

# Biomedical applications of gut stem cells: gaining first-hand insights for developing therapy for the future

## Abstract

Understanding developmental biology holds the key to the future of medicine as virtually every disease can be viewed as a failure of development. Broadly put, developmental biologists seek to understand the biology of stem cells to answer the immense complexity in the developmental process. If we can decipher the molecular cues important for the maintenance of stem cells it will boost our ability to understand how these cues may go away in disease states and create effective therapeutics to fight against them. Moreover, in the era of personalized medicine cell therapy using stem cells hold greatest prospect of changing the face of human diseases and alleviating suffering in the near future. Use of stem cell therapy now hold a promising impact to treat various degenerative and genetic diseases including certain type of cancers, neurological diseases, autoimmune diseases, restoration of sight, wound healing, cardiac diseases, liver diseases, metabolic disorders, spinal cord injury and bone disorders etc. There are currently a number of stem cell sources that are being investigated for use in biomedical applications, including adult stem cells, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs), where in each presents its own unique advantages and disadvantages. Stem cells have the unique ability to continuously self-renew and differentiate into intermediate and mature cells of a variety of lineages. Though therapeutic potential of adult stem cells is the main focus of our research group; use of ESCs and iPSCs have also been regarded promising candidates for future therapies considering their pluripotency and personalized therapeutic possibilities. Over the years gut stem cells has been used in analyzing adult stem cell behavior, identifying niche components, modelling pathogen-epithelia interactions, gene editing, disease modelling and orthotopic transplantation etc. The present review highlights the use of intestinal stem cells in various biomedical applications, and how it has helped us to understand many of the complex diseases including various genetic diseases, obesity and cancer and design effective therapy against them

**Keywords:** gut stem cells, adult stem cells, embryonic stem cells, induced pluripotent stem cells, biomedical applications, gastrointestinal tract

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Upendra K Kar, Martin Hauer Jensen,  
Daohong Zhou

Department of Pharmaceutical Sciences, University of Arkansas  
for Medical Sciences, USA

**Correspondence:** Upendra K Kar, Division of Radiation  
Health, Department of Pharmaceutical Sciences, University of  
Arkansas for Medical Sciences, 4301 W Markham, #522-10,  
Little Rock, AR 72205, USA, Tel (501) 686-6964, Fax (501) 526-  
6599; Email ukar@uams.edu

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## Introduction

### Gut the complex organ

The gut (gastrointestinal tract) is a series of hollow organs joined in a long, twisting tube from the mouth to the anus.<sup>1</sup> The GI tract comprises the foregut, midgut, and hindgut, and each region gives rise to different tissues and organs. For example the foregut endoderm gives rise to the epithelium of the oral cavity, pharynx, esophagus, stomach, liver, pancreas, and proximal duodenum. The midgut and hindgut endoderm gives rise to the epithelium of the distal duodenum, jejunum, ileum, colon, rectum, and anal canal along with the epithelial lining of the bladder and urethra.<sup>2</sup> The role of the gut is not restricted to process food. Over the years, researchers have successfully deciphered many of the key aspects of gut i.e. gut is endowed with a range of sensory receptors that activate four major effector systems i.e. the enteroendocrine system, the nervous system, the gut immune system, and the non-immune defense systems.<sup>3</sup> With all its folds, villi, and microvilli, gut is arguably the largest surface in the body.<sup>4</sup> Gut has been described as ‘the largest endocrine organ in the body (gastrin, cholecystokinin or CCK, secretin, somatostatin, ghrelin, bombesin, and gastrin-releasing peptide (GRP)). These hormones released by the

enteroendocrine cells which can act locally as well as on other cells (including immune cells), nerve endings, or organs at remote sites including pancreatic islets and the CNS.<sup>5</sup> Around 40 neurotransmitters of the same classes as those found in the brain also been reported in Gut. Most importantly; in the era of personalized medicine we have given lot of emphasis to both of our genome and the second genome i.e. “Microbiome”. The human body consisting of >100 trillion (10<sup>14</sup>) microbial cells moreover, it has been estimated that <10% of DNA found in the human meta-organism derives from Homo sapiens origin.<sup>6</sup> Recent studies suggest microbiome in our digestive tract can play a profound role in our overall health including depression, anxiety, autism, schizophrenia, obesity, cancer and irritable bowel syndrome etc. Considering the importance of the microbiome Human Microbiome Project (HMP)<sup>7</sup> and the European Metagenomics of the Human Intestinal Tract (MetaHIT) Consortium has been established.<sup>8</sup> The recent findings suggest human gut microbiome altered by antibiotic use, with age, host genetics, chronic dietary patterns, and other environmental exposures.<sup>9-14</sup> There are some excellent reviews has been written on types of microbes present and their dynamic nature in the human gut.<sup>15,16</sup>

## The self-renewing epithelium of stomach, intestine and colon

The self-renewing epithelium of gut particularly; the resident stem cells of stomach, small intestine and colon has been studied in great detail.<sup>17,18</sup> These are actively cycling stem cell populations have offered a great opportunity to study on adult stem cells as well as their relationship with various disease progression. Now we know a small reservoirs of tissue-specific specialized cells i.e. adult stem cells, responsible for tissue homeostasis and repair of injured organs in mammals.

To maintain and repair the tissues during the lifespan of the animal, adult stem cells use three different types of cell division:

- I. Asymmetric divisions, generating one stem cell and one progenitor cell.
- II. Self-renewing symmetric division, generating two daughter stem cells to expand the stem cell population.
- III. Non self-renewing symmetric division, resulting in the generation of two progenitors.<sup>19</sup>

The hierarchical organization of adult tissue consists of stem cells, progenitors and terminally differentiated cells. In recent times cellular plasticity has been explained i.e. certain terminally differentiated adult cells can revert back to become pluripotent and retain the capacity to either de-differentiate or transdifferentiate.<sup>20,21</sup>

The human stomach contains a complex, three-dimensional epithelium organized into two distinct functional domains: the fundus (corpus), which is the major source of peptidases and acid responsible for the digestive functions of the stomach, and the antrum (pylorus) comprising mucus-secreting cells and hormone-producing endocrine cells.<sup>18</sup> In both areas, the epithelium is comprised of tubular-shaped invaginations called gastric units. Each gastric unit is divided into two parts: 1) the pit with mucus-secreting cells and 2) the gland, composed of various cell-types located within three distinct regions denoted the isthmus, the neck and the base. The cellular composition of these gastric units varies depending on the anatomical region of the stomach. In the corpus, the gastric units are formed by (1) surface mucous cells also known as pit cells (2) Parietal cells or acid secreting cells (3) chief cells, which contain zymogen granules and secrete the enzyme pepsinogen and (4) endocrine or hormone producing cells (e.g. Somatostatin, Histamine, and Leptin). In contrast, in the pyloric-antrum, the gastric unit composition is more simple, mainly consisting of mucous cells, secreting protective gastric mucin (MUC5AC), enteroendocrine cells (Gastrin and Somatostatin), and occasional Parietal cells.<sup>18</sup> The turnover rate of individual gastric units differs extensively, depending on their anatomical region and cellular composition. The adult pyloric epithelium, comprising mostly mucous-secreting cells, is estimated to self-renew in every 10-14 days. Multiple actively proliferating Lgr5-positive cells have recently been identified to reside at the base of each pyloric gland. *In vivo* lineage analysis characterized these cells as self-renewing, multipotent stem cells involved in long-term renewal of the pyloric epithelium under normal homeostasis conditions.<sup>18,19</sup> In a recent study Li et al demonstrated that Lgr5+ stem cells are the cancer-initiating cells and might contribute to malignant progression.<sup>22</sup>

The innerepithelial lining of the intestinal tract plays an integral role in its function and is anatomically subdivided into the small

intestine and colon.<sup>21</sup> It continuously monitors the composition of its contents i.e. food and drink, as well as the contaminants they might bring with them, microorganisms (gut has approximately 10 fold more bacteria cells); microbial products; complex biomolecules; toxic chemicals etc. The intestinal epithelium has crypt-villus structures which play an important role in the function of the gut. Nearly 90% of the intestinal epithelium is lost every 4-5 days and is replaced by cells newly generated from the crypt.<sup>17,22</sup> The crypts are considered to be the real “engine” of the self-renewal process which is fueled by the resident stem cells, thought to be located close to the crypt base. To replenish the large amount of disposable functional epithelium, ISCs produce rapidly cycling progenitor cells, referred to as transit-amplifying (TA) cells.<sup>23,24</sup> As they proliferate, TA cells migrate up the crypt-villus axis and differentiate into mature epithelial cells that are eventually shed off into the lumen. Stem cells differentiate into four major cell types; Absorptive enterocytes: play a central role in the digestion and absorption of luminal nutrients, Goblet cells: produce mucin to lubricate the luminal surface, Enteroendocrine cells: produce various hormones to control digestive enzyme secretion, metabolism, and bowel movements and Paneth Cells: helps in stem cells maintenance.<sup>25</sup> In contrast to these three differentiated cell types, Paneth cells are long-lived (2-3 months) differentiated cells that migrate downward to the crypt bottom; there they sterilize the crypt lumen through the production of antibacterial peptides. The colonic epithelium is devoid of Paneth cells but harbors Paneth-like cells, named deep crypt secretory cells or crypt base goblet cells.<sup>26</sup>

## The debate over gut stem cells

Over the years, “pure population” and “precise number” of true stem cells in each crypt has been a matter of intense debate. Earlier studies postulated four to five ISCs per crypt however Lopez Garcia et al.<sup>27</sup> and Snippert et al.<sup>28</sup> suggested 14-16 ISCs per crypt.<sup>27,28</sup> More recently using marker-independent approach together with mathematical modelling it has been showed that the number of functional stem cells per crypt in the small intestine is in the order of six.<sup>29</sup> Originally crypt base columnar (CBC) cells, wedged between the Paneth cells, were proposed to serve as stem cells. However later Clevers and colleagues demonstrated Leucine-Rich Repeat Containing G Protein-Coupled Receptor 5 (Lgr5) as a specific marker of CBCs, and these cells are able to generate all differentiated lineages of the small intestinal epithelium.<sup>30,31</sup> Subsequently, multiple studies have described an alternative crypt stem cell i.e. quiescent cells, able to retain DNA label and located just above the Lgr5+ stem cell niche, at the +4 position. Lineage tracing based on Bmi1, mTert, Lrig1 or Hopx promoter-driven expression implied the role of quiescent cell in the intestinal homeostasis.<sup>27,32</sup> Moreover, Cells that express Bmi1 can expand following ablation of Lgr5+ cells to compensate for the loss of the actively cycling stem cell pool.<sup>32</sup> Ritsma et al.<sup>33</sup> showed there are about 16 LGR +ve cells in a crypt, some of which are located towards the center of the crypt and others which are higher up, located at the border of the crypt base. Importantly, the probability of these center and border stem cells to stay in the crypt over time and function as a stem cell was different. The ‘central cells’ were more likely to retain stem cell capacity compared with the ‘border cells’. This was not absolute as there is a constant transfer of cells between these two regions. Therefore the functionality of an ISC is defined by its position rather than the expression of a specific protein marker.<sup>33</sup> In an elegant study Yan et al.<sup>34</sup> demonstrated that Bmi1- expressing cells can replace the Lgr5+ cells lost by irradiation.<sup>34</sup> Though Metcalfe et al.<sup>35</sup> later demonstrated that depletion of Lgr5+ stem cells strongly

affects the regenerative response, demonstrating that Lgr5<sup>+</sup> stem cells are indispensable for radiation-induced intestinal regeneration.<sup>35</sup> The relationship of +4 cells with Lgr5<sup>+</sup> stem cells is of intense debate. Following injury and loss of CBC stem cells, a “reserve” population (or populations expressing Troy or Mist1) of cells that reside outside the crypt base may act as facultative stem cells, moving down to the crypt niche, can regenerate the entire tissue.<sup>36–40</sup> In recent times plasticity in lineage selection has been revealed i.e. Sox9<sup>Hi</sup>, Ngn3 precursors, and DCKL1- positive tuft cells and DLL1-positive cells or other differentiated population can dedifferentiate to produce functional stem cells.<sup>41,42</sup> Most importantly, delta- like 1 (DLL1)-positive cells (which normally give rise to secretory precursors) and DCKL1-positive tuft cells have the capacity to repopulate the epithelium after injury.<sup>43,44</sup>

### Three-dimensional cell culture: a breakthrough discovery

The recently developed *ex vivo* 3 dimensional culture method (started with intestinal organoid culture system) allowed us to maintain and expand adult stem cells while retaining their multi-lineage potential *in vitro*.<sup>45</sup> In fact, the invention of the method galvanized the field of stem cells and regenerative medicine. The three-dimensional organoids retain a stem cell hierarchical organization, wherein self-renewing stem cells continuously produce diverse differentiated cell types. Over the years it has been emerged as a promising experimental tool because it reflect the natural state of organs better than conventional tissue culture models. An explosion of studies have used organoids to explore questions regarding stemness, physiology, and oncogenic transformation etc.<sup>46,47</sup>

The culture system developed by Clevers and colleagues is simple, using Matrigel as an ECM substitute, supplemented with growth factors constituting key endogenous niche signals: EGF (epithelial growth factor), an EGFR ligand, to promote cell proliferation; Noggin, a BMP (bone morphogenetic protein) inhibitor, R-spondin, an LGR4/5 (leucine-rich repeat-containing G-protein-coupled receptor 4/5) ligand, a WNT agonist and WNT, a Frizzled/LRP (lipoprotein receptor-related protein) ligand to maintain and expansion of stem cells.<sup>48</sup> Several modifications has also been proposed to enhance the success, including supplementation of valproic acid, nicotinamide, A83-01 (Alk inhibitor), Prostaglandin E2, p38 inhibitor (SB202190), Rho kinase inhibitor (Y-27632) and GSK3 $\beta$  kinase inhibitors.<sup>49</sup> These methods allow the growth of ever-expanding small intestinal organoids, which display all hallmarks of the original tissue in terms of architecture, cell-type composition, and self-renewal dynamics. We have summarized the use of organoids generated from gut stem cells to answer many of the fundamental biological questions particularly in disease modeling, diabetes, cancer and their applications in clinic.

### Drug discovery (toxicity and diseases model)

Our understanding of human health is constantly evolving. Over the years animal models such as mice, rats and non-human primates have been used as invaluable tools for modelling human disorders by enabling the dissection of disease mechanisms at different developmental stages and in a variety of cell types *in vivo*. Particularly the advent of 1) genetic editing tools like Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) genes, originally discovered in *E. coli*, now known to be essential to eliminate invading genetic material by editing the genome<sup>50</sup> 2) patient- derived xenograft (PDX) models:

tumor tissue implanted directly into immunocompromised (NOD scid gamma) mice. These technologies have contributed to understand the underlying mechanism of many diseases including cancer. However; though translating information from mouse into the more complex system like human is exciting, yet it is discouraging. Understandably so as, we evolutionarily diverged from our ancestors almost 108 years ago and acquired considerable developmental, anatomical, genetic, physiologic and metabolic differences. On the genomic level, although the majority of human and mouse genes are orthologous, about 20% do not have an identifiable singular orthologue, and 1% lack a homologue.<sup>51</sup> On the physiological level, mice and humans differ in many organ functions. For example, heart size and resting cardiac rate are substantially different. Similarly the anatomy of the mouse and human intestinal tract also have prominent differences, which might be shaped by their diverging diets, feeding patterns, body sizes and metabolic requirements etc. Moreover, considering the increasing number of clinical trials failure, scientists are giving more attention to conduct biomedical research with human sample. Though patient-derived cells is tremendously useful for studying disease aetiologies at the molecular and cellular levels, however, isolation of specific cell types and subtypes are difficult, moreover most of the pathophysiology experienced in multicellular and tissue level. Thus, the use of stem cells disease models has become increasingly favored for purposes of drug discovery.

Organoids generated from the epithelium of gastrointestinal tissues such as stomach, intestine and colon has been widely used in both basic and translational research.<sup>52,53</sup> Over the years multiple approaches has been followed to generate organoids from primary tissue, ESCs or iPSCs. Regardless of their source, organoids are compared with their primary tissue, in their composition and architecture, harboring small populations of genomically stable, self-renewing stem cells that give rise to fully differentiated progeny comprising all major cell lineages at frequencies similar to those in living tissue. Similarly; Human stem cells and their derivatives has provided the ideal platform, to screen compounds for safety and efficacy.

Use of Gut stem cells has helped us in our understanding on numerous genetic diseases of the GI tract. Cystic fibrosis (CF) is an inherited disorder that causes severe damage to the lungs and digestive system. CF is caused by a spectrum of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel that is normally expressed in epithelial cells of many organs including liver, pancreas, and small bowel.<sup>54,55</sup> A lack of appropriate animal model made it difficult to study the disease.<sup>56</sup> Dekkers and colleagues identified forskolin- induced swelling of normal epithelial intestinal organoids as a physiological function of nonmutant CFTR.<sup>57</sup> Though epithelial organoids generated from patients with CF did not undergo forskolin-induced swelling it was restored in upon incubation with pharmacological agents and CRISPR/Cas9 genome editing system.<sup>57</sup> Recently; GI epithelial organoids have also been used to study microvillus inclusion disease (MVID) and multiple intestinal atresia (MIA).<sup>58,59</sup> MVID is frequently caused by mutations in myosin Vb (MYO5B) that result in inappropriate localization of apical proteins and enterocyte polarization. Wiegerinck et al.<sup>60</sup> identified a mutation in syntaxin 3 (STX3) by whole-exome sequencing in patients with MVID but who did not have a mutation in MYO5B. Duodenal epithelial enteroids from these patients had partial loss of microvilli, inappropriate accumulation of vesicles, and absence of STX3 protein.<sup>60</sup> Using Intestinal epithelial organoids generated from a subset of MIA patients with a combination of genome-wide



linkage analysis and whole-exome sequencing, Bigorgne et al.<sup>59</sup> & Avitzur et al.<sup>61</sup> identified previously uncharacterized mutations in the tetratricopeptide repeat domain 7A (TTC7A) of patients with MIA and combined immunodeficiency.<sup>61</sup> Enteric anendocrinosis is an extremely rare disease characterized by severe malabsorptive diarrhea and a lack of intestinal enteroendocrine cells. Recently, Spence et al.<sup>62</sup> demonstrated that knockdown of *NEUROG3* in PSC-derived intestinal organoids resulted fewer enteroendocrine cells, similar to patients with anendocrinosis.<sup>62</sup> Grabinger et al.<sup>63</sup> demonstrated that intestinal organoids grown out of primary intestinal crypts, are an interesting and suitable model to study toxicity and cell death induction in *ex vivo* cultured primary epithelial cells.<sup>63</sup>

## Diabetes

The recent advent of stem cell technologies have also provided the ability to dissect a complex disease like diabetes (is actually a group of diseases). Diabetes is a progressive and complex disease affecting millions of people worldwide.<sup>64</sup> The three main types of diabetes are type 1 (T1D; progressive  $\beta$  cell destruction mostly due to autoimmunity progressive  $\beta$  cell destruction mostly due to autoimmunity), type 2 (T2D; insulin resistance and inadequate insulin secretion), and gestational diabetes (gestation; second or third trimester of pregnancy). T1D, which occurs from autoimmune-mediated beta-cell depletion, is caused by insulin deficiency, and leads to weight loss. T2D, on the other hand, often arises as a consequence of obesity-driven metabolic syndrome and is characterized by insulin resistance. Both T1D and T2D are associated with hyperglycemia and diabetic patients require intensive disease management as over time, it leads to multi-organ degenerative complications involving the nervous system (diabetic neuropathy), the eyes (diabetic retinopathy), the kidneys (diabetic nephropathy), and the GI tract (intestinal enteropathy) etc.<sup>64,65</sup> Though management of insulin is the primary option of treatment, for decades, diabetes researchers have been searching for ways to replace the insulin-producing cells of the pancreas to treat diabetes. Being the largest endocrine organ of the body and its shared ancestral origin with pancreas i.e. endoderm several attempts have been made to produce insulin from Gut.<sup>66</sup> In the pancreas, endocrine cells are clustered into islets of Langerhans, however in the gut (stomach and intestines), these cells are scattered throughout the epithelium. In a developmental perspective; there are numerous transcription factors responsible in embryonic development of beta cells.<sup>67</sup> Examples include the homeobox protein *Pdx1*, which is required both at early stages of pancreas development and for adult beta cell function, *Neurogenin 3* (*Neurog3*), which is required for specification of all endocrine lineages, and *MafA*, which is involved in maturation of committed beta cells.<sup>68</sup>

Over the years although insulin production has been achieved in several other tissues including muscle and the liver, the difficulty in insulin secretion from these surrogate cells limits their potential for insulin replacement therapy. For example; IEC-6 (rat small intestine epithelioid) cell line was shown to produce and secrete insulin after transfection with transcription factor pancreatic and duodenal homeobox protein 1 (*PDX1*) with and without the need for betacellulin (an epidermal growth factor) or after transplantation in diabetic rats.<sup>69–71</sup> However, as gut enteroendocrine cells shared several features of  $\beta$ -cells they have drawn attention to many in the field. Specifically, the G-, K- and L cells, three types of enteroendocrine cell located in the epithelium of the small intestine producing gastrin, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1, respectively,

all carry sophisticated glucose/nutrient-sensing machinery. Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretins released from enteroendocrine cells into the circulation in response to ingested nutrients, such as glucose and fat. GIP is secreted from K-cells in the duodenum and upper small intestine, and GLP-1 is secreted by L-cells in the lower small intestine and colon. In addition to its glucose-dependent insulinotropic effect, GLP-1 has other beneficial effects that help achieve targeted blood glucose levels, such as inhibition of glucagon secretion, delayed gastric emptying and appetite suppression etc. A series of elegant studies has demonstrated the production of Insulin is feasible in G, K and L cells.<sup>71,72,73</sup>

As enteroendocrine cells arise from the pluripotent intestinal stem cells located in intestinal crypts they are under active investigation. For Example L-cell could potentially evolve as novel, effective therapeutics for type 2 diabetes. Recently; Peterson et al.<sup>74</sup> demonstrated that L-cell differentiation can be selectively increased by short-chain fatty acids (SCFAs) in mouse and human.<sup>74</sup> Moreover, they demonstrated that the earliest cell fate decisions are regulated by the NOTCH signaling pathway, and blocking the NOTCH signaling pathway can increase the number of L-cells, and secretion of glucose-stimulated GLP-1. Furthermore, in high-fat diet-fed mice with impaired glucose tolerance, administration of c-secretase inhibitor improved the early insulin response to glucose, and restored glucose tolerance in association with a significant increase in L-cell number and GLP-1 secretion.<sup>74</sup> NOTCH receptor interacts with ligand anchored in the membrane of neighboring cells, an intrinsic c-secretase cleaves the receptor, releasing the NOTCH intracellular domain (NICD). NICD translocates into the nucleus, and stimulates the expression of hairy and enhancer of split 1. Hairy and enhancer of split 1 has been shown to repress expression of protein atonal homolog 1 and neurogenin 3. Atonal homolog 1 is the first factor known to be involved in endocrine specification, inducing cells to the secretory lineages, goblet, paneth and enteroendocrine cells. Neurogenin 3 is expressed in the precursor cell to all enteroendocrine cells, and loss of neurogenin 3 in mice results in a loss of most enteroendocrine lineages. Thus, activation of NOTCH signaling induces intestinal stem cells to differentiate into absorptive epithelial cells, and blocking the NOTCH signaling pathway increases the number of cells differentiating into enteroendocrine lineages including L cells. Intestinally; gamma-secretase inhibitor was found not only to increase the number not only of L-cells, but also of K-cells with increased GIP secretion. The glucose-dependent insulinotropic effect of GIP has been explored to develop as a potential glucose-lowering drug.

It has been reported that the insulinotropic action of GIP is impaired in diabetic patients and a ameliorated GIP improved the glycemic condition.<sup>75</sup> In a recent study Ariyachet et al.<sup>76</sup> has demonstrated a new approach to harness the intrinsic regenerative capacity of the stomach epithelium to replenishing  $\beta$  cell mass *in vivo*. Moreover, reprogramming suppressed hyperglycemia in diabetic mice, suggesting the potential for development of engineered stomach tissues as a renewable source of functional  $\beta$  cells for glycemic control.<sup>76</sup> The authors developed a triple-transgenic mouse model where *Neurog3*, *Pdx1*, and *MafA* transcription factors (NPM) were expressed in *Neurog3*<sup>+</sup> enteroendocrine progenitor cells. NPM factors caused extensive reprogramming of enteroendocrine cells into insulin<sup>+</sup> cells throughout the GI tract, with the greatest efficiency (~40%) in the gastric antrum. Induced GI insulin<sup>+</sup> cells were sufficient to suppress hyperglycemia and restore near-normal glucose tolerance in streptozotocin (STZ) treated diabetic mice.

These studies suggest that Gut stem cells can be induced to express insulin and feasibility of using gut stem cells for development of an autologous cell-based therapy for diabetes. In a recent study D'Addio et al.<sup>77</sup> demonstrated that patients with T1D- end stage renal disease (T1D-ESRD) have reduced colonic stem cell numbers with impaired function<sup>77</sup> and altered levels of insulin-like growth factor 1 (IGF-I) and its binding protein 3 (IGFBP3). When it binds to IGF-1, it induces apoptosis and suppresses the pro-growth effects of IGF-1. Though, IGFBP3 can also act independently to induce caspase-8-dependent apoptosis using its own receptor, TMEM219. Interestingly, treatments with either caspase 8 or caspase 9 inhibitors can reverse these effects. Furthermore, ecto-TMEM219 recombinant protein able to normalize the circulating IGF-I/IGFBP3 levels and reestablish the homeostasis of Colonic Stem Cells.

Recently, Gut stem cells have also helped to reveal the underlying mechanism on obesity. Obesity has become a worldwide health problem associated with premature death along with reduced life quality. The underlying molecular mechanisms explaining how obesity negatively affects the function of multiple organs are not fully understood. Recently; changes in the function of the intestinal stem cells is closely examined to explain the deleterious effects of obesity on organ function. Using Diet-Induced Obesity Model (DIO) Mah et al.<sup>78</sup> demonstrated that DIO decreased Paneth and goblet cell number with increase ISC number. Interestingly, it correlates with elevated plasma insulin. However insulin and/ or insulin-like growth factor 1 (IGF1), has the ability to reverse the impairment, suggesting the link between DIO-ISC-insulin/IGF1.<sup>78</sup> Similarly; high-fat diet (HFD)-induced obesity which leads to many life-threatening diseases, including cardiovascular disease and cancer is well known. In a recent study Beyaz et al.<sup>79</sup> demonstrated how stem and progenitor cells adapts to pro- obesity diets (HFD) and the molecular mechanism behind the initiation of tumors. They fed mice with HFD which increased the number of ISCs and lowered the paneth cells. ISCs from HFD mice had an increased ability to support regeneration and the formation of intestinal organoids in culture. Moreover, addition of fatty acids that are present in the HFD to the culture medium enhanced the organoid formation capability of the ISCs of control mice. ISCs from HFD mice had elevated expression of peroxisome proliferator-activated receptor (PPAR- $\delta$ ). Interestingly; treating mice with PPAR- $\delta$  agonist recapitulated many of the ISC phenotypes induced by HFD suggesting PPAR- $\delta$  is required for fatty acids to stimulate ISC activity in organoids generated from control mice. The study further showed, ISCs from mice fed a HFD or treated with the PPAR- $\delta$  agonist had increased Wnt signaling, which promotes ISC proliferation and had an increased incidence of spontaneous intestinal tumors.<sup>79</sup> Clearly, these studies demonstrates investigating the biology of gut stem cell of Diabetes patients we can uncover novel mechanisms i.e. how Diabetes contributes to intestinal enteropathy and find ways for developing potential new therapeutic approach.

## Cancer

Over the years intensive research on successive stages of cancer has been dissected i.e. initiation, promotion, progression and malignant transformation. Clinical and experimental data have proved that physical, chemical and biological factors plays an integral role in each stage of cancer. The hallmarks of cancer comprises: sustenance of proliferative signaling, evasion of growth suppressors, resistance to cell death, enablement of replicative immortality, induction of angiogenesis, and activation of invasion and metastasis. This list was

further expanded to include reprogramming of energy metabolism, evasion of immune destruction, and two characteristics that appear to enable the acquisition of cancer hallmarks, genome instability/mutation and tumor-promoting inflammation.<sup>80</sup> Capturing this incredible phenotypic diversity is essential to the understanding of neoplastic transformation and requires complex cancer models that can recapitulate the homo-and heterotypic cellular interactions that drive tumorigenesis. Though patient-derived xenograft (PDX) models, in which fragments of tumor tissue are implanted directly into immunocompromised mice is considered as the ideal method PDX models have several crucial failings that limit their effectiveness in a clinical setting. In recent times Organoid methods (generating organoids from the patient tissue) have become more popular considering their potential application to assess drug candidate toxicity for developing precision medicine.

In a broad sense tumors develop in those tissues in which cellular homeostasis has been disturbed by hyperplastic, dysplastic or regenerative changes. Now there is no doubt that studying the biology of stem cells would allow us to understand the insight of pathophysiology of these complex diseases because disruption of the normal homeostatic processes of stem cells i.e. differentiation and maturation is responsible for carcinogenesis. The tumor cell interacts with its microenvironment (niche) which influence and promote various steps of tumor development. Tumor-associated stroma includes a wide variety of cell types including bone-marrow derived immune cells, cancer-associated fibroblasts and myofibroblasts, stem and endothelial cells.<sup>81,82</sup> In a cancer tissue, aberrant driver pathways delimit the niche-restricted growth of the cancer cells and permit their dominant overgrowth in the hostile environments of the remote tissues they invade or metastasize. A recent large-scale deep sequencing study revealed that the majority of the recurrently mutated genes in sporadic colorectal cancer (CRC) are ascribed to five common signaling pathways: WNT, RAS/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), transforming growth factor (TGF), and TP53 signals.<sup>83</sup> Interestingly, most of these driver pathways are coincidentally essential to regulate the self-renewal of the human intestinal stem cells (ISCs), in which the surrounding niche environments either activate or inactivate these pathways *in vivo*.<sup>84</sup> Gregorieff et al.<sup>85</sup> has demonstrated that Yap, a downstream transcriptional effector of Hippo signaling pathway, is critical for recovery of intestinal epithelium after exposure to ionizing radiation and driving cancer initiation. Yap transiently reprograms Lgr5(+) ISCs by suppressing Wnt signaling and excessive Paneth cell differentiation, while promoting cell survival and inducing a regenerative program that includes Egf pathway activation. Moreover, Yap is required for progression of early Apc mutant tumor-initiating cells, suppresses their differentiation into Paneth cells, and induces a regenerative program and Egfr signaling. In tissue injury, Yap reprograms Lgr5(+) ISCs by inhibiting the Wnt homeostatic program, while inducing a regenerative program that includes activation of Egfr signaling.<sup>85</sup> Matano et al.<sup>86</sup> used CRISPR-mediated disruption of the tumor suppressors APC, TP53, and SMAD4, combined with CRISPR-mediated knock-in of dominant-active alleles of the oncogenes KRASG12V and PIK3CAE545, to model multistep carcinogenesis.<sup>86</sup> Using niche factor manipulation in their culture media, they managed to establish pure cultures of organoids carrying oncogenic multigene modules of varying complexity comprising up to five simultaneous alterations. Interestingly; mutation in APC, KRASG12V, SMAD4, TP53, and PIK3CAE545K organoids grew independently of niche

factor supplementation and formed tumors when implanted into the kidney capsule of NOG mice.<sup>86</sup> In another study Drost and colleagues used a similar CRISPR-mediated approach to target the tumor suppressors APC, TP53, and SMAD4 in combination with CRISPR-mediated knock-in of a dominant-active KRASG12D allele in human small intestine and colon organoids. These organoids were also grown independently of niche factor supplementation and were capable of generating carcinoma upon subcutaneous xenotransplantation with localized invasion into the underlying muscle tissue.<sup>87</sup> Using the three dimensional ex-vivo method recently Van de Wetering et al.<sup>88</sup> generated organoids both from cancerous tissue and adjacent non-cancerous tissue and screened 83 experimental and approved cancer drugs.<sup>88</sup> Furthermore they correlated their results with genotypic characterization of the tumor organoids. They found varying responses to drugs across the colon tumor organoids and identified predicted genotype drug interactions, such as sensitivity to the MDM2-inhibitor Nutlin-3a in p53 inactivated tumor organoids, and sensitivity to anti-EGFR treatment in tumor organoids lacking RAS mutations.<sup>88</sup> In a recent study, Fujii et al.<sup>89</sup> generated a biobank of 55 colorectal tumor organoid lines and 41 matching normal colorectal organoids. They investigated the association between oncogenic pathway mutations with the niche factors. For example, tumor organoids acquired mutations in the Wnt and TGF- $\beta$  signaling pathways did not require Wnt pathway stimulation or TGF- $\beta$  pathway inhibition. Interestingly, Fujii et al.<sup>89</sup> found that organoids that required EGF for growth also frequently required inhibition of p38, which was associated with ligand mediated EGFR internalization. However a subset of KRAS mutant tumor organoids that required EGF for growth, suggesting that anti-EGFR therapy may provide some benefit to those patients. They also established renal subcapsular xenografts for 21 colorectal cancer organoids and observed tumor organoids can grow in the absence of niche factors. Furthermore; on cancer metastasis they demonstrated that tumor organoids derived from liver metastases showed greater metastatic capacity in xenografts compared to the matched primary tumor derived organoid, despite the mutational and gene expression signatures of these organoid pairs being indistinguishable. Therefore, oncogenic driver mutations probably has a selective advantage and improved growth potential to colorectal tumors.<sup>89</sup> Though there are certain limitations using stem cells derived organoids in cancer research i.e. absence of blood vessels, immune cells and microbiome etc there use will continue to grow. These studies not only led us to have more understanding on the biology of carcinogenesis but also the promise of personalized medicine.

*Helicobacter pylori* is the causative agent of many gastric diseases and the main risk factor in the development of gastric adenocarcinoma. Considering its threat World Health Organization classified as a class 1 carcinogen. *Helicobacter pylori* attaches to host cells using a variety of adhesins including VacA and CagA and with close proximity promotes the delivery of virulence factors which is not fully understood.<sup>90</sup> *H pylori* infections induce chronic inflammation and intestinal metaplasia (IM) through genetic and epigenetic changes and activation of intracellular signaling pathways that contribute to gastric carcinogenesis. However, the precise mechanism of IM in gastric carcinogenesis has also not been fully elucidated. So far no vaccine against *H. pylori* has been developed and many current drugs have begun to fail due to resistance mechanisms of the bacteria. Recently stem cells and the three dimensional *ex vivo* culture has been used to find more details about the input signals *H. pylori* requires to efficiently colonize, as that holds a great promise for understanding the molecular mechanism behind *H pylori* mediated

carcinogenesis and developing effective therapeutic measures to treat gastric cancer. In a recent study; Schlaermann et al.<sup>91</sup> developed a robust and quasi-immortal 3D organoid model to understand the underlying mechanisms of infection, mucosal immunity and cancer of the human stomach.<sup>91</sup> Wroblewski et al.<sup>92</sup> used the organoid method and showed that Infection of *H pylori* cagA<sup>+</sup> wild-type or isogenic mutant strains increase epithelial cell proliferation and  $\beta$ -catenin nuclear translocation. They further identified a previously unreported mechanism through which *H pylori* may heighten the risk of carcinogenesis: increased snail expression, which was confirmed in human gastric tissue specimens.<sup>92</sup> Furthermore, selective interaction of *H pylori* with undifferentiated cells i.e. stem or progenitor cells in comparison to the differentiated epithelial cells.<sup>93</sup> These studies suggest *H. pylori* has the ability to penetrate and manipulate the protected epithelial stem and progenitor cell compartments is a critical step in *H pylori*-induced gastric pathology.<sup>93,94</sup> Over all studying gut stem cells from patients has led us to discover the underlying genetic mechanisms in identifying tumor subtype, oncogenic drivers, tumor growth, engraftment, metastasis and new targets for developing effective drugs. More over the patient driven and patient specific personalized medicine is not a dream rather a reality in clinics.

## Conclusion

Over the years stem cells technology has generated a great deal of interest in this new era of medical research due to its potential clinical applications. In this review, we have attempted to describe how gut stem cells has enabled us to provide basic insights in many scientific discoveries in biomedicine and helped us to design effective therapeutic strategies against adverse pathophysiological conditions. Organoid generated from gut stem cells has close resemblance to human gut both in health and disease, moreover it is easy to generate in research laboratories. Organoids generated from normal and tumor specimens can be used to characterize the events that occur during transformation, growth, and progression of malignancies such as colorectal or gastric cancers. Many researchers across the world is trying to transform gut endocrine cells to produce insulin which will be a realistic solution to curtail the burden of diabetes. Finally, organoids are the best choice to screen pharmacological agents to assess the efficacy and safety of specific drugs which hold a great appeal in personalized medicine.

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## Conflict of interest

The author declares no conflict of interest.

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