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Stem cell pathology

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Abstract

Rapid advances in stem cell biology and regenerative medicine have opened new opportunities for better understanding disease pathogenesis and development of new diagnostic, prognostic and treatment approaches. Many stem cell niches are well defined anatomically, thereby allowing their routine pathological evaluation during disease initiation and progression. Evaluation of consequences of genetic manipulations in stem cells, and investigation of the roles of stem cells in regenerative medicine and pathogenesis of various diseases, such as cancer, require significant expertise in pathology for accurate interpretation of novel findings. Therefore, there is an urgent need for developing stem cell pathology as a discipline to facilitate stem cell research and regenerative medicine. This review provides examples of anatomically defined niches suitable for evaluation by diagnostic pathologists, describes neoplastic lesions associated with them, and discusses further directions of stem cell pathology.

Keywords

cancer; mouse models; disease pathogenesis; stem cell biology; stem cell niches; tissue evaluation

Introduction

Stem cells are defined by two key properties: their ability to self-renew and to produce differentiated progeny (1). There are two main types of stem cells: embryonic stem cells and adult or tissue stem cells. Embryonic stem cells are pluripotent cells derived from the inner mass of blastocysts. Adult stem cells are found in various tissues, such as mammary, prostate, intestinal, follicular and ovarian surface epithelia, blood, and tissues of musculoskeletal and nervous systems. These cells sustain turnover and repair throughout life and their potency is limited to cells of that tissue. Tissue stem cells reside in a microenvironment known as the stem cell niche. Niche components interact with stem cells and play a role in their protection and cell fate decisions (2; 3).

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Stem cell research covers a broad variety of topics, ranging from fundamental aspects of embryonic stem cell regulatory programs, to the basic biology of adult stem cells, to clinically-relevant aspects of tissue regeneration and disease pathogenesis. Recent years have been marked by the development of revolutionary approaches in stem cell research, such as establishment of embryonic stem cells, somatic nuclear transfer, induced cell reprogramming and interspecies chimeras (4–6). Numerous breakthroughs have been also observed in the area of translational medicine, including in vitro fertilization, tissue and organ regeneration, and uncovering the pathogenesis of many diseases, such as cancer, diabetes, neurodegeneration and many others (7; 8).

Advances in genomics, as well as the development of sophisticated tools for manipulating the vertebrate genome, have greatly enhanced our ability to develop robust animal models necessary for our understanding of fundamental mechanisms controlling stem cells and their niches (9–11). Recent development of accurate gene editing technologies has further accelerated development of relevant model systems in various species. Studies of disease pathogenesis are increasingly reliant on cell fate mapping by lineage tracing analysis in genetically modified mice (1; 3). This approach has definitively shown that many stem cell niches are well defined anatomically, thereby allowing their pathological evaluation during disease initiation and progression (12–14). Such studies require significant expertise in pathology for accurate interpretation of novel findings. Various topics of regenerative medicine and stem cell biology, such as in situ contributions of stem cells to normal and pathological regeneration, and organismal reactions to transplantations of various bioengineering constructs, remain relatively unsupported by pathologists. At the same time, stem cell research programs rarely seek out the participation of pathologists. Therefore, there is an urgent need for developing stem cell pathology as a discipline able to match the rapid advances of stem cell research and regenerative medicine. We define **stem cell pathology as an area of pathology which focuses on studying the roles of stem cells in disease pathogenesis, identifies pathological consequences of stem cell transplantation, and evaluates side effects of genetic and epigenetic manipulations of stem cells.**

Given appropriate training and having accurately collected and oriented material, pathologists may be able to provide their informed evaluation of potential stem cell niche defects even on routine sections stained with hematoxylin and eosin. Such preliminary evaluation can be further corroborated by immunohistochemistry, thereby providing a basis for further functional studies performed in collaboration with stem cell researchers.

A number of immunohistochemical markers of putative stem cells have been identified. Also, functional properties of stem cells, such as enzymatic activity (e.g., ALDH) or compound efflux (e.g., side population detected by exclusion of the DNA-binding dye Hoechst 33342) are used for detection of putative stem cells (Table 1). Unfortunately, very few, if any, of those markers are uniquely specific for stem cells. For example, expression of the detoxifying enzyme aldehyde dehydrogenase (ALDH) family of proteins and their corresponding enzymatic activity have been identified as useful markers of stem/progenitor cells in mammary, prostate, colon, hematopoietic, neural, ovarian surface epithelium and mesenchymal cell lineages (14–18). However, this marker is also highly expressed in the liver (19) and theca cells of the ovary (14). Another approach for detecting putative stem

cells is to label slowly cycling cells based on the assumption that many stem cells exhibit slow cycling. Unfortunately, it has become clear that some stem cells, such as leucine-rich-repeat-containing G protein-coupled receptor 5 (LGR5)⁺ cells in the intestine are highly proliferative (1). Other approaches based on functional characterization of cells ex vivo such as sphere and organoid formation are also broadly used (20). Although valuable, such approaches may not always reflect biological behavior of stem cells in vivo. This problem is being addressed by the development of orthotopic transplantation assays and by the introduction of refined genetic tools for lineage tracing, thereby allowing evaluation of stem cell behavior and fate in their native environment (1; 3). Both approaches have their own challenges (Table 1), but currently represent the golden standard of stem cell identification and characterization.

This review provides some examples of anatomically defined adult stem cell niches and shows their contributions to pathological processes, such as cancer. It should be noted that the concept of cancer-prone stem cells can be traced to the work of Dr. Julius Cohnheim (21), a pathologist, who proposed that malignancies may arise from “embryonic rests”, preceding the currently accepted notion that many cancers arise from stem cells and their niches by nearly a century (12; 22; 23).

Gastric epithelium

The stomach can be divided into three anatomically distinct regions. The cardia, most proximal to the esophagus; the corpus (fundus/body), which is the main part of the stomach; and the pylorus, the distal part of the stomach linking it to the duodenum (Figure 1a).

Cardia

The cardia includes the “epithelial transitional zone” (TZ) connecting the squamous and glandular epithelia (Figure 1b). In humans a TZ delineates the junction between the esophagus and stomach. In mice, the same TZ divides squamous and glandular regions of the stomach. In mice, expression of LGR5 marks the distinct stem cell population which resides at the base of the first gland and contributes to homeostasis of the epithelium in this region (Figure 1c and d) (24; 25). Barrett’s esophagus (BE), which is an intestine-like columnar metaplasia occurring in the stratified squamous epithelium of the distal esophagus, has been considered the precursor of gastro-esophageal adenocarcinoma over the past 3 decades (26). This precancerous lesion has been suggested to result from the migration of stem cells and their progeny from the first gland of the TZ toward squamous epithelium in response to the gastroesophageal reflux (25; 27). Our studies indicate that inactivation of tumor suppressor genes *Trp53* (*p53*) and *Rb1* (*Rb*) in LGR5⁺ stem cells of the TZ leads to early dysplastic lesions progressing to metastatic gastric cancers (Figure 1e and Fu et al., unpublished observations).

Corpus

The glands of the corpus region are subdivided into four segments, pit (foveolar) surface, isthmus, neck and base, from the top to the bottom of the glands (Figure 1f). The putative stem cell population was first identified in the isthmus region of corpus glands which is

characterized by highly proliferative cells lacking differentiation markers (28). Some studies have suggested that these isthmus stem cells are marked by the transcription factors, Rnt Related Transcription Factor 1 (RUNX1) and Muscle, Intestine and Stomach expression 1 (MIST1) (29; 30). Deregulation of WNT signaling and constitutive activation of oncogenic *Kras* in the isthmus stem cells can induce diffuse-type gastric carcinoma and precancerous foveolar metaplasia potentially progressing to intestinal-type gastric carcinoma, respectively (29; 30). In addition to the isthmus stem cell zone, the corpus glands contain distinct differentiated Troy⁺ chief cells at the gland base, which serve as a “reserve” stem cell population able to give rise to all corpus lineages during injury (31). Some Troy⁺ chief cells express the isthmus stem cell markers MIST1 and RUNX1 suggesting considerable plasticity between stem/progenitor and differentiated cells (29; 30).

Pylorus

The pyloric stem cells are marked by LGR5 expression, located at the base of glands, and contribute to daily epithelial homeostasis (Figure 1g–i) (13). Deregulation of WNT signaling activity by loss of its inhibitor APC can initiate adenoma formation in the pyloric epithelium (13). Additionally, combined inactivation of *Smad4* (TGF- β mediator gene) and *Pten* (well-known tumor suppressor gene) in LGR5⁺ stem cells result in aggressive pyloric adenocarcinoma with muscular invasion (32). Interestingly, inactivation of *p53* and *Rb* in pyloric LGR5⁺ cells results in adenoma formation but is insufficient for the progression to overt malignancy (Figure 1j and Fu et al., unpublished observations).

Beside the LGR5⁺ stem cells, Villin⁺ cells identified at the isthmus of pyloric glands are considered to be rare, quiescent stem/progenitor cells that become highly proliferative in response to inflammatory injury (33). The gastrin receptor, cholecystokinin B receptor (CCK2R), marks the other distinct stem/progenitor cells in the pylorus that are partially overlapping with LGR5-low-expressing (LGR5^{low}) and LGR5-negative (LGR5^{neg}) cells in the isthmus (34). Different from the Villin⁺ stem cells, the CCK2R⁺ isthmus cells are characterized by rapid proliferation and ability to maintain the epithelial renewal for at least 12 months (34). Progastrin, which is a precursor of gastrin secreted by neuroendocrine G-cells in the pylorus, can convert CCK2R⁺ isthmus cells into LGR5^{high} base stem cells and also facilitate pyloric cancer development (34). Additional putative stem cell markers, such as SOX2 and RUNX1, are also expressed by isthmus cells of pyloric glands (30; 35).

Intestinal epithelium

The intestinal epithelium is one of the most regenerative tissues in the body being entirely renewed every 3–5 days (36). This rapid turnover process is fueled by the stem cell components residing close to the base of crypts of Lieberkühn (aka intestinal crypts, Figure 2a). The intestinal stem cells near the crypt base continuously produce rapidly proliferative progenitors called transit-amplifying (TA) cells, which are able to migrate out of the crypts onto villi and simultaneously differentiate into various types of cells responsible for food digestion, nutrition absorption, and disease defense.

Two types of adult intestinal stem cells have been proposed over the past four decades. In the “+4 model” introduced in 1965 (37), the putative stem cells are located at the forth position

(+4) from the crypt base (Figure 2a), which reside directly above the Paneth compartment. These cells were later described as “quiescent stem cells” due to their infrequent or asymmetric division properties (38; 39). The biomarkers, such as B lymphoma Mo-MLV Insertion region 1 homolog (BMI1) (40), Mouse Telomerase Reverse Transcriptase (mTERT) (41), Homeodomain-only protein (HOPX) (42), and Leucine Rich Repeats and Immunoglobulin like Domains 1 (LRIG1) can be used to identify these slow-cycling +4 cells (43).

In the “stem cell zone” model introduced in 1974, active rapidly cycling “Crypt Base Columnar” (CBC) stem cells represent a population of slender columnar cells interspersed between Paneth cells at the intestinal crypt bases (44; 45 and Figure 2a). These CBC stem cells are long-lived and multipotent, and divide once every day to maintain the daily epithelial homeostasis (46; 47). Some biomarkers, including LGR5 (Figure 2b) (48), Achaete Scute-Like2 (ASCL2) (49), Olfactomedin-4 (OLFM4) (50), Prominin 1 (PROM1/CD133) (51), Activated Leukocyte Cell adhesion Molecule (ALCAM/CD166) (52), SPARC Related Modular Calcium Binding 2 (SMOC2) (53), and Tumor Necrosis Factor Receptor Superfamily member 19 (TNFRSF19/Troy) (54) have been used for the identification of CBC cells in intestinal crypts.

Recent cell lineage tracing and ablation experiments support the existence of both the +4 and CBC stem cells. The regular epithelial homeostasis is maintained by the active CBC cells. However, during tissue injury, the damage-resistant +4 cells become activated and contribute to the entire crypt regeneration including restoration of CBC cells that are lost by damage (42; 55).

The adult intestinal stem cell has been thought of as the cell of origin of intestinal cancer for decades. Recently, a variety of cell type-specific marker-driven *Cre* recombinase mouse models suggest that inactivation of tumor suppressor genes, such as *Apc* and *p53* in the intestinal stem/progenitor cells (i.e. LGR5⁺, BMI1⁺, CD133⁺ or LRIG1⁺ cells) is sufficient to initiate multiple tumor formation or epithelial hyperplasia (40; 43 and Figure 2c; 51; 56). However, similar mutations occurring in the differentiated intestinal cells are unable to initiate carcinogenesis. These experiments suggest a greater susceptibility of intestinal stem cells to cancer in comparison to their differentiated progenies.

Ovarian surface epithelium

The ovarian surface epithelium (OSE) undergoes extensive damage and regeneration during ovulation. Using label retention assays based on incorporation of 5-bromo-2'-deoxyuridine/5-iodo-2'-deoxyuridine (BrdU/IdU) and histone-GFP fusion protein retention in H2B-GFP transgenic mice, Szotek and colleagues identified putative stem cells as slowly-cycling cells in the OSE (57). These cells proliferated in response to the estrous cycle, showed enhanced colony forming ability in cell culture, and were able to exclude Hoechst 33342. Another putative OSE stem cell population was reported based on expression of stem cell marker LY6A (SCA-1) (58). Unfortunately, it has been uncertain if these cells have potential for long-term self-renewal and contribute to OSE regeneration in vivo, key features of stem cells. Furthermore, the anatomical location of such cells remained unknown. The

issues have been addressed in a more recent study which identified the OSE-stem cell population able to efficiently form clonal spheres, to display extended self-renewal properties in serial sphere generation assay, and to contribute to OSE regeneration in long-term lineage tracing assays (14). These cells express stem cell markers ALDH1, LGR5, LEF1, CD133, and CK6B. Furthermore, OSE-stem cells have low levels of microRNAs of the miR-34 family (miR-34a, b and c), which negatively regulate stem cell properties of adult stem cells (59). Interestingly, these cells are mainly located in the hilum area of the mouse ovary (Figure 3). This area represents the transitional/junctional zone between the OSE, the tubal epithelium (TE) and the mesothelium. At the same time, lineage tracing analysis in *Lgr5^{EGFP-Ires-CreERT} Gt(ROSA)26Sor^{tm9(CAG-tdTomato)}Hze/J* (Ai9) mice also determined that LGR5⁺ OSE stem cells do not contribute to the regeneration of the TE, thereby suggesting presence of a separate stem cell niche in charge of this function (14). Similar results confirming location of LGR5⁺ cells in the OSE of the hilum area but not in the TE of adult mice were recently reported by the Barker group (60).

Inactivation of *p53* and *Rb* tumor suppressor genes, whose pathways are frequently altered in high-grade serous ovarian carcinoma (61), results in preferential proliferation, immortalization, and malignant transformation of the hilar OSE cells. These results provide direct experimental evidence that this most common and aggressive type of ovarian cancer may arise from the stem cell niche, and neoplasms originating from stem/progenitor cells have a particularly aggressive behavior.

Prostate epithelium

Normal prostate epithelium is composed of three main cell types: basal, luminal neuroendocrine. Luminal cells express Keratin (KRT) 8, KRT18, Prostate specific antigen (PSA) and high levels of androgen receptor (AR), and are dependent on androgen for survival. Basal cells express p63, KRT5, KRT14 and low level of AR, and are androgen responsive but androgen independent for survival (62; 63). Neuroendocrine cells express chromogranin A and synaptophysin, and lack AR and PSA (64).

Although the overall organization of the rodent prostate differs from that of the human gland, it provides a unique opportunity to study many important features of the prostate, including the localization and properties of prostate stem/progenitor cells. The mouse prostate is composed of a series of branching ducts, each containing distal, intermediate, and proximal regions relative to the urethra (65 and Figure 4a and b). Proliferating, transit-amplifying cells are preferentially located in the distal region of the prostatic ducts, whereas cells with stem cell-like properties, such as low cycling rate, self-renewal ability, and high ex vivo proliferative potential, mainly reside in the proximal region of the prostatic ducts (59; 66–69). Furthermore, the latter cells were shown to share with stem cells of other organs the expression of specific antigens such as SCA-1, α 6 integrin, and BCL-2 (69 and Figure 4c; 70; 71), and to survive prolonged androgen deprivation (72; 73). Thus, approaches based on the isolation of cells according to their displayed stem cell-specific markers can be complemented by careful evaluation of stem cell compartments in situ. Interestingly, the majority of neuroendocrine cells are located in the proximal regions of prostatic ducts and their ablation results in prostate hypotrophy (74). Their potential role in the regulation of

prostate stem cells remains to be investigated. The adult prostate epithelium is mainly sustained by unipotential luminal and basal progenitors (75–77). However, bipotential Nkx3.1-expressing luminal population has also been identified in regenerating prostate epithelium (78).

Experimental evidence based on mouse models suggest that prostate cancers arise from stem/progenitor cells (59; 69; 70; 78–81). The earliest lesions and invasive neoplasms were detected in the proximal regions of the prostatic ducts in several mouse models of prostate cancer. For example, the earliest morphological precursors of metastatic carcinomas were detected in the proximal regions of prostatic ducts of mice with prostate epithelium-specific inactivation of *p53* and *Rb* by postnatal day 60 (69). In this model, androgen withdrawal-independent neoplasms were also detected in the proximal regions of prostatic ducts in mice castrated at postnatal day 60 and sacrificed 100 days afterwards (69). Numerous dysplastic foci (prostatic intraepithelial neoplasia) were also found in the distal region of prostatic ducts of these mice. However, these lesions never progressed to invasive or metastatic carcinoma by the time of death caused by rapidly growing carcinomas arising from the proximal regions of prostatic ducts. The absence of functional *p53* and *Rb* genes in the cells of both proximal and distal prostatic intraepithelial neoplasia was confirmed by microdissection-PCR (69). Thus, differences in biological behavior of PIN as a function of their location cannot be explained by incomplete inactivation of either of these genes.

Similarly, early invasive adenocarcinomas in the proximal but not distal regions of the prostatic ducts were observed in mice with prostate epithelium-specific inactivation of *mir-34* and *p53* (59 and Figure 4d and e). Accordingly, inactivation of these genes resulted in expansion of the prostate stem cell niche. Taken together these results indicate that transformation of stem cells may result in particularly aggressive prostate cancers.

Skin

The skin is a complex organ which contains several types of stem cells, including epithelial and melanocytic stem cells. The epithelial stem cells are found in the hair follicle, interfollicular epithelium and sebaceous gland. Melanocytic stem cells are found in the hair follicle in mouse and in hair follicle and epidermis in human.

The epithelial hair follicle stem cells are the best understood and are confined to a region of the hair follicle known as the lower permanent portion or bulge (82 and Figure 5a and b). Hair follicle growth occurs in cycles, and prior to the growth activation phase, stem cells migrate outside the bulge for the beginning of the new bulb of the hair follicle, known as hair germ. These hair germ cells divide and differentiate to form the hair shaft (cuticle, cortex and medulla) and the inner root sheath. The outer root sheath is also derived from the stem cells in the bulge during the growth phase. Cells in the dermal papilla secrete growth factors, including fibroblast growth factors (FGF)-7 and 10, transforming growth factor (TGF)- β 2, and noggin, which are important in initiating proliferation of the primed cells. WNT signaling also plays a role in activation of primed stem cells in the hair germ, while sonic hedgehog signaling is a potent mitogen for stem cells in the bulge promoting their self-renewal. Markers of follicular stem cells in mice and humans include Tenascin C, CD200,

KRT15, and KRT19 (83; 84). Additional markers in mice include MACF1, $\beta 1$ integrin and $\beta 6$ integrin, $\alpha 6$ integrin and CD34 (82; 85; 86).

Within the intra-follicular epidermis, stem cells are located in the stratum basale (or basal layer), and their identity and precise organization remains unclear. Originally it was thought that the stem cells are rare and produce transit-amplifying rapidly dividing cells that differentiate into hexagonal columns of cells called epidermal proliferative units. In human these stem cells express high levels of $\beta 1$ integrins (87) and MCSP, Delta1, and LRIG 1 (87–89). Recent work in mice, including lineage tracing and live imaging challenged this view, proposing instead that most cells in the basal layer are stem cells, which stochastically may choose to differentiate or self-renew (90; 91). The recent discovery of multiple populations of independent stem cells, which are spatially segregated, complicates matters even more (92). The mature keratinocytes are suprabasal, and sequentially populate the stratum spinosum (expressing KRT1 and 10), stratum granulosum (expressing involucrin and loricrin), and stratum corneum. Cells in the stratum basale are attached to the basement membrane by integrins $\alpha 3\beta 1$ and $\alpha 6\beta 4$ and proliferate in response to growth factors secreted by fibroblasts in the dermis. These factors include FGF7 and 10, insulin-like growth factor, epidermal growth factor receptor ligands and TGF α . Differentiation of epidermal stem cells is dependent on NOTCH signaling. Specifically NOTCH1, 2 and 3 receptors are expressed in the suprabasilar keratinocytes of mice. NOTCH ligand Jagged1 is also expressed in the suprabasilar keratinocytes, while Jagged2 is expressed in the stratum basale cells. Notch activation promotes asymmetric division of stratum basale cells. This division along a plane parallel to the basement membrane ensures upward stratification of the differentiating cells.

In addition to the follicular and inter-follicular stem cells, some studies suggest that sebaceous glands also contain a population of epithelial stem cells. These stem cells are located in the basal layer of the gland and express BLIMP1 (93). The interfollicular, follicular and sebaceous gland stem cells are considered interchangeable and functionally identical under stress conditions (94). Recently, LGR6⁺ cells were found in the region located directly above the follicle bulge, known as the infundibulum. Prenatally these cells established the hair follicle, sebaceous gland, and interfollicular epidermis. Postnatally, LGR6⁺ cells mainly generated sebaceous gland and interfollicular epidermis, but were able of formation of new hair follicle during long-term wound repair (95).

Epidermal tumors include basal cell carcinoma, squamous cells carcinoma, follicular and sebaceous neoplasia. Selective expression of oncogenes in differentiated cells leads to formation of benign tumors, such as papillomas, while expression in the proliferating cells of the follicle results in malignant carcinoma (96; 97). Similarly, inactivation of tumor suppressor genes, such as *p53* and *Pten*, in LGR5⁺ hair follicle stem cells results in formation of squamous cell carcinomas (Figure 5c and Fu et al., unpublished results).

Melanocytes, along with peripheral neurons and endocrine cells, are derived from neural crest cells. Melanoblasts, the precursor cells for melanocytes, migrate from the neural crest to the epidermis and hair follicles, and to other areas in the body including the iris of the eye and cochlea of the ear. In hair follicles, the melanoblasts are located in the hair matrix and the bulge region. Following injury, these stem cells migrate from the bulge to the epidermis,

where they proliferate to produce melanocytes. Melanocyte stem cell migration and differentiation is regulated by binding of ligands to the melanocortin-1 receptor. Self-renewal of these stem cells is dependent on expression of collagen XVII by adjacent epithelial stem cells. Migratory melanoblasts express DCT, MITF1, KIT, PAX3, SOX10, SI, TYR and TYRP1, while melanocyte stem cells in the bulge express DCT and PAX3, with downregulation of other markers (98). Differentiated melanocytes express DCT, MITF1, KIT, PAX3, SOX10 (98). A population of neural crest stem cell (NCSC)-like cells is maintained in the dermal papilla and replenish melanocytes in the epidermis after age-related loss of stem cells in the bulge. These cells express neural crest markers such as Nestin, Twist and Slug (99), and will differentiate into melanocytes following UV damage and increased production of WNT ligands by keratinocytes.

Melanomas, neoplasms of melanocytic cells, may arise from de-differentiated melanocytes, melanocyte stem cells, or multipotential NCSC-like cells. The proliferation and basilar location of melanocytes are controlled by interactions with keratinocytes. Removal of melanocytes from this microenvironment results in increased cell division and altered adhesion molecule expression, characteristics similar to those of melanoma cells (100). Following UV damage, keratinocytes secrete growth factors for melanocytes (101), as well as pro-inflammatory cytokines (102), both of which can contribute to malignant transformation.

The nervous system

The coordinated development of neurons, glia, and supporting cells is fundamentally controlled and impacted by the role of neural stem cells (NSCs). While limited in the adult animal, coordinated neurogenesis still occurs in certain regions of the brain, namely the hippocampus, subventricular zone (SVZ), and olfactory cortex, where NSCs drive neurogenesis. NSCs are multipotent cells that give rise to neurons as well as neuroglia (oligodendrocytes and astrocytes) (103). In the developing animal, abundant proliferation of NSCs occur in the peri-ventricular tissue where initial NSC division occurs. From this population, precursor cells derived from NSCs migrate to the SVZ and then to the gray matter structures of the CNS where they mature (103). While neurons are the initial result of NSC replication, glial cells are also abundantly produced and migrate to populate the entire CNS during development. The spatial production, development, and maturation of neurons and glia from the NSCs requires a coordinated effort of large numbers of transcription factors and trophic factors, many of which remain to be elucidated.

The SVZ is a primary niche for NSCs in the adult mammalian brain (Figure 6a and b). They are located immediately subjacent to the ependyma where they are referred to as B1 astrocytes, identified via advanced microscopy as having chromatin clumping close to the nuclear membrane and a cilium that communicates with the lateral ventricle (104). These cells proliferate and give rise to intermediate progenitor cells that eventually form neuroblasts. As the mammalian subject ages, there is a profound decline in the proliferative capabilities of NSCs; however, small numbers of NSCs are found in the dentate gyrus and the SVZ of the adult aged brain (104). The role that aging has in changing the plasticity of NSCs is not fully understood; however, this is an area of active interest.

A second emerging concept in CNS stem cell pathology, is the role that stem cells play in tumor formation (104). Most of the research on this topic has focused on high-grade astrocytomas due to their increased frequency in the human population and their significant morbidity and mortality. The glioma stem cells share some similarities with the NSCs including expression of NSCs markers (i.e. CD133, SOX2, nestin) and the ability to self-renew and proliferate (105). These glioma stem cells respond to their own set of cues derived from the cancer niche and include select transcription factors, local hypoxia, and epigenetic alterations. Little is known regarding the stem cell niche of glioma in animal species; however, the dog is an attractive model to explore (106 and Figure 6c). The neuroanatomical localization of canine gliomas (oligodendroglioma and astrocytoma specifically) often to the peri-ventricular tissue suggests a potential role for emergence from the SVZ. Further research into the role of stem cell proliferation in canine glioma is justified; however, reagents commonly used to identify either NSCs or glioma stem cells rarely work in the dog making identification more challenging. Numerous rodent models have been employed to study gliomagenesis with respect to stem cell pathology; (107; 108 and Figure 6d).

Transitional zones, stem cells and cancer

Transitional zones (TZ)/junction areas are anatomically defined regions of organs where two different types of epithelial tissue meet. It is well known that many TZs, such as the gastro-esophageal, anal canal, uterine cervical and corneal limbus junctions are highly susceptible to cancer (109–112). The presence of adult stem cells in such junctions has been definitively demonstrated for the corneal limbus region (113; 114 and Table 2), the gastric squamo-columnar junction (45; 115; 116 and Figure 1) and the ovarian hilum region (14 and Figure 3). Putative stem/progenitor cells have been identified in the anal canal (117) and the uterine cervix (118; 119).

For the most of these locations, the definitive proof that TZ stem/progenitor cells are more susceptible to malignant transformation as compared to their more differentiated progeny remains to be shown. Indeed, it is possible that some neoplasms may originate from differentiated cells which have acquired some stem cell properties (120; 121). However, recent work has provided the first direct experimental evidence that high-grade serous ovarian carcinomas may develop from the hilum area, which is TZ between OSE, mesothelium and TE, in the mouse (14). Another study demonstrated that the majority of serous tubal intraepithelial carcinoma, the likely precursor of high-grade serous ovarian carcinoma, are frequently located in the vicinity of tubal-peritoneal junctions, which are TZs between TE and mesothelium (122). Importantly, both tubal-peritoneal junctions and serous tubal intraepithelial carcinoma express stem cell marker LEF1 (123). These finding may offer an equivalent to the cancer-prone stem cell niche in the mouse hilum area. They also support the notion that cancer susceptibility of TZs in other organs may be explained by cancer-prone stem cell niches therein.

Another important question is whether TZ stem cells are more prone to malignant transformation than stem cells located in other regions of the same tissue and organ. As

shown in the Stomach section of this manuscript, our studies suggest that this is the case. The mechanisms responsible for this uneven susceptibility remain to be understood.

Cancer propagating/stem cells

Cancer cells frequently acquire stem cell-like properties. In many cases, such properties are specifically associated with subpopulations of cancer cells known as cancer propagating cells (CPC), cancer stem cells or cancer-imitating cells (124–127). Such cells are characterized by their ability for long-term self-renewal, high potential for proliferation, high tumorigenicity, and capacity to recreate the complexity of the original tumors. They also frequently show increased chemoresistance; therefore, they may play a significant role in cancer recurrence (125; 127). Thus it is tempting to attempt detection of CPCs in tissue sections. However it should be taken into account that stem cell marker expression is frequently unstable due to the phenotypical plasticity of cancer (128–130). Thus, the use of immunostainings for detection of putative CPCs should be performed in conjunction with their functional characterization.

Future issues and challenges

The majority of studies of stem cell niches are focused on mice and other experimental animals. Since anatomy and physiology of humans are frequently quite distinct from other animals delineating stem cell niches in human tissues is of the utmost importance. At the same time, new animal models need to be prepared to allow specific targeting of not only a specific cell type, but also a specific stage along the continuum of cell lineage development, from stem cells to their differentiated progeny.

With increase in the development of regenerative medicine approaches, pathology needs to be more focused on systematic assessment of complications of stem cell transplantation. Furthermore, side effects of genetic and epigenetic manipulations of gametes, ES, iPS and adult stem cells remain to be better evaluated. Discovery of novel stem cell niches in the TZs and other anatomically defined areas is another task requiring pathologists. It is also important to understand why some stem cell populations reside in specific locations such as TZa and how such locations contribute to pathogenesis of various diseases, including cancer.

Addressing the growing needs of pathological support in stem cell research and regenerative medicine studies, specialized training of pathologists in stem cell pathology is required. It would be also helpful to establish sections/units of diagnostic pathology specializing in stem cell pathology. In case of research institutions, such sections should be staffed by comparative pathologists who are also experienced in animal models. Such pathologists should be in charge of the development of approaches for accurate collection and orientation of specimens allowing evaluation of stem cell niches. We provide an example of such approaches for some mouse tissues used by the Cornell Stem Cell Pathology Unit (Table 3). Since the majority of stem cells can be detected only by a combination of several markers, development of multiplexed immunohistochemical and molecular biological approaches should be another task of such sections. We anticipate that close integration of stem cell pathology with animal modeling and in vivo imaging will significantly accelerate our

progress towards understanding the pathogenesis of diseases associated with stem cell niche disorders.

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Figure 1. Stomach of adult mouse

a. Longitudinal section of the stomach through squamous epithelium (SE), cardia including transitional zone (TZ), corpus and pylorus, followed by the proximal part of duodenum (Du). **b.** Transitional zone (TZ). SE, squamous epithelium. **c.** LGR5⁺ stem cells (arrow) detected by GFP immunostaining in the gastric TZ gland of *Lgr5^{EGFP-Ires-CreERT2}* mouse stomach. **d.** Lineage tracing of LGR5⁺ stem cells in the gastric epithelium at the TZ of 244 day-old *Lgr5^{EGFP-Ires-CreERT2}* Ai9 mouse stomach (14) exposed to tamoxifen at 44 days of age. All cells of the TZ gland express tdTomato (red) indicating their origin from LGR5⁺ stem cells. **e.** Dysplasia (arrows) at the TZ of *Lgr5^{EGFP-Ires-CreERT2} p53^{loxP/loxP} Rb^{loxP/loxP}* 114 day-old mouse stomach exposed to tamoxifen at 54 days of age. **f.** The corpus glands of the stomach. B, base. N, neck. I, isthmus. P, pit surface. **g.** The pyloric glands of the stomach. **h.** LGR5⁺ stem cells (arrow) detected by GFP immunostaining at the base of the pyloric glands *Lgr5^{EGFP-Ires-CreERT2}* mouse stomach. **i.** Lineage tracing of LGR5⁺ stem cells in the pyloric epithelium of 244 day-old *Lgr5^{EGFP-Ires-CreERT2}* Ai9 mouse stomach exposed to tamoxifen at 44 days of age. All cells of a gland express tdTomato (red) indicating their origin from LGR5⁺ stem cells. **j.** Adenoma (arrows) in the pylorus of *Lgr5^{EGFP-Ires-CreERT2} p53^{loxP/loxP} Rb^{loxP/loxP}* 382 day-old mouse stomach exposed to tamoxifen at 73 days of age. Hematoxylin and eosin staining (a, b, e, f, g, and j). ABC Elite method with hematoxylin counterstaining (c and h). Fluorescence, counterstaining with DAPI (d and i). Scale bar, 1.2 mm (a), 40 μ m (b – d, and g – i), 30 μ m (e), 50 μ m (f), and 140 μ m (j).

Figure 2. Small intestine of adult mouse

a. The epithelium of small intestine. The +4 slow cycling stem cell (arrow) is located above the stem cell zone (SCZ) at the base of the intestinal crypts (C). V, villous. **b.** LGR5⁺ stem cells (arrows) detected by GFP immunostaining in the intestinal crypts of *Lgr5^{EGFP-Ires-CreERT2}* mouse small intestine. **c.** Epithelial hyperplasia (arrow) in the small intestine of *Lgr5^{EGFP-Ires-CreERT2} p53^{loxP/loxP} Rb^{loxP/loxP}* 276 day-old mouse exposed to tamoxifen at 41 day of age. Hematoxylin and eosin (a and c). ABC Elite method with hematoxylin counterstaining (b). Scale bar, 25 μ m all images.

Figure 3. Ovary of adult mouse

a. Longitudinal section of the ovary and adnexa. The hilum area (H) contains the transitional zone between the ovarian surface epithelium (OSE) and mesothelium or tubal epithelium (arrow in the bottom panel). **b.** ALDH1 (brown) is expressed preferentially in the OSE (arrows) of the hilum region (H) as compared to other regions. ALDH1 staining is also present in the theca cells (TC) of the ovary (OV). **c.** Early atypical OSE lesions (arrow) in the hilum region of $p53^{\text{loxP/loxP}} Rb^{\text{loxP/loxP}}$ mouse ovary 60 days after trans-infundibular intrabursal injection of AdCre. The lesions are characterized by increased nuclear to cytoplasmic ratio, nuclear size and shape variability, more columnar appearance, and cell crowding. OV, ovary. B, bursa, U, uterus. UT, uterine tube. Rectangles in the top panels indicate respective location of the bottom panels. Hematoxylin and eosin (a and c). ABC Elite method with hematoxylin counterstaining (b). Scale bar, 600 μm (top panels) and 120 μm (bottom panels).

Figure 4. Prostate of adult mouse

a and b. Transverse section of the prostate. The periurethral part of the prostate is located inside of the muscular layer (M) and contains the proximal region of the prostatic ducts (P in a, arrow in b). D, distal region of the prostatic ducts. **c.** Epithelial cells of the proximal region (P), but not those of the distal region (D) of the prostatic ducts, highly express SCA-1 (arrows). **d and e.** Adenocarcinoma invading the surrounding stroma (arrow) and filling up the lumen in the proximal regions of the prostatic ducts of $p53^{PE-/-} mir-34^{PE-/-}$ mouse prostate (59). The rectangles in a and d indicate respective locations of b and e. Hematoxylin and eosin (a, b and d). ABC Elite method with hematoxylin counterstaining (c). Scale bar, 200 μ m (a, c and d) and 60 μ m (b and e).

Figure 5. Skin of adult mice

a. Longitudinal section of the skin and hair follicles. E, epidermis. H, hair shaft. HG, hair germ. D, dermal papillae. B, bulge. S, sebaceous gland. **b.** Immunostaining of CD34 (red) and H2B label-retaining stem cells (green). **c.** Squamous cell carcinoma (S) at the back skin of *Lgr5^{EGFP-Ires-CreERT2} p53^{loxP/loxP}* 538 day-old mouse exposed to tamoxifen at 55 days of age. HF, remnants of hair follicle. Hematoxylin and eosin staining (a and c). Immunofluorescence with DAPI counterstaining (b). Scale bar, 55 μ m (a), 30 μ m (b) and 100 μ m (c).

Figure 6. Canine and mouse brain

a. Canine brain, Cross section through frontal cortex of the canine brain. Subventricular zone (SVZ) containing stem/progenitor cells is indicated by the arrow. **b.** The SOX2-expressing stem/progenitor cells (arrowheads in the inset) at the subventricular zone (arrow). The rectangles in b and c indicate respective locations of the insets. C, frontal cortex, V, lateral ventricle (C). **c.** Canine glioma (arrow). The neoplastic glial cells are strongly immunoreactive for SOX2. **d.** Glioblastoma multiforme (arrow) in the SVZ of $p53^{+/-}$, $Nf1^{+/-}$ mouse brain. Hematoxylin and eosin staining (a and d). ABC elite method with hematoxylin counterstaining (b and c). Scale bar, 5 mm (a – c), 100 μ m (insets in b and c, and d).

Table 1

Stem cell identification.

Assay	Pitfalls
Immunodetection (e.g., CD44, CD49 ^f)	Insufficiently specific
Functional properties, such as enzymatic activity (e.g., ALDH) or compound efflux (e.g., side population)	Insufficiently specific
Label retention (e.g., BrdU, H2B-GFP)	Not all stem cells are slowly cycling (e.g., LGR5 ⁺ intestinal stem cells)
Formation of monoclonal spheres/organoids in several consecutive rounds of dissociation and regeneration (e.g. prostaspheres)	Cell culture assays are not sufficient for the unambiguous identification of stem cells
Formation of a complete tissue after single cell transplantations in several consecutive rounds of dissociation and regeneration (e.g., mammary stem cells)	Under physiological conditions stem cells may have more restricted potential (e.g., prostate epithelium stem cells)
Cell lineage (fate) tracing (e.g., intestinal cells)	Identification of stem cell-specific promoters is a major challenge

Table 2

Examples of stem cell niches in transitional/junction zones

Location	Species	Assays	Niche markers	References
Anorectal junction	Mouse	Label retention, IHC	CD34, integrin α 6, SOX2, p63, Tenascin C	(117)
Corneal limbus	Human Mouse	Histology, IHC, cell culture, transplantation, wounding	ABCG2, KRT14, p63, ABCB5	(114; 131; 132)
Gastric squamo-columnar junction	Human Mouse	Histology, IHC, label retention, chemical random mutagenesis, lineage-tracing	LGR5, CD44	(13; 27; 115; 133)
Cervical squamo-columnar junction	Human	Gene-expression arrays, histology, IHC, western blotting	AGR2, CD63, GDA, KRT7, MMP7	(118; 119)
Ovarian hilum	Mouse	FACS, label retention, lineage-tracing, sphere/clonal formation, gene expression arrays, IHC, qRT-PCR, laser microdissection, transplantation	ALDH1, LGR5, LEF1, CD133, KRT6B	(14)

Abbreviations: IHC, immunohistochemistry; FACS, fluorescence-activated cell sorting; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

Table 3

Specimen collection for pathological evaluation of stem cell niches in the mouse

Organ	Location of interest	Recommended orientation of section	Tissue preparation	Ref.*
Stomach	Base and isthmus of glands	Longitudinal vertical section from forestomach through TZ, corpus, pylorus to duodenum	The stomach is opened along the greater curvature, spread and pinned on cork, and placed into fixative. The flat, fixed stomach tissue is longitudinally dissected into 1 mm- wide strips followed by routine tissue processing. The gastric strips are embedded cut side down.	(134)
Intestine	Intestinal crypts	Longitudinal horizontal section of "Swiss rolled" intestine	The intestine is cut into several segments, opened longitudinally and rolled up on a wooden stick. The rolled intestine tissue is removed from stick and fixed.	(135)
Prostate	Proximal regions of prostatic ducts	Transverse section of all lobes including periurethral area	Entire genitourinary block is removed after transection of the urethra. Transverse sectioning through urethra should include periurethral area, and dorsolateral and ventral prostatic lobes	(136)
Ovary	Hilum region	Longitudinal section	The ovary is removed from the abdomen and left attached with a small segment of oviduct. The specimen is gently sandwiched between foamy biopsy sponges or filter paper without any compression, and fixed.	(14)
Brain	Sub ventricular zone	Serial transverse section from middle to caudal of cerebrum	The whole cerebrum is removed from skull and fixed. After fixation, the cerebrum is transversely dissected into several slices.	(137)
Eye	Limbus region	Longitudinal vertical section	The excess tissue is trimmed off the eye globe. The globe is fixed in modified Davison's fixative.	(138)
Skin	Hair follicle	Longitudinal section along with the hair flow	A square of skin flap is removed from the body, pinned out and fixed. The fixed skin flap is trimmed into strips and embedded cut side down.	(139)

* Abbreviation: Ref. Reference