription Factor 6
SOX9	SRY-Box Transcription Factor 9
SRC	Sarcoma Protein Tyrosine Kinase
STAT3	Signal Transducer and Activator of Transcription 3
STC1	Stanniocalcin 1
STK11	Serine/Threonine Kinase 11
STRAD	STE20-related Kinase Adaptor Alpha
TA	Transit-Amplifying Cells
TAGLN	Transgelin
TAZ	Transcriptional coactivator with PDZ-binding motif
TCF	T cell transcription factor
TGFb	Transforming growth factor beta
TREG	Regulatory T cell
WAE	Wound-associated epithelial cells
WNT	Wingless-related integration site
YAP	Yes-associated protein

INTRODUCTION

The central protein of this thesis is a kinase known as LKB1, short for Liver Kinase B1. Initially overlooked and deemed uninteresting due to its elusive function, LKB1 experienced a pivotal turning point in 1998 when a laboratory in Helsinki made a groundbreaking discovery, identifying it as the genetic driver of Peutz-Jeghers Syndrome (PJS) (Hemminki et al., 1998). Since then, Helsinki has been a research hub for LKB1 biology, providing invaluable insights that form the foundation of our knowledge today. This thesis project aims to contribute a modest yet significant piece to the ever-evolving research of LKB1 biology.

Previous work in the lab revealed that deletion of *Lkb1* in smooth muscle cells can induce PJS polyposis in mice, with enrichment of myofibroblast cells within the polyps (Katajisto et al., 2008). This finding challenged previously held hypotheses suggesting that the growth advantage in proliferative epithelial cells was solely attributed to epithelial *Lkb1* loss and shifted the focus from epithelial cells to the underlying mesenchyme. Building upon this discovery, in this thesis project, I demonstrate that *Lkb1* loss in gastrointestinal fibroblasts alters their function, disrupting the homeostasis of the epithelial niche and leading to PJS polyposis. Furthermore, I show the dependence of *Lkb1*-deficient fibroblast activity on a positive feedback loop driven by the inflammatory cytokine IL11 and that disrupting this feedback loop holds therapeutic potential for PJS patients.

Moreover, highlighting the importance of comprehending the effects of *Lkb1*-deficient fibroblasts on epithelia, we utilized organoids, that provide a unique platform for studying organ development, disease modeling, and drug testing. Additionally, this project contributes to advancing organoid research by applying artificial intelligence to automate gastrointestinal organoid classification.

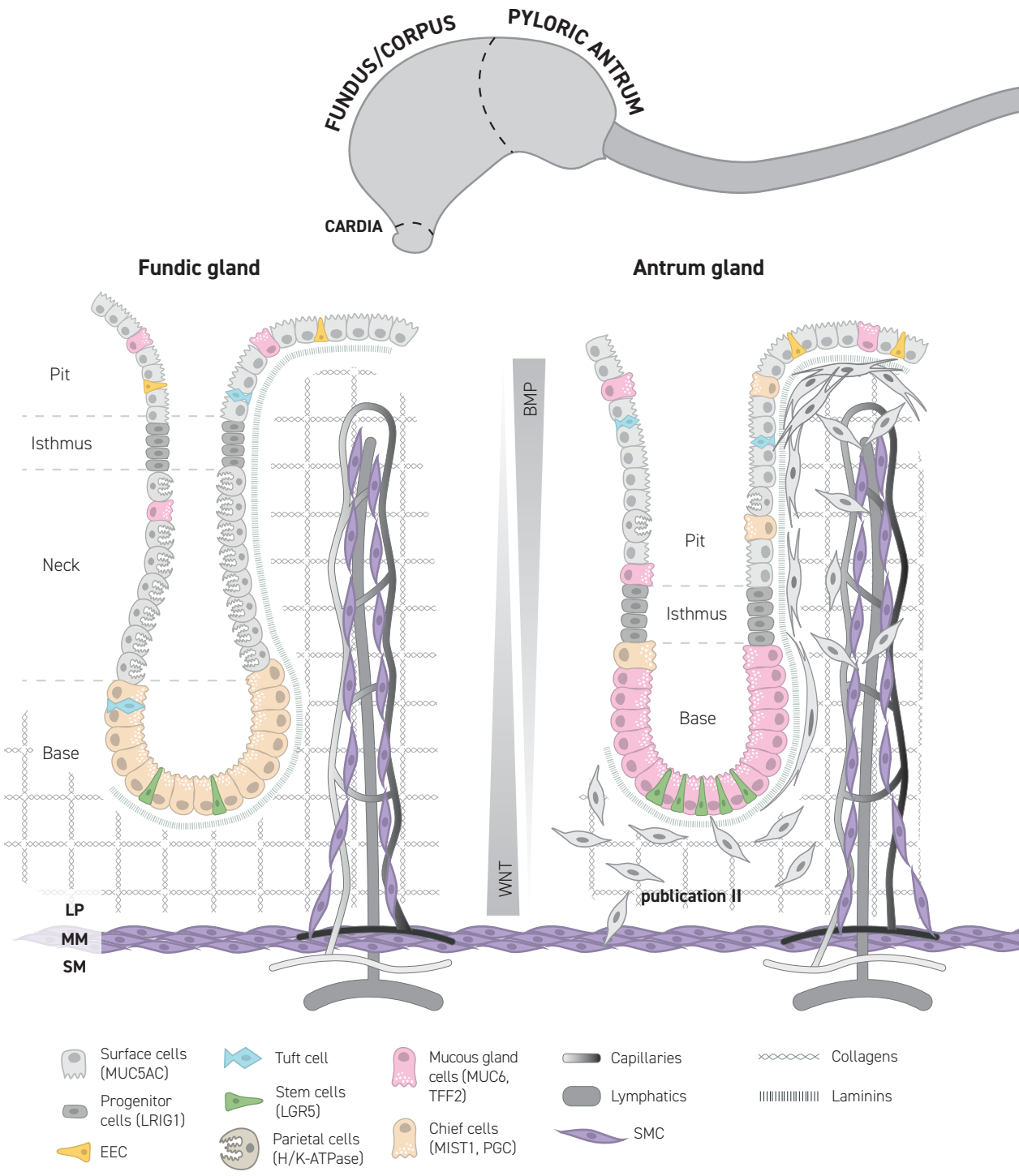
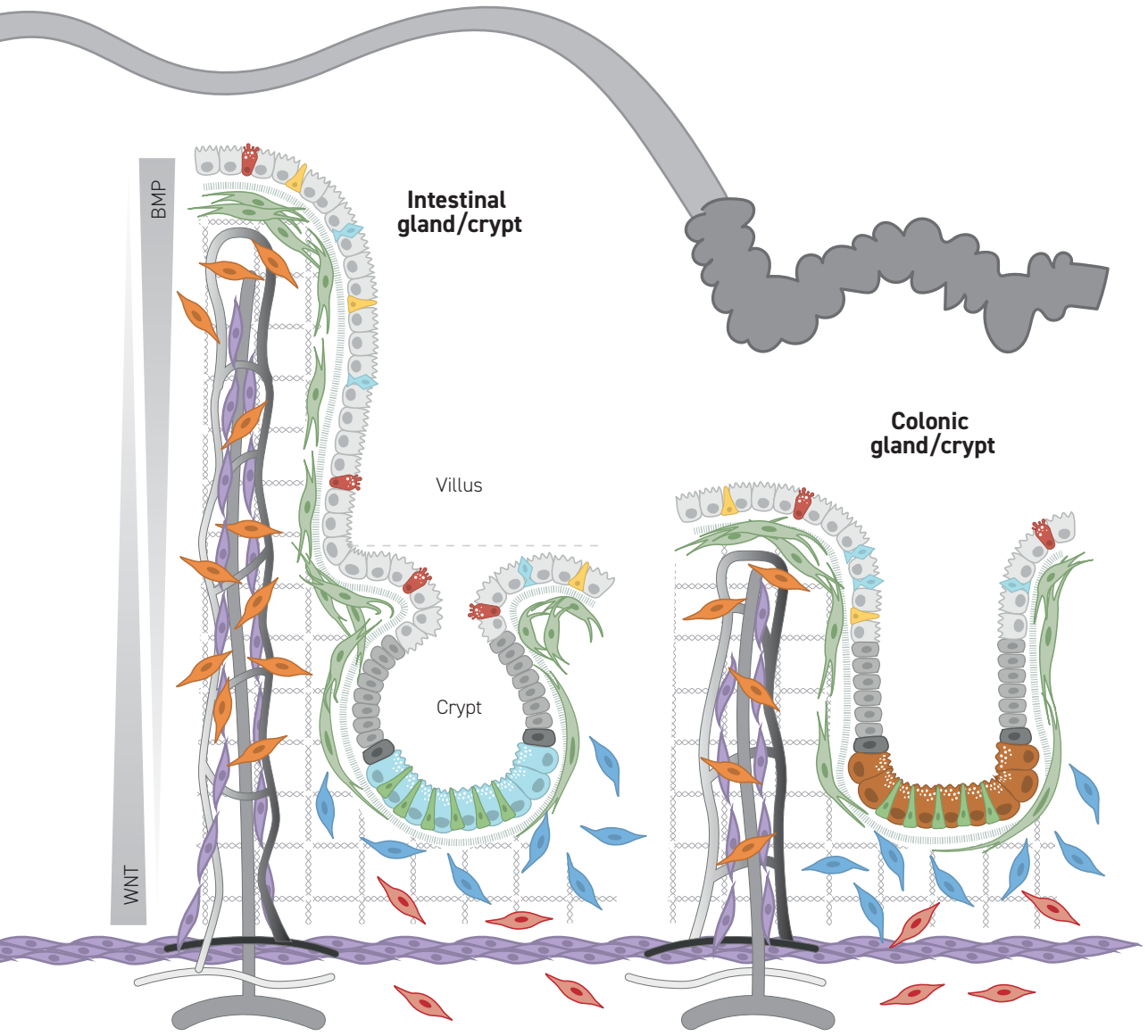


Figure 1. The Mucosal Architecture of the GI Tract (2-pages figure). Diagram illustrating the regional differences in epithelial composition across the GI tract and the fibroblast heterogeneity of the lamina propria. Please refer to the accompanying text for a comprehensive description and detailed explanation of the GI mucosa differences depicted in this diagram. LP = Lamina Propria, MM = Muscularis Mucosae, SM = Submucosa, CTF = Crypt Top Fibroblast, CBF = Crypt Bottom Fibroblast, SEMF = Subepithelial Myofibroblast, SMC = Smooth Muscle Cell, EEC = Enteroendocrine Cell. The diagram was created based on illustrations and information from (Kinchen et al., 2018; Engevik et al., 2020; McCarthy et al., 2020a; Kayisoglu et al., 2021; Melissari et al., 2021; Deng et al., 2022; Hoffmann, 2022; Pasztoi & Ohnmacht, 2022; Kraiczy et al., 2023; Pærregaard et al., 2023; Sylvestre et al., 2023)


BMP
WNT
Intestinal gland/crypt
Villus
Crypt
Colonic gland/crypt
Enterocyte/ colonocyte
+4 Reserve stem cell (BMI1)
Tuft cell
Paneth cells
Goblet cell
Transit-amplifying progenitors
Stem cells (LGR5)
EEC
Deep secretory cell (REG4)
Fibroblasts
Pi16+CBF
Lepr+CBF
Wnt4+ CTF
SEMF
Fibroblast subsets characterised in publication II

REVIEW OF THE LITERATURE

1. The Gastrointestinal Tract

The digestive tract, also known as the gastrointestinal (GI) tract, consists of a large tube starting at the mouth and ending at the rectum. The primary function of the GI tract is to facilitate the breakdown and absorption of nutrients from food while also eliminating waste materials from the body (Sensoy, 2021). The tube consists of several layers of tissue, each serving a specific function (Figure 2). The outermost layer provides structural support and helps attach the rest of the gastrointestinal organs to the surrounding tissue. In the stomach and the small intestine, where the layer is covered by the peritoneum, it is called serosa, whereas in the absence of peritoneum, such as in the colon, the layer is referred to as the adventitia (Rao and Wang, 2010). The next layer, called muscularis externa, consists of two layers of smooth muscle fibers: an inner circular layer and an outer longitudinal layer. The muscularis externa is responsible for peristalsis, the rhythmic contractions that move food along the digestive tract. Above the muscularis externa lies the submucosa. This layer contains connective tissue, blood vessels, lymphatic vessels, and nerves. The submucosa plays a role in transporting absorbed nutrients away from the digestive tract and supporting and nourishing the innermost layer, the mucosa (Rao and Wang, 2010). The mucosa is in direct contact with the lumen of the digestive tract, where the food travels, and its functions vary depending on the location along the GI tract (Figure 2). In the stomach, the mucosa is specialized in the secretion of enzymes and acids to break down and process food. In the small intestine and colon, the mucosa is specialized in absorbing nutrients and water (Rao and Wang, 2010).

The mucosa consists of three different cell layers. Lining the GI tract lumen lies the epithelium, a single-cell layer of polarized cells specialized in organ-specific functions (Figure 2). Beneath the epithelial layer is the lamina propria, consisting of loose fibrous connective tissue providing structural and vascular support to the epithelium and modulating immune responses (Rao and Wang, 2010; Powell et al., 2011). Finally, the third layer of the mucosa at its base is the muscularis mucosae, a thin double layer of smooth muscle fibers that aid in the movement and folding of the mucosa, allowing for efficient absorption and digestion (Rao and Wang, 2010). Together, the lamina propria and muscularis mucosae compose and modulate the stem cell niche, an essential environment for the regulation of GI homeostasis as well as modulation of tissue regeneration upon damage (Pinchuk et al., 2010; Powell et al., 2011).

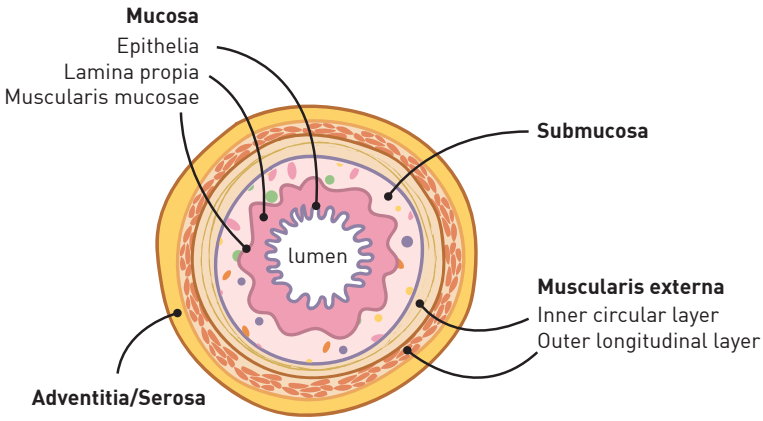


Figure 2. Tissue Layers of the Gastrointestinal Tract (GI). Transverse cut of the GI tract depicting the different tissue layers. These layers can be found throughout the entire GI tract. Adventitia/serosa layer provides support, the muscularis externa is responsible for peristaltic movements, and the submucosa, being highly vascularized and innervated, connects the organ with the rest of the body. The innermost layer, the mucosa, is specialized in the digestion and absorption of nutrients.

1.1. The Composition of the Epithelium

The GI epithelium undergoes constant turnover due to exposure to a harsh environment, including low pH conditions, a thriving microbiota, and mechanical stress from luminal flow (Creamer et al., 1961). The epithelial stem cells residing at the bottom of the crypts proliferate actively to renew all the cells in the epithelium every five to seven days (Creamer et al., 1961; Barker et al., 2007). As portrayed in Figure 1, the epithelial layer shows regional compartmentalization and structural differences across the GI tract. In the small intestine, to increase the absorption surface, the epithelial layer contains luminal protrusions, called villi, and invaginations into the lamina propria called intestinal glands or crypts. There are no villi in the stomach and colon, but the glands with the stem cells at the bottom persist. This very particular organization with the stem cells at the bottom of the glands, in addition to the increase in absorption surface, has a protective role, as it keeps the stem cells away from the constant stress of food transit and microbiota that resides in the lumen of the digestive tract (Gehart and Clevers, 2018).

The epithelium of the GI tract also exhibits variation in the terminally differentiated cell types across the different GI organs, aligning with the specialized functions of each organ (Figure 1). In the small intestine, enterocytes play a vital role in nutrient absorption. At the same time, goblet and Paneth cells secrete mucus and antimicrobial peptides, respectively, providing protection and lubrication to the lumen. Additionally, enteroendocrine cells secrete hormones that regulate digestion, appetite, and metabolic processes, facilitating