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The role of stem cell niche in intestinal aging

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ABSTRACT

Like most tissues, intestine shows multiple alterations during aging. While the main function of nutrient absorption is relatively well maintained, capacity of the intestine to respond to abrupt changes or damage declines with age. The reduction in renewal and regeneration capacity results from alterations in the stem cells that renew the epithelium, and in the complex interactions stem cells have with their microenvironment, or the Niche. This review highlights recent evidence on age-associated changes in the intestinal stem cell function, and focuses on stem cell extrinsic mechanisms. Strategies targeting niche interactions have already shown promise in alleviating problems associated with intestinal aging in animal models, and may provide means to protect the elderly for example from chemotherapy induced gastrointestinal side-effects.

1. Introduction

Gastrointestinal tract (GI tract) is responsible for our ability to harvest external resources. It forms a tube where functions of break-up, absorption, and waste disposal occur as food and liquids pass through esophagus, stomach, small intestine, colon and rectum. GI tract also hosts large quantities of microbes that exist by virtue of generous availability of nutrients, but also contributes to processing of food in to metabolites that are necessary for the host. Together with the microbial flora, the harsh acidic conditions required to dissolve food in the stomach, and the mechanic abrasion resulting from peristaltic passage of material impose a special challenge to the main function of the intestine. Intestine must at the same time provide a robust protective barrier, while allowing effective extraction of nutrients and water from the luminal content. The majority of nutrient absorption occurs in the small intestine, where combination of effective absorption and protection is achieved by a single layer of epithelial cells that is so rapidly renewed that damage has little time to accumulate. Consequently, most of the small intestinal epithelium is turned over in approximately 5 days. The cells responsible for this life-long rapid renewal are intestinal stem cells. However, for stem cells to accurately conduct their tissue renewing function, they are nurtured, protected, and guided by their microenvironment – also called the stem cell Niche. Consequently, the life-long maintenance of intestinal function is subject to age-induced changes in both stem cells and their niche. Stem cell intrinsic changes are reviewed in depth by Jasper (2020) in this special issue. Here, we focus on how the stem cell niche impacts tissue functions and renewal during aging.

2. Aging associated changes in intestinal function

Aging is associated with a multitude of alterations in the intestine. Loss of proper absorptive function by intestinal epithelium can quickly result in malnutrition and cachexia, which can be particularly harmful for elderly and frail individuals (reviewed in (Drozdowski and Thomson, 2006; Gariballa and Sinclair, 1998)). Early reports provided evidence for reduced absorptive capacity in the elderly (Feibusch and Holt, 1982), and reports on decreased epithelial surface area suggested a possible mechanism (Warren et al., 1978). More recent studies indicate specific defects in absorption of lipids (Woudstra et al., 2004) and glucose (Drozdowski et al., 2003; He et al., 2020). Surprisingly effects of aging on intestinal amino acid/peptide uptake has not been systematically studied in humans, but old mice are less capable to adapt uptake to a diet high in protein (Ferraris and Vinnakota, 1993). Moreover, adaptive responses to alterations in food availability are blunted during aging at least in mice (Gebert et al., 2020). However, other studies on elderly humans contradict the findings on reduced absorption (D'Souza, 2007; Dumic et al., 2019; Lipski et al., 1992; Remond et al., 2015). Moreover, studies on GI tract motility indicate that aging does not directly impact the transit time of food in the small intestine (Anuras and Sutherland, 1984; Madsen and Graff, 2004; Sarosiek et al., 2010). Taken together,

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the extent to which absorptive decline may contribute to the common malnourishment and cachexia of the elderly remains unclear.

Even though the absorptive capacity may withstand aging relatively well due to its large functional reserve (Firth and Prather, 2002), the thin intestinal epithelium must also create a robust barrier for large quantities of microbes. Indicating that the intestinal barrier deteriorates with age (reviewed in (Man et al., 2014)), rate of gastrointestinal infections increases with age (Duncan and Flint, 2013). The barrier can be compromised by multiple mechanisms. Proteins of tight junctions are reduced in the gut of old rats (Ren et al., 2014) suggesting that changes in the physical cell-to-cell adherence can contribute to microbial entry. On the other hand, genes involved with mucus production and the thickness of the protective mucus layer is reduced in old mice (Gebert et al., 2020; Sovran et al., 2019), possibly promoting access of microbes. While the intestinal "leakiness" to solutes is not increased (Saltzman et al., 1995; Valentini et al., 2014), bacterial entry and inflammation in both colon and small intestine is indeed increased with age (Sovran et al., 2019; Elderman et al., 2017; Steele et al., 2014). Moreover, aging may be associated with excessive production of proinflammatory cytokines (Steegenga et al., 2012), particularly in the distal ileum (Gebert et al., 2020; Man et al., 2015). Intestinal epithelium also continuously monitors the intestinal content and secretes antimicrobial peptides capable of influencing the microbiome composition (Ayabe et al., 2000). In fact, the reported absorptive dysfunction is likely to in part reflect the bacterial over growth associated with aging (Holt, 2001, Riordan et al., 1997). Aging is also associated with microbial dysbiosis that can induce permeability and induce inflammation (Thevaranjan et al., 2017), and aging linked complications such as insulin resistance, and the overall frailty are associated with alterations in the microbiome (Claesson et al., 2012; De Bandt et al., 2011). Interestingly, number of cells that produce antimicrobial peptides such as defensins and cryptdins – called Paneth cells – is increased in old intestines (Gebert et al., 2020; Moorefield et al., 2017; Nalapareddy et al., 2017; Pentinmikko et al., 2019), but whether this indicates impairment of Paneth cell function with age or possibly a secondary adaptation to the altered microbiome is not known.

The compromised ability to isolate microbes, the resulting inflammation, and the higher incidence of ulceration (Lewis, 2000) in the elderly requires robust epithelial repair and regeneration. Moreover, pathogen induced gastroenteritis and diarrhoea can acutely and critically challenge the repair capacity of old and frail individuals (Majowicz et al., 2010; Marshall and Bruggink, 2011; Tate et al., 2012). Finally, intestinal epithelium proliferates actively, making it particularly damage prone to common chemotherapeutic agents and radiation. Even though intestinal lining has impressive renewal capacity due to its stem cells, the ability to repair severe damage is reduced with age. Potten was the first to show unequivocally that upon irradiation 6–7 month old mice recover quicker than 28–30 month old mice (Martin et al., 1998). In the elderly humans the decline in intestinal regenerative capacity reduces their tolerance of chemotherapeutic and radiation therapy (Margalit et al., 2011), impacts dosing during cancer treatments (Chang et al., 2017; Browner, 2020), and although old patients would benefit from chemotherapy they are less likely to be administered due to risk of comorbidities (Karaca et al., 2018; Kim et al., 2016).

Taken together, both protective and regenerative function of intestine declines with age with clinically relevant consequences.

3. Intestinal stem cells

Intestinal stem cells are the subject of many excellent reviews (Barker, 2014; Beumer and Clevers, 2016; Gehart and Clevers, 2019) and this issue has a dedicated review on their aging related changes by Jasper (2020). We describe stem cells here only briefly and in the context of their niche.

Throughout the small intestine, epithelium is compartmentalized to food absorbing finger like protrusions, villi, and invaginations called crypts of Lieberkühn. New cells are constantly made by actively cycling intestinal stem cells (ISCs) that are marked by expression of Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) (Barker et al., 2007), and located at the bottom of crypts. By dividing approximately once a day, $Lgr5^+$ cells produce progenitors named transit-amplifying (TA) cells. TA cells proliferate few times to expand the cell pool before differentiating and exiting the crypt. In the small intestine, ISCs are wedged between specialized secretory cells, Paneth cells, that are post-mitotic and have both immune and niche functions (see 4.1). In addition to multiple rapidly dividing $Lgr5^+$ cells in each crypt, a less abundant and slower cycling cell type is present in the crypts above the crypt bottom that is filled with Paneth cells and ISCs. These cells are often named +4 cells due to their location in crypts, and they express various markers associated with stemness (Hopx, Tert, Bmi1, Lrig1) (Spit et al., 2018). Experimental data indicates, that these more quiescent cells are able to regenerate the intestine upon dramatic loss of actively cycling ISCs (Buczacki et al., 2013; Metcalfe et al., 2014; Tian et al., 2011; Barriga et al., 2017).

3.1. Epithelial cell plasticity

Interestingly, more differentiated cells within the crypt are also able to compensate for the loss of ISCs. $Dll1^+$ secretory precursor cell, Paneth cells, and $Alpi^+$ enterocyte precursors have all been shown to replenish the ISC pool and reconstitute the differentiated epithelium if ISCs are lost (Roth et al., 2012; Tetteh et al., 2016; van Es et al., 2012). Permissive chromatin state in the crypt cells likely underlines the plasticity of these progenitor cells (Kim et al., 2014). As epigenetic alterations are seen in other aged tissues (Zhang et al., 2020), changes in the chromatin state of intestinal precursors could potentially impact intestinal plasticity and repair with age.

The remarkable plasticity of crypt cells highlights two features of the ISC environment. First, the surrounding niche likely induces the dedifferentiation of cells that can occupy the niche location after ISCs are lost. Second, cellular architecture, chessboard pattern of ISCs and Paneth cells, is restored via communication between the ISCs and other cells of the crypt. As this self-organizing ability involves ISCs, they can in a way be considered as an active part of their own niche. Whether aging affects the self-organizing capability of crypt cells is not directly tested, but stem cells in old crypts rearrange slower after cell loss (Choi et al., 2018). Moreover, the imbalanced ratio of Paneth and ISCs at the base of old crypts (see 4.1) suggests that intercellular interactions are altered during aging.

3.2. Differentiation of aged ISCs

Once ISC progeny exit the crypt bottom they begin to differentiate towards one of the epithelial cell lineages. Three major cell signalling pathways, Wnt, Notch, and MAPK, are able to dictate the lineage commitment of the differentiating ISCs (Basak et al., 2017; Yin et al., 2014) (reviewed in (Gehart and Clevers, 2019)). While high activity in all three maintains ISC fate, reduction in Notch results to initiation of secretory cell program (Sancho et al., 2015). In Notch low cells, high-Wnt and low-MAPK promotes Paneth cells, and low-Wnt drives Goblet and Enteroendocrine cell differentiation. MAPK activity dictates between the Goblet and Enteroendocrine lineages.

Several reports have indicated changes in the cellular composition of intestinal epithelium of aged mice (Gebert et al., 2020; Moorefield et al., 2017; Nalapareddy et al., 2017; Pentinmikko et al., 2019; Igarashi et al., 2019; Mihaylova et al., 2018). Increased number of secretory cell lineage has been reported in aged mouse and human tissues. Nalapareddy and colleagues reported increased number of Paneth and Goblet cells in the old mouse intestine (Nalapareddy et al., 2017). Igarashi and colleagues noted increase in Goblet and Enteroendocrine lineages and reduced number of ISCs (Igarashi et al., 2019), while Gebert and colleagues reported reduced Goblet cells in the distal ileum (Gebert et al., 2020) and Sovran and Eldermann reported reduced Goblet cell numbers

in the colon (Sovran et al., 2019; Elderman et al., 2017) suggesting region specific phenotypes of intestinal aging. Moreover, Mihaylova and colleagues reported reduction in ISCs but unaltered frequencies of Goblet and Enteroendocrine cells in crypts (Mihaylova et al., 2018). Moreover, they observed increased number of Paneth cells. Pentinmikko and colleagues recently noted a reduction in ISC number that was accompanied by increase in the number of Paneth cells (Pentinmikko et al., 2019). On the contrary, Moorefield and colleagues reported increased number of ISCs and Paneth cells in crypts of old mice (Moorefield et al., 2017). Discrepancies between these reports can reflect the differences in mouse lines, environmental factors of the housing facility, the age of analysed animals, as well as location of the analysed area. Gebert and colleagues conducted a comprehensive proteomic analysis of 12 small intestinal regions and discovered clearly region specific aging patterns (Gebert et al., 2020). Regional phenotypes of the aged intestinal epithelium extend also to villus morphology as villous blunting is reported in the jejunal region (He et al., 2020) while increased length is observed in the distal small intestine (Gebert et al., 2020). However, old epithelium seems to have an overall bias to produce more secretory cells (Fig. 1). The increased number of Paneth cells is one of the most consistent changes in the intestinal crypt, and also observed in human tissue (Pentinmikko et al., 2019). While the functional outcome of secretory lineage bias is not known, it might impact the absorption capacity as discussed above. Given the plasticity of differentiated cells upon acute loss of ISCs, changes in differentiation may potentially also influence tissue regeneration.

4. Intestinal stem cell niche

The large number of actively cycling ISCs are tightly controlled by the surrounding microenvironment, the stem cell niche. Niche in the small intestine provides protection from the harsh luminal environment of the gut and supplies ISCs with growth factors necessary for maintenance of stemness. The niche is not just a passive safe harbour for stem cells, but can guide stem cell behaviour remarkably dynamically. However, the role of niche in age-related functional decline has only recently been addressed. We discuss the age-associated alterations separately for various components of the intestinal niche.

4.1. Epithelial niche

ISCs reside at the bottom of the crypts, intermingled between specialized secretory cells that participate in their maintenance (Rothenberg et al., 2012; Sasaki et al., 2016; Sato et al., 2011). These are called Paneth cells in the small intestine, whereas in the colon Deep Crypt Secretory (DCS) cells possess similar niche function (Rothenberg et al., 2012; Sasaki et al., 2016). The intimate contacts between Paneth and ISCs allows efficient signalling between these cells. Paneth cells are known to produce Wnt3, Dll4 and Egf to maintain stem cell capacity in ISCs (Sato et al., 2011). In addition, Paneth cells actively create part of the metabolic niche by secreting lactate that is used by the ISCs (Rodriguez-Colman et al., 2017). Interestingly, increased number of Paneth cells in old mice and humans have been reported by several laboratories (Moorefield et al., 2017; Nalapareddy et al., 2017; Pentinmikko et al., 2019; Mihaylova et al., 2018). Simultaneously, majority of these reports highlight reduced or equal number of ISCs, insinuating that the niche function of aged Paneth cells is compromised (Fig. 1).

Organoid culture technology (Sato et al., 2011; Sato et al., 2009) has proven its usefulness in assessing the regenerative capacity of intestinal epithelium. Isolated crypts derived from old mice and healthy human donors, show reduced capability to grow organoids (Nalapareddy et al., 2017; Pentinmikko et al., 2019). As functional crypts contain both ISCs and their epithelial niche, generation of organoids from isolated ISCs and Paneth cells provides an assay to dissect their respective roles in the aging phenotype (Pentinmikko et al., 2019). Such coculture assays have illuminated that the function of both cell types is reduced with age. Strikingly, even young ISCs show reduced organoid forming capacity when cultured together with the old Paneth cells, suggesting alterations in signals emanating from the old Paneth cells (Pentinmikko et al., 2019). Notably, reduction in the crucial Wnt pathway activity has been indicated to underlie the reduced regenerative capacity of old epithelium. Nalapareddy and colleagues detected reduced Wnt signature in aged ISCs (Nalapareddy et al., 2017). Utilizing in vitro organoid cultures, they were able to show that supplementation of excess Wnt ligands improved regenerative capacity of old epithelium. Moreover, their analysis indicated reduced Wnt3 expression in the epithelial niche. Pentinmikko and colleagues found no change in Paneth cell Wnt expression, but identified a novel niche factor, secreted Wnt inhibitor Notum, to be produced at higher level in old Paneth cells (Pentinmikko

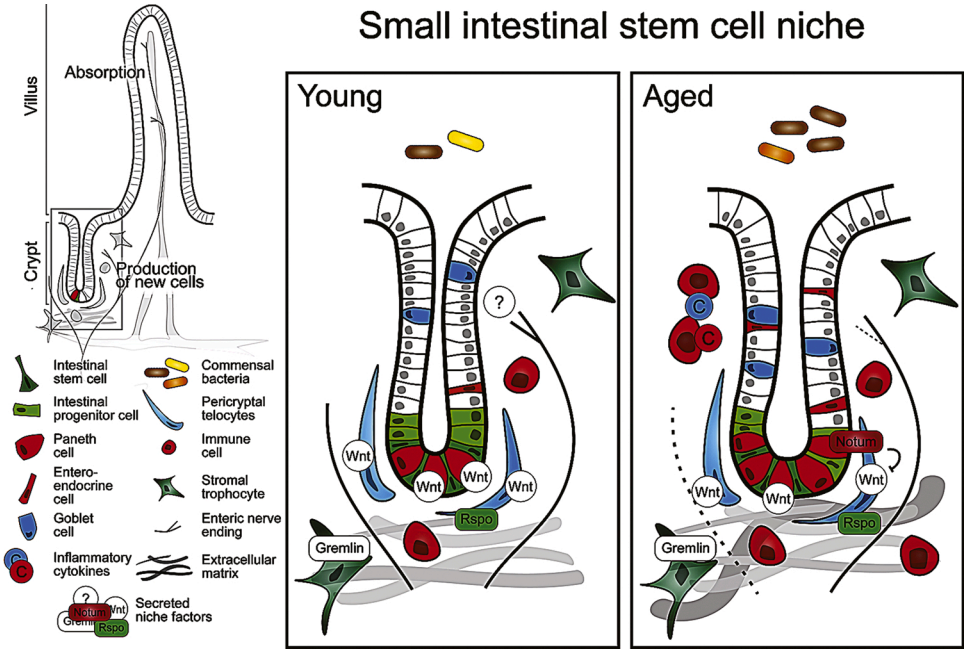

Fig. 1. Age-associated changes in the small intestinal stem cell niche. Intestinal stem cells (ISC, dark green) are maintained by their niche composed of multiple cell types and acellular components. In old individuals, stem cell niche undergoes alterations that reduce the regenerative capacity of intestinal epithelium. Proportion of secretory cell types, such as Paneth cells (red), increase with age. Decrease in Wnt signaling activity due to production of Wnt inhibitor Notum and reduced amount of Wnt ligands affects ISC function. Changes in the microbial content of the gut lumen as well as cytokine profile in the microenvironment likely contribute to altered behavior of ISCs. Mechanical properties and signaling of the enteric nervous system in the tissue changes, that likely alters the functionality of the epithelium. Wheter the number or function of stromal trophocytes and pericryptal telocytes is altered is not known (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

et al., 2019). Importantly, inhibition of Notum activity with a small molecule ABC99 was able to improve intestinal regeneration of old animals when treated with chemotherapy agent 5-Fluorouracil.

Expression of Notum is regulated by the activity of canonical Wnt-signalling, placing it to a negative feedback loop (Kakugawa et al., 2015). However, Wnt-activity is reduced in the old niche (Nalapareddy et al., 2017) suggesting alternative ways of Notum regulation in old Paneth cells. In old animals, Paneth cells experience high mTORC1 activity which alleviates Ppara mediated inhibition of Notum (Pentinmikko et al., 2019). Therefore, activity of the key nutrient sensor mTORC1 in the niche, can attenuate ISC function in the old intestine. Correspondingly, reduction in nutrient intake promotes the ISC supporting function of Paneth cells via mTORC1 inhibition (Yilmaz et al., 2012). While the lifespan extending Caloric Restriction (CR) increased self-renewal of ISCs via Paneth cells in a paracrine fashion, the dietary effects in young animals were mediated by an ectoenzyme Bst-1 instead of reduction in Notum - whose expression in young tissue is anyway minimal. Similarly, knockout of Bst-1 in young Paneths did not recapitulate the whole aging phenotype (Pentinmikko et al., 2019; Yilmaz et al., 2012). Therefore, low (CR) and high (Aging) activity of mTORC1 guides Paneth cells to enhance or attenuate ISC function via paracrine mediators Bst-1 and Notum respectively. Whether dietary interventions promote regeneration via Notum regulation in the aging niche is not yet addressed. Moreover, whether the metabolic niche function of Paneth cells via lactate production is changed during lifespan extending dietary restrictions or aging remains to be studied.

Taken together, Paneth cells compose the main part of epithelial niche for ISCs and their function is altered with age. This attenuates the regenerative capacity of the tissue. Whether the decreased Wnt-activity in old ISCs contributes to organismal aging and to related complications in other organs is yet unresolved. However, low Wnt activity may contribute to tumor initiation, as shown in mouse model of intestinal adenomas (Huels et al., 2018).

The role of other epithelial cell types in the maintenance of ISCs or as reserve stem cells upon acute damage in old intestine (see above) is not known. Hormone producing Enteroendocrine cells are capable of long-distance signalling and also respond to nutritional status of the animal (Gribble and Reimann, 2019). Moreover, upon experimental loss of Paneh cells, Tuft cells together with Enteroendocrine cells highjack the niche function of Paneth cells and are able to support functional ISCs (van Es et al., 2019). Therefore, also other epithelial cells can regulate ISCs, and may contribute to intestinal aging.

4.2. Subepithelial mesenchymal cells

While Paneth and DCS cells are an important source of Notch ligands regulating ISCs number and differentiation, subepithelial stromal cells provide substantial amounts of other stem cell regulating niche factors. Mesenchymal cells that closely envelope the crypt epithelium are particularly relevant in this regard, and generate for example the differentiation regulating gradient of bone morphogenetic protein (BMP) -signaling (McCarthy et al., 2020) by localized secretion of BMP-ligands (He et al., 2004) and their antagonists, such as Gremlin1 (Fig. 1) (McCarthy et al., 2020; Kosinski et al., 2007). Recently, multiple marker genes were shown to identify subsets within these pericryptal cells, and to demonstrate the critical role they have in maintaining ISCs by production of Wnt-ligands (Degirmenci et al., 2018; Greicius et al., 2018; Shoshkes-Carmel et al., 2018). Moreover, these cells produce R-spondins, the ligands of Lgr-family receptors, which enhance Wnt-signals in the ISCs (de Lau et al., 2014) and maintain self-renewal (Yan et al., 2017). Whether composition of these important mesenchymal cells in the niche changes with age is not known. Suggesting that epithelial aging is not due to changes in pericryptal fibroblasts, Nalapareddy and colleagues did not observe changes in expression of Wnt2b - the main canonical Wnt-ligand produced by the stroma (Nalapareddy et al., 2017; Farin et al., 2012). Instead, they noted reduction in the mesenchymal production of Wnt3 in old the intestine, but majority of Wnt3 is produced by Paneth cells suggesting that stromal Wnt3 has only modest impact on epithelial growth (Gregorieff et al., 2005). Interestingly, the lifespan extending inhibitor of mTORC1, Rapamycin, improved ISC and Paneth cell function in old animals, and altered the expression of stromally produced Wnt-ligands (Pentinmikko et al., 2019). Whether this underlines some of the anti-aging effects of systemic mTORC1 inhibition remains to be studied.

Senescent, damaged but viable and irreversibly post-mitotic, cells accumulate with age (Gorgoulis et al., 2019). These cells often display the so-called Senescence Associated Secretory Phenotype (SASP), associated with secretion of a plethora of proinflammatory and growth regulating factors (see 4.4). Such signals produced in the stem cell niche could dramatically impact tissue renewal. While eradication of senescent cells by 'senolytic' agents has emerged as one of the most promising strategies to rejuvenate tissue stem cell function (Chang et al., 2016), relatively little is known on impact of senescent cells in the intestine. Cell culture experiments suggest, that stromal SASP can influence colon cancer growth, possibly contributing to increased tumor incidence among the elderly (Guo et al., 2019). However, whether the number of SASP cells increases in the old ISC niche is not well understood. While number of β-galactosidase (β-gal) positive cells, a proxy for senescence, was not increased in the aged crypts (He et al., 2020), the analyzed mice were only 16 months of age and focus was on β-gal positive cells of the epithelium. Even if ISC senescence is not involved with intestinal aging, potency of SASP cells warrants detailed studies on their potential accumulation in the pericryptal mesenchymal niche.

4.3. Niche resident enteric nerve cells

The stroma, in to which the epithelium invaginates, also inhabits enteric nerve cells that are critical for the intestinal function. Whereas myenteric plexus controls the contractions of the two muscle layers and peristalsis, submucosal enteric nerves are connected with the epithelium, and mediate sensory inputs from the enteroendocrine cells. Enteroendocrine cells can detect changes in the nutrient status, pH, and even respond to mechanical cues sent by the passing food (reviewed in (Gribble and Reimann, 2016; Worthington et al., 2018)). Subsequently, enteroendocrine cells stimulate basal nerve ends by secreting neurotransmitters, for example 5-HT, and initiate a reflex in the submucosal plexus that regulates water balance in the lumen, and gut motility via the myenteric plexus (Xue et al., 2007).

The abundance of enteric nerve cells has been reported to decrease with age in mouse and rat models as well as in some human studies (reviewed in (Saffrey, 2013)). While impact of aging on gut motility is debated, reduction in enteric nerves is suggested to participate in the functional decline of the aged intestine (Saffrey, 2013; Patel et al., 2017). Subepithelial enteric nerve cells may also regulate ISC function. Puzan and colleagues noticed that coculture of ISCs with enteric nerve cells promoted differentiation towards the endocrine lineage (Puzan et al., 2018). Moreover, the epithelial barrier function was improved, suggesting that reduced nerve innervation in the old intestine could contribute to the leakiness of the aged gut (Puzan et al., 2018; Parrish, 2017). The precise mechanism how enteric nerve cells affect ISC function is not fully clarified. However, ISCs express receptors for neurotransmitters and upon stimulus may alter their proliferative state (Davis et al., 2018). Moreover, ISC function is impaired upon blockade of cholinergic signalling from the nerve cells resulting to precocious production of tuft cells in the niche (Middelhoff et al., 2020). Furthermore, enteric glial cells can contribute to the colon cancer growth by secreting factors in response to tumour cell produced signals (Vales et al., 2019). These results indicate, that enteric nerve cells communicate with the epithelial ISCs and niche cells to modify intestinal function (Fig. 1). How much aging changes these regulatory circuits and how reduction in subepithelial nerves contributes to epithelial aging is not known.

Interestingly, some reports show that CR may prevent the age-

associated reduction in the enteric nerve cells (Saffrey, 2013). CR also improves the barrier function in *Drosophila* intestine (Akagi et al., 2018), similarly to the factors produced by the enteric nerve cell (Puzan et al., 2018). It is therefore possible that some of the beneficial effects of CR for the barrier function during aging could be driven via enteric nervous system. However, so far evidence from mammals is not consistent with this notion. Ma and colleagues reported that CR does not prevent loss of barrier function of the old intestine in laboratory rats (Ma et al., 1992), whereas Ott and colleagues reported beneficial effects in obese human patients (Ott et al., 2017). CR mimetic Rapamycin has been shown to improve barrier function in *Drosophila* intestine suggesting that possible CR effects are affecting via mTORC1 (Schinaman et al., 2019). On that note, activation of low-energy sensor AMPK with Metformin has shown similar effect of barrier function in mouse models and diabetic human patients (Deng et al., 2018). In any case, whether CR, or other metabolic intervention can improve the barrier function of old epithelium via enteric nerve cells is not directly studied, and differences between model organisms and humans should be considered for conclusions.

4.4. Immune cells, inflammation and microbiota of the niche

As discussed above, the intestinal microbiome, barrier function, and prevalence of inflammatory cytokines are altered during aging (Fig. 1). Suggesting that the myriad age-associated changes in the microbial composition (reviewed in (O'Toole and Jeffery, 2015; Biagi et al., 2017)) may impact the longevity of the whole organism in multiple ways, a mutant screen in a single species (*E.coli)* already identified 29 genes whose deletion extends lifespan of their *C. elegans* hosts (Han et al., 2017). However, it remains unclear if the alterations in microbiota are a consequence or possibly also a partial cause of aging in mammals.

Regarding the intestinal niche function, the microbiota produces multiple metabolites that can influence stem cells (reviewed in (Xing et al., 2020)). Among the best studied is butyrate, a short-chain amino acid that was originally reported to increase intestinal proliferation (Sakata, 1987), but later to specifically inhibit proliferation of intestinal stem cells via HDAC inhibition and Foxo3 (Kaiko et al., 2016). Interestingly, Kaiko et al. also found that the shape of the colonic crypt positions stem cells "behind" the butyrate consuming differentiated cells, suggesting that the anatomy of the gastrointestinal tract - and of the stem cell niche - may reflect co-evolution with the microbiota. However, considering the inhibitory effect of butyrate on stem cell function, and that microbial and dietary alterations result in reduction of butyrate levels during aging (Biagi et al., 2010), modulation of butyrate levels as the means to increase intestinal health in elderly remains a complicated issue. Gallic acid is emerging as another microbially produced metabolite with potentially striking effects on ISCs. In a model where gastrointestinal tumors develop from ISCs, gallic acid is responsible for blunting the tumor suppressive ability of mutant p53, and thereby implicates changes in the microbiome along the gastrointestinal tract as the cause for differing tumor incidences in the small and large intestine (Kadosh et al., 2020). Suggesting that such mechanism could also influence stem cells during aging, gallic acid boosts tumorigenesis via Wnt signalling by preventing p53 from blocking TCF4-chromatin interactions (Kadosh et al., 2020). However, further studies are needed to illuminate whether alterations in the microbiome contribute to the reduction in Wnt-activity and ISC decline with age via gallic acid production. Finally, lactate produced both by Paneth cells (Rodriguez-Colman et al., 2017) and by microbiota (Lee et al., 2018) promotes ISC self-renewal and divisions. Interestingly microbially produced lactate appears to promote also the non-metabolic niche functions of Paneth cells and results in increased Wnt-signaling in stem cells (Lee et al., 2018). Taken together, as the microbiota has many metabolic interactions with the ISCs and other parts of the niche, the complex evolution of microbiota during aging is likely to impact stem cell function via multiple mechanisms. Targeting the microbiota by selective diets and probiotics may therefore provide low-risk avenues to promote intestinal health in the elderly.

Aging is associated with general increase of inflammatory signature in most tissues, and as intestine houses majority of the microbiota, it is tempting to speculate how the combination microbial composition, barrier function and "inflammaging" are linked, and influence aging of the whole organism. Specifically in the small intestine, increase in inflammation related proteins is one of the most consistent age-induced changes irrespective of the analysed region (Gebert et al., 2020). Suggesting causal links between the altered microbiome and the age-associated inflammation, transplantation of microbiota from old animals increases inflammatory status in young recipient mouse (Fransen et al., 2017). Moreover, profiles of cytokines IL-6 and IL-8 in human centenarian intestines correlates with their gut microbiota composition (Biagi et al., 2010). Furthermore, age-associated accumulation of SASP-cell could result to local increase of inflammatory signals. Setting off a vicious cycle, cytokines attract additional tissue resident and circulatory lymphocytes, but they can also directly affect the ISC function. Lindemans and colleagues reported that IL-22 can promote regenerative function of ISCs independent of the stromal myofibroblasts or Paneth cells (Lindemans et al., 2015). IL-22 is commonly produced by the innate lymphoid cells, suggesting that age associated myeloid bias might reduce the availability of IL-22 in the old niche (de Haan and Lazare, 2018; Sonnenberg and Artis, 2015). On the other hand, proinflammatory cytokines can stimulate differentiation while regulatory T-cells are able to repress it (Biton et al., 2018). Despite the strong correlations between age associated changes in microbiota, intestinal barrier function, and inflammatory cells and cytokines, their causal relationships are not fully understood. However, targeting the inflammation in the niche may provide attractive intervention strategies for promoting function of old intestine. Whether such result could be obtained with senolytics targeting inflammation inducing cells is not yet known.

4.5. Acellular niche

The systemic environment changes with age dramatically. Blood circulating components serve as biomarkers of age, but some of these may contribute to local decline in tissue function (Castellano et al., 2017; Lehallier et al., 2019; Villeda et al., 2011). Heterochronic parabiosis experiments have shown, that young plasma is able to rejuvenate functionality of aged stem cells in the muscle and brain (reviewed in (Conboy and Rando, 2012)). While not experimentally shown, systemic milieu likely affects also the highly vascularized intestinal epithelium. Among older humans, circulating glucose levels are higher possibly due to reduced function of pancreatic beta-cells (Chia et al., 2018). Therefore, nutrient sensing cells of the epithelium, such as Paneth and Enteroendocrine cells, might behave differently in the aged environment. Whether these alterations affect their ISC supporting function is not known.

Composition, topology and mechanical properties of the extracellular matrix (ECM) can regulate stem cell function (Bao et al., 2017; Engler et al., 2006; McBeath et al., 2004; Swift et al., 2013; Totaro et al., 2017). In epithelial tissues, specialized layer of ECM called the basement membrane forms the mechanical basis for epithelium, but it can also regulate cell proliferation and differentiation (Mahoney et al., 2008). If a key component of the basement membrane, laminin, is removed, intestinal epithelium degenerates quickly (Fields et al., 2019). Thus, ECM components can affect the ISC function and participate in the formation of the niche.

The undulating topology of intestinal epithelium, crypts and villi, increases absorptive surface area and keeps ISCs protected from the luminal content. Moreover, the topology guides differentiation during intestinal development (Shyer et al., 2015), and due to apical constriction, the niche topology in adult intestine is extremely curved (Sumigray et al., 2018). Progenitor cells that exit the crypt undergo a dramatic but transient morphological change that influences the correct spacing of

villi and crypts (Sumigray et al., 2018). How much the curvature at the bottom of crypts affects ISC function is not known. However, columnar shape of the crypt resident cells leads to separation of the two daughter cells after cells division (McKinley et al., 2018), insinuating, that shape might participate in the differentiation kinetics mediated by the lateral-inhibition machinery (Gehart and Clevers, 2019; Sancho et al., 2015).

Whether changes in the ECM composition, mechanical properties or tissue topology affect the stem cell behaviour in the aging intestine is currently not known. Suggesting that ECM provided adhesion may change with age, stem cells rearrange slower in old crypts after laser ablation of a single cell (Choi et al., 2018), but this may also reflect stem cell intrinsic changes. On a tissue level, mechanical properties of the human colon have been reported to change, while some studies do not observe differences between the age groups (Christensen et al., 2015; Watters et al., 1985). However, inflammation in the human intestine increases stiffness (Stewart et al., 2018), suggesting that under some age-associated pathological conditions, mechanical properties of the tissue are changed. Interestingly, stiffening of the stem cell niche in the aging brain has been shown to reduce the function of neural progenitor cells (Segel et al., 2019). Therefore, altered mechanical properties in the aged ISC niche might change the capacity to regenerate or to produce functional tissue via the mechanosensing YAP/TAZ pathway which is reported to modulate cellular fate and regenerative capacity of the ISCs (Barry et al., 2013; Gregorieff et al., 2015).

5. Conclusions

Intestinal function declines with age, which increases infections and possibly contributes to malnutrition. Moreover, intestinal regenerative capacity declines with age and poses a challenge for common first-line cancer therapies. As mucosal health and regenerative responses depend on ISC function, age-induced alterations in the stem cell regulating niche can dramatically impact intestinal health.

Recent findings on the epithelial Paneth cells highlight the impact of aging niche on tissue function. Inhibition of the age-induced niche factor Notum presents a proof of principle for strategies aiming at increasing tissue function via niche targeting. However, current knowledge on aging of the intestinal niche is limited. The role of stromal myofibroblasts in homeostasis and regeneration of the young intestine is undisputable, but whether they contribute to alterations of old intestine is not known. Similarly, the niche functions of enteric nerve cells and immune cells are subject to ongoing studies, but their role in stem cell biology during aging is not yet understood. Providing exciting opportunities, removal of senescent cells with senolytics can have system wide beneficial effects, but impact on intestine is still unclear.

Organoid cultures have allowed reductionistic and mechanistic studies on the impact of aging on ISCs and their epithelial niche. More complex coculture systems with nonepithelial niche cells and tunable biophysical properties are necessary to conduct decisive studies on other compartments of the niche. Moreover, organoids recapitulate the regenerative growth, but in vitro assays addressing homeostatic renewal and responses in the intestine are still lacking.

Systemic signals and the whole-body metabolism change with age and may impact stem cells directly or via the niche including the microbiota. In the intestine, long term dietary restriction regulates stem cells via the niche (Pentinmikko et al., 2019), but acute starvation influences stem cells via intrinsic mechanisms (Mihaylova et al., 2018). It is therefore likely that lifestyle factors such as diet and exercise influence intestinal health and function via multiple mechanisms. Understanding of causal relationships and order of events in intestinal aging will allow development of strategies targeting the actual aging process, but already now the identified age-associated alterations in niche interactions offer opportunities for experimental approaches that rejuvenate intestinal stem cells and tissue function.

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