The Truth Behind Esophagus: The Stem Cells' Significance

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Introduction

The esophagus presents the unique feature of involving stem cells (SCs) in a wide spectrum of events even from its embryonic development to the complicated esophageal diseases, as well as the esophageal tumorigenesis. The scientific progress during years in the field of stem cells (SCs) was taking place in strong relationship to the evolution of our experience about esophageal pathophysiology. For instance, an observation about stem cells behavior could be a stimulus for a therapeutic implication in an esophageal disease or vice versa, and an expression of molecular markers in an esophageal disease could be extremely crucial for a discovery of a pathway in stem cells signaling. This strong association has been continued for years, and it seems that it will be maintained in the future too.

In this chapter, we will present all the recent data about the contribution of stem cells to the esophageal development and maintenance of esophageal homeostasis. In addition, we will demonstrate the regulatory effect of stem cells on the benign diseases of the esophagus as well as their role in esophageal tumorigenesis and message transportation between cancer stem cells. Finally, we will show several possible molecular therapeutic targets that are based on the SCs metabolic pathways as well as the new applications of SCs technology in creating tissue-engineered esophageal scaffolds that could replace natural esophagus due to a variety of reasons that lead to esophageal destruction.

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Stem Cells Are the Main Factors of Esophageal Homeostasis

The esophagus is derived from the anterior portion of the developmental intermediate foregut, which is a structure that also gives rise to other organs like the trachea, lung, and stomach. The separation of the esophagus from the trachea is ensured by Sox2, which is a key family member of SRY (sex-determining region Y)-related transcriptional factors and is essential for maintaining self-renewal and pluripotency in ESCs [56]. The absence of Sox2 expression has been associated with esophageal atresia and tracheoesophageal fistula [122]. In addition, except from its role in esophagus separation from the respiratory organs, Sox2 contributes to the esophageal basal progenitor cells proliferating and differentiating into squamous superficial epithelial cells in adult esophagus [33].

Nevertheless, a homolog of the tumor suppressor and transcription factor p53, p63 is the most potent regulator of the differentiation of simple columnar into squamous epithelium [17]. The esophageal epithelium in mouse models with negative expression of p63 remains columnar, exactly like the skin epithelium. Furthermore, a large portion of the esophageal epithelial cells with negative p63 expression is characterized by the presence of multi-cilia. It has been reported that ciliated epithelial cells are present in developing the esophagus, highlighting that epithelial differentiation is arrested when there is a mutation in p63 expression in esophageal cells.

Two transcriptional factors of the Krüppel-like factor (Klf) family also contribute to the maintenance of homeostasis in the adult esophageal epithelium. The expression of Klf5 seems to be restricted to the basal layer and regulates progenitor cells proliferation. If a transgenic overexpression of Klf5 is caused in the esophageal epithelium, a twofold increase in the proliferation rate would take place, without any other disturbance of the esophageal epithelium functionality [93]. On the contrary, Klf4 is expressed in the suprabasal cell layer of the esophageal epithelium and plays a crucial role in cell differentiation. Klf4 deficient mice present impaired differentiation and increased proliferation leading to dysplasia.

Another very important factor to the esophageal epithelium homeostasis is the Notch signaling pathway. In vitro organotypic cultures and in vivo mouse models have shown that Notch signaling through the transcriptional factor CSL is required for human esophageal epithelial differentiation, especially factors NOTCH1 and NOTCH3 [43]. The key role of Notch signaling has been also underlined by studies that have demonstrated a relationship between mutations in the Notch signaling pathway and ESCC. Upregulation of Notch pathway components (Dll3, Jag2, and Hes5) was observed in a mouse model that leads to increased esophageal precursor cell differentiation after chemical inducement of endoplasmic reticulum (ER) and following unfolded protein response (UPR) activation due to thapsigargin treatment [94]. Thapsigargin is a plant-derived inhibitor of cell proliferation through ER stress inducement that leads to increased cell differentiation and upregulation of different Notch signaling pathway components. In addition, UPR after increased ER stress

serves as a regulatory mechanism that forces progenitor cells with accumulated unfolded proteins to initiate differentiation.

The basal layer of the esophageal progenitor cells is not homogeneous. Mouse models demonstrate that the esophageal basal epithelial cells present a scaled potential of stemness depending on the expression of two SCs markers: α_6 integrin and transferrin receptor CD71. According to these models, there are three subpopulations of basal cells: one that presents α_6 integrin^{high} and CD71^{low} expression, which is a minor subpopulation of small and undifferentiated cells that are full of label-retaining cells and represent a putative esophageal stem cell population. On the other hand, basal cells that express both α_6 integrin^{high} and CD71^{high} levels are the majority of the esophageal basal cells and represent a transit-amplifying population as it is enriched of actively cycling cells. Finally, basal cells that present α_6 integrin^{low} and CD71^{high} leave the basal cell layer and differentiate [15].

However, there is an arguing statement against the heterogeneous hypothesis, which claims that the normal esophageal epithelium is generated by a single and homogeneous population of progenitor cells. More specifically, every basal cell possesses equal potential of self-renewal and differentiation into squamous cell. During periods that esophageal epithelium is guided by homeostatic mechanisms, cell production and cell loss are balanced as proliferating basal cells create equal proportion of dividing and nondividing cells. On the contrary, when an injury happens, basal cells that are adjacent to the site of injury generate more proliferating cells until the injury is repaired (Fig. 1) [24].

The distribution of esophageal epithelial cells into layers and functional groups does not seem to be of great importance, due to the proven remarkable plasticity for self-renewal that the esophageal epithelial cells present in ex vivo wounding response models and in vivo mouse models. Undoubtedly, proliferation and mitotic activity are higher in the interpapillary basal layer and lower superficially toward the tip of the papilla. On the other hand, the orientation of mitosis is random linearly through the basal layer, and the cell divisions are not restricted to specific cell compartments. The expression of epithelial and progenitor cell markers such as EpCAM and CD34 determines the accumulation of epithelial cells into distinct populations, but there is no difference in self-renewal ability depending on the presence of each cell as unique or into a population. In 3D organotypic cultures, all esophageal epithelial cells were capable of restoring the architecture of the tissue they came from, and the main factor of successful result was the number of cells plated in the culture rather than the cell type [4].

Our attempt to investigate the principles of esophageal stem cells has led to the development of mouse models in order to make research easier, but we have to keep always in mind that there is a number of obvious differences between mouse and human esophageal epithelia [16]. Firstly, human esophageal epithelium has more cell layers than mouse epithelium. Secondly, the basement membrane of the human esophagus is thrown into folds by submucosal projections, called papillae, just like the human skin. In addition, there are mucosal and submucosal glands in the human esophagus that are not observed in the mouse esophagus. Furthermore, the transition from the proliferating compartment to the differentiating compartment is more

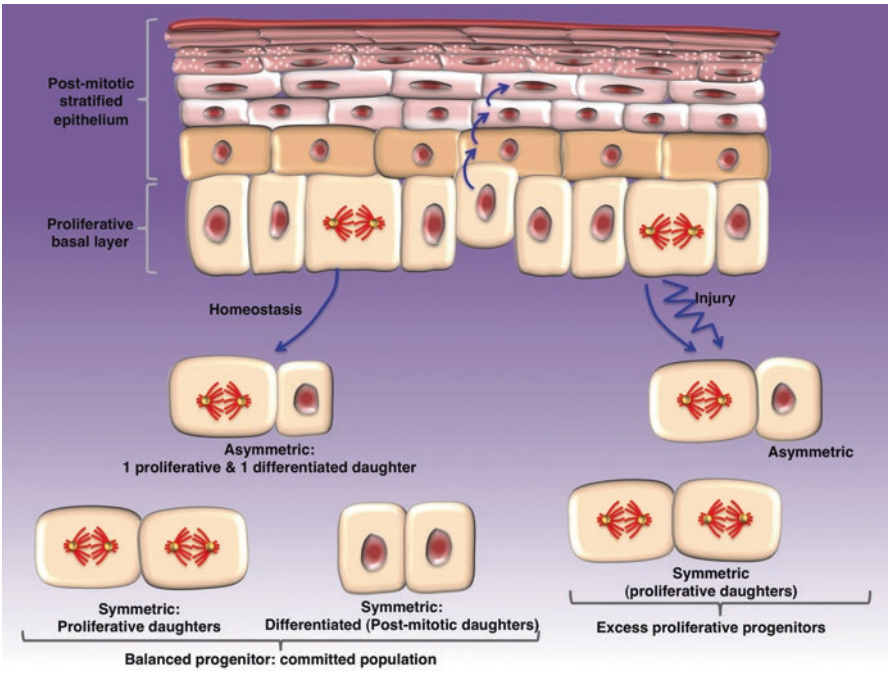

Fig. 1 The contribution of basal progenitors to epithelial self-renewal during homeostasis and in response to injury in the esophagus [5]

abrupt in the mouse esophageal epithelium than in human epithelium, in which mitoses are taking place five layers above the basement membrane. Finally, cell turnover in the human esophagus seems to be slower compared to mice. All these differences show that the distribution of the esophageal epithelial cells into the three layers of stem cells, transit-amplifying cells, and differentiating cells seems to be easier in the human esophagus than in mice or rats.

An extremely useful marker for distinguishing human esophageal cancer stem cells is the low-affinity neurotrophin receptor p75ntr, which is usually expressed in neural stem cells. Human esophageal epithelial cells with a high expression of that marker were found to present increased proliferative ability in vitro in comparison with those with low expression [80]. However, such measures need to be repeated due to the utilization of passage 2 cells rather than the use of freshly isolated esophageal cells, because in vitro cultivation influences cell surface markers.

SCs research in adult tissues revealed another quite interesting fact: the ESCs pluripotency transcriptional factor NANOG is selectively expressed in mouse stratified epithelia presenting a lineage-restricted mitogenic activity. More specifically, mouse NANOG is expressed in adult esophageal epithelium, where its promoter is hypomethylated [84]. Generally induced overexpression of NANOG in mouse models provokes hyperplasia especially in esophageal epithelium, accompanied by increased cell proliferation through the following mechanism: the exogenously

overexpressed NANOG activates the mitogenic pathways of the stratified epithelia via transcriptional factors such as Aurora A kinase (AURKA), and the endogenous NANOG binds to the AURKA promoter in the primary keratinocytes. Consequently, overexpression of NANOG or AURKA in mouse models causes increased proliferation and aneuploidy in esophageal basal cells. Finally, inactivation of NANOG in cell lines from ESSC results in decreased AURKA expression and diminished proliferation of both basal cells and keratinocytes; hence NANOG and AURKA are correlated with increased cancer cell proliferation in ESCC.

All the molecular SC mechanisms stated above conserve the goal of maintaining the homeostasis of the esophagus in embryonic and adult tissues. Sometimes though, the balanced self-renewal and proliferation of stem and progenitor cells that are required especially in quickly replenished tissues like the esophagus for achieving homeostasis are disrupted. A pathological condition that is associated with such a disruption is eosinophilic esophagitis (EoE), in which basal progenitor cells become hyperplastic due to proinflammatory stimulation. Once again, a stem cell mechanism seems to be responsible for the progenitor basal cells' irregular reaction to the inflammatory stimulation. Bone morphogenetic protein (BMP) signaling pathway is essential for epithelial morphogenesis in embryonic esophagus; however BMP signaling pathway seems to regulate tissue homeostasis and EoE development in the adult esophagus [37]. BMP signaling was activated in differentiated squamous cell epithelium, on the contrary to basal progenitor cells that express the BMP antagonist follistatin. Nevertheless, in mouse models BMP signaling was increased in basal progenitor cells and promoted squamous epithelial cells differentiation. In addition, BMP activation induced the production of intracellular ROS, initiating an NRF2-mediated oxidative response during the progenitor basal cell differentiation. On the other hand, both in human biopsies and in EoE mouse models, high levels of follistatin and disrupted BMP pathways led to reduced levels of differentiation. Consequently, BMP signaling pathway is responsible for basal cell differentiation into squamous epithelium, and EoE is related to a dysfunction of this mechanism which leads to decreased esophageal squamous differentiation with a consequent progenitor basal cell hyperplasia.

Except from intrinsic dysregulations that lead to esophageal dysfunction, there are several exogenous agents that could cause esophageal injury and activate a repair process by the esophageal epithelium. Severe caustic injury by alkali is a very common cause of esophageal injury, for the repair of which the intrinsic esophageal reaction sometimes is not enough. For that reason, ovine esophageal models have been developed that are utilized for testing the conditions under which viable autologous esophageal cells could be isolated in order to be used in tissue-engineering models for the replacement of the injured esophagus by caustic substances. It has been proven that an esophagus which has been exposed to low concentrations (2.5%) of NaOH would maintain a relatively large population of viable cells for tissue-engineering applications [65]. On the other hand, esophagi exposed to greater concentrations (15–25%) of NaOH could not provide tissue-engineering models with the required number of viable esophageal cells; thus alternative sources of esophageal cells should be searched, such as stem cells.

Even in less extensive injuries than caustic injuries, the esophageal epithelium presents a regenerative capacity which is based on its feature to maintain a balance between proliferation and differentiation. The progenitor basal cells proliferate, and then they migrate outward near luminal surface where they differentiate in squamous cells. An esophageal stem cell population, which is accumulated in the basal layer, is responsible for this process. This population maintains its capacity for self-renewal and epithelial reconstruction in both 3D organotypic culture models (in vitro) and in mouse models (in vivo). The esophageal stem cells both in vivo and in vitro give rise to undifferentiated and differentiated cells, uncovering the mechanism through which the adult esophagus faces injury insults and repairs itself [38].

Stem Cells in Esophageal Cancer

Esophageal cancer is the eighth most common cancer worldwide and the sixth most common cause of cancer death in the world. Signs and symptoms of esophageal cancer rarely present in early stages, so most of the time, this malignancy presents in advanced stages; thus it is related to low rates of survival (5-year survival 10–25%). In addition, the two main histological types are squamous cell carcinoma (ESCC) and adenocarcinoma (EAC). Interestingly, the incidence of the two types of esophageal cancer presents geographic patterns, with ESCC being the most common type worldwide presenting an incidence of 90%, but it mainly appears in Asian-belt, including Turkey, Iran, China, Japan, India, and Bangladesh. ESCC has been associated with tobacco and alcohol consumption, as well as diet traditions that include ingestion of spicy foods and hot beverages. On the other hand, EAC is the predominating type of esophageal cancer in the developed continents of the West, such as Europe and Northern America. EAC has been correlated with abnormal columnar metaplasia of the squamous cell esophageal epithelium and formation of Barrett's esophagus (BE) due to chronic gastroesophageal reflux disease (GERD), which is caused by the life trends of the Western societies such as obesity and maldietary habits [1].

During the recent years, the observations about esophageal tumor behavior in animal and human studies led to the development of the cancer stem cells (CSCs) hypothesis. Esophageal cancer is one of the most common death-related cancers worldwide. CSCs are considered to give esophageal cancer all the features that lead to greater mortality rates, such as tumor initiation, drug and radiation resistance, invasive growth, metastatic potential, and tumor relapse [70]. The next goal of the scientific society is to reveal specific markers in order to distinguish CSCs from non-CSCs. Esophageal CSCs derived from ESCC are related to increased β-catenin, Oct3/4, β1-integrin, miR-296, and miR-200c expression. In addition, aldehyde dehydrogenase-1 (ALDH1), Lgr5, and CD44 are useful for sorting esophageal CSCs [105]. Another very interesting observation is that the esophageal CSCs and the SCs of the normal embryonic developing esophagus follow the same trait of both upregulation and suppression of specific genes, so they express the same

molecular markers, making specific pharmaceutic targeting of esophageal CSCs extremely difficult [126]. Nevertheless, a fluorescent vector consisting of fluorescein ZsGreen fused to the carboxyl-terminal region of ornithine decarboxylase (cODC) has been used for targeting three chemotherapeutic drugs, AKT inhibitor XI, ERK inhibitor II, and JAK inhibitor I, which contribute as markers of esophageal CSCs [40]. Finally, except from the correlation of CSCs with the development of the two most common esophageal cancer types (EAC and ESCC), a less common type, small-cell esophageal cancer, seems to arise from a pluripotent esophageal progenitor cell [73].

CSCs development and consequent rise of esophageal carcinoma seems to initiate from a clonal region of paraneoplastic epithelium, a phenomenon called "field change." The quantitative analyses of scattered single esophageal epithelial progenitor cells expressing a mutation that inhibits the Notch signaling pathway, which is frequently inactivated in squamous cancers, demonstrate that cell divisions that produce two differentiated daughters are no longer present in mutant progenitors. In addition, mutant clones are maintained and become immortal, promoting the differentiation of neighboring wild-type cells, which are then lost from the tissue. As a result, the entire normal epithelium is replaced by mutant cells, in which Notch signaling has been disrupted and carrying p53 mutations has been established. Consequently, the phenomenon of "field change" is considered to be a result of imbalanced differentiation of individual esophageal progenitor cells [2]. Moreover, genetic lineage tracing has been used to quantify cell behavior during neoplastic transformation. It demonstrated that dividing esophageal tumor cells were characterized by an abnormality: more dividing than non-dividing daughters were produced in every division cycle. Furthermore, in invasive cancers induced by KRAS expression, a greater portion of the produced cells were dividing than nondividing, indicating that agents that determine proliferating cells' fate are the ideal targets for effective control of tumor growth [24]. The interpretation of the esophageal tumor growth could be achieved by bioinformatics and computational models, which have outlined the contribution of the ornithine metabolic pathway in the survival of chemotherapy-resistant CSCs, indicating possible targets for effective treatments against developing esophageal cancer [48].

It is well known that dietary habits are strongly associated with esophageal cancer. Alcohol consumption has been related to ESCC appearance. Nevertheless, the molecular events behind this association had never been clarified until the invention of the CSCs theory. More specifically, consumption and high concentration of ethanol in the squamous epithelial cells causes cell damage that usually leads to the cell death [59]. As a result, esophageal SCs are triggered to proliferate and differentiate in order to replace missed esophageal epithelial cells. The high rate of esophageal basal cells proliferation in combination with the carcinogenic effect of acetaldehyde, a liver-produced metabolite of ethanol, raises the possibility of a mutation to the proliferating esophageal progenitor cells and their transformation to esophageal CSCs, which give rise to invasive and usually fatal esophageal carcinomas [119]. In addition, another unclarified risk factor that seemed to be correlated with the development of esophageal cancer, although the exact mechanisms of that correlation

have not been specified yet, is the esophageal microbiota. The increase of esophageal adenocarcinoma during the last decades seems to be correlated with the radical treatments against *Helicobacter pylori*, because of the protective effect of *H. pylori* via IL-1b and TNF-a production against high levels of acid secretion and the antagonism against other pathogens that raised their population after *H. pylori* extinction. Moreover, specific bacteria, like *Bacteroidetes*, *Proteobacteria*, *Fusobacteria*, and *Spirochaetes*, have been correlated with esophagitis and Barrett's esophagus [118].

Following CSCs hypothesis described above, after CSCs formation at a specific tissue in the body, tumor dissemination takes part after insertion of CSCs from their tumor niche to the bloodstream. CSCs maintain the unique ability of epithelial-mesenchymal transformation. Mesenchymal cells are circulating in the bloodstream and when they disseminate to specific organs undergo transition again giving rise to primary tumors in a second tumor niche [14]. This theory of cancer generation is supported by observations that patients developed esophageal cancer after bone marrow-derived stem cells (BMSCs) transfusion [39]. In addition, abnormal esophageal alterations compatible with BE caused by providing a rat model with BMSCs and GERD products, bile and acid [53]. However, identification of the circulating CSCs in the bloodstream remains still a challenge, despite the fact that there is a hematopoietic growth factor, called stem cell factor (SCF), which is diagnostic for EAC presenting higher diagnostic sensitivity for EAC diagnosis than carcinoembryonic antigen (CEA), which is an ordinary esophageal tumor marker [62].

Esophageal cancer develops from CSCs that are located among basal cells and presents shorter telomeres than adjacent normal tissue, as well as chromosomal instability in the absence of histological inflammation [103]. This observation has been used to create immortalized epithelial models that simulate esophageal cancer. In such a model, in which immortalization of human esophageal epithelial cells was maintained by human papillomavirus type 16 and human telomerase reverse transcriptase (hTERT), cyclooxygenase-2 (COX-2) seemed to play a crucial role for the inducement and the conservation of the immortalized cells; thus it would be an ideal therapeutic target against esophageal carcinoma even in precancerous stage [124]. Furthermore, nestin, which is a member of the class VI family of intermediate filament proteins and was firstly identified as a protein expressed in progenitor cells of the central and peripheral nervous system, is another molecule that demonstrated an elevated expression in ESCC cell lines and an association with poor prognosis in ESCC patients, as well as a contribution to malignant proliferation and apoptosis of ESCC cell lines [123]. Finally, epithelial-mesenchymal transition (EMT), which has been implied in esophageal cancer morphogenesis as mentioned above, is regulated by TGF-β family molecules such as activin A that is strongly associated with colony formation, increased invasiveness, and cell migration of BE [106].

Several expression products and molecular biomarkers characterize the esophageal CSCs, however, without proven value as diagnostic markers. The most important is SOX2, which is a protein belonging to the family of high-mobility group transcription factors and is pivotal for early development and maintenance of undifferentiated ESCs [90]. Overexpression of SOX2 is responsible for development of ESCC through a complicated regulatory network of microRNAs, kinases, and

signaling molecules. In addition, high expression levels of SOX2 are associated with poor clinical prognosis of ESCC and increased proliferation rates of CSCs [56]. One possible pathway through which SOX2 promotes in vivo tumor growth of ESCC is activation of AKT/mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, which enhances cell proliferation [28]. Furthermore, it has been shown that ESCC arises from esophageal stem/progenitor cells that are located in basal layer, and esophageal tumorigenesis driven by SOX 2 requires the interaction between SOX2 and microenvironment-activated STAT3 [55]. On the other hand, molecules that take part in SOX2-regulated oncogenic signaling pathways could be possible targets for pharmaceutic interventions against esophageal tumorigenesis. LSD1 (also known as KDM1, AOF2, or BHC110) which is a highly conserved flavin adenine dinucleotide (FAD)-dependent, lysine-specific demethylase that was initially found to specifically remove mono- and dimethyl groups from methylated histone H3 at lysine 4 (H3K4) to downregulate gene expression could serve as a selective epigenetic target for therapy in SOX2-expressing cancers [121]. Finally, except from the two most common types of esophageal cancer, SOX2 is highly expressed in small-cell esophageal cancer too. SOX2 overexpression in both small-cell esophageal cancer and esophageal embryogenesis highlights that esophageal small-cell carcinoma may arise from embryonic-like stem cells in the esophageal epithelium. Moreover, the two distinct differentiation patterns (neuroendocrine and glandular) of small-cell esophageal cancer is an indicator of the crucial role that SOX2 plays in the differentiation of pluripotent esophageal stem cells into esophageal small-cell carcinoma cells [36].

Notch signaling pathway which is responsible for multiple developmental activities, including stem cell survival, stem cell fate decision, and regulation of stem cell self-renewal by cross talk with other cell signaling pathways such as Wnt and Hedgehog, has been correlated with ESCC initiation, invasiveness, and metastatic potential. Moreover, Notch signaling has a fundamental role in controlling stem cell numbers through transcriptional activation of HEY (Hairy/enhancer of split related with YRPW motif) gene family members. Overexpression of specific products of Notch signaling pathway, such as HEY1 and HEY2, in ESCC seems to be associated with poor clinical prognosis, as well as increased progression and invasiveness of esophageal tumorigenesis [23]. In addition, Notch1 expression in clinical specimens was located in basal cells of esophageal epithelia and was associated with short survival intervals and high pathological grades of ESCC. On the other hand, in vitro expression of Notch1 in ESCC cell lines was correlated with increased cell aggressiveness and 5-FU drug resistance [54]. Wnt/β-catenin signaling pathway, which is parallel to Notch signaling pathway and has similar activity in maintenance of CSCs leading to poor clinical outcomes, has been associated with ESCC development. More specifically, microRNA-942 (miR-942), which is a crucial contributor to the Wnt/β-catenin signaling pathway, is overexpressed in ESCC leading to poor prognosis for the ESCC patients. Furthermore, miR-942 promotes esophageal tumor spheres formation, $CD90^{+}$ subpopulation cells development, and pluripotency markers expression. Another special role of miR-942 is upregulation of Wnt/β-catenin signaling activity via direct inhibition of sFRP4, GSK3β, and TLE1,

which are multiple-level negative regulators of the Wnt/β-catenin signaling cascade; thus miR-942 could be an ideal therapeutic target for future treatment attempts against esophageal cancer [26]. Additionally, WNT10A, which is another product of Wnt signaling pathway, is overexpressed in ESCC leading to poor clinical outcomes; promotes migration, invasion, and proliferation of transformed esophageal cells; induces a greater $CD44^{high}/CD24^{low}$ population, which are putative markers of cancer stem cells; and increases self-renewal capability of ESCC cells [57].

Aldehyde dehydrogenase-1A1 (ALDH1A1) is overexpressed in esophageal CSCs that initiate and develop ESCC and is associated with the pathological stage and clinical status of the ESCC patients; thus ALDH1A1 could serve as a biomarker for diagnostic and follow-up purposes in ESCC patients and as a prognostic factor too [108]. In addition, Oct4, which is a member of POU-domain transcription factors and is expressed normally by pluripotent cells of embryonic tissue and adult stem cells, seems to play an important role in identifying putative CSCs in esophageal tumor tissue, as well as determining response to treatment. Nevertheless, it has not been yet clarified if the Oct4-positive putative cancer stem cells exist in ESCC or the CSCs properties are acquired by tumor cells as a response to treatment given, resulting immediately in an uncontrolled cell proliferation and consequent treatment failure [109]. Moreover, H3K4 demethylase Jumonji/Arid1b (Jarid1b), which is an epigenetic factor that is required for continuous cell growth in melanoma, seemed to play an important role in maintaining CSCs in the esophagus; thus its continuous inhibition has been under investigation for providing a possible therapeutic option against esophageal and other squamous cell cancers [41]. Furthermore, CD44 and CD117 have been proven to have an important role in esophageal cancer progression; thus they could serve as reliable markers for undifferentiated malignant squamous cells of the esophagus and possible therapeutic targets [29, 89]. Finally, the expression of low-affinity neurotrophin receptor (p75NTR) in the infiltrative margin of ESCC indicates a crucial regulatory molecule of esophageal carcinogenesis and invasiveness with obvious therapeutic potential, the same as Hesca-2, a monoclonal antibody (mAb) IgM raised to the human embryonic stem cells (hESCs), which characterizes esophageal cancer as well [98].

Barrett's esophagus (BE) is the transformation of the original squamous esophageal epithelium into columnar epithelium, a process called intestinal metaplasia, which is considered to be a result of gastroesophageal reflux disease (GERD) and chronic injury caused by exposure to intestinal bile salts and gastric acid. There are three types of columnar epithelial cells metaplasia that substitute for squamous epithelium: (i) the intestinal type, which includes intestinal mucin (MUC2)-expressing goblet cells, as well as other intestinal cells, and is strongly associated with progression to EAC; (ii) the cardia type, which includes mucus cells; and (iii) the gastric fundic type, which includes mucus, parietal, and chief cells. The exact molecular events that lead from normal squamous esophageal epithelium to intestinal metaplasia (BE) and progression to EAC remain unclear. There are four theories about the orientation of a progenitor cell that gives rise to intestinal epithelium among squamous cells. Firstly, an esophageal squamous cell could be converted in an intestinal

columnar cell, a phenomenon called transdifferentiation. In addition, a native esophageal progenitor cell could diverge from its normal fate and differentiate into an intestinal columnar cell (esophageal progenitor cell transcommitment). Moreover, a circulating bone marrow-derived stem cell in the bloodstream could attach to the esophageal epithelium and differentiate into an intestinal columnar cell (circulating stem cell transcommitment). Finally, an adjacent columnar cell from gastroesophageal junction or gastric cardia could shift to replace a gap in esophageal squamous epithelium due to an injury and then undergoes intestinal differentiation (columnar progenitor cell transcommitment). There have been no indications yet about which theory is most possible to exist; however there are no indications that all four theories are not true [113].

Transdifferentiation is supported by the presence of multilayered epithelium (MLE) that contains both squamous and columnar esophageal cells. In addition, MLE in BE is characterized by a "transitional zone" of epithelial cells that demonstrate morphological features of both squamous and columnar epithelial cells, such as intercellular ridges, distinct microridges, microvilli, and bulging mucus. Nevertheless, microscopic assessment of normal gastroesophageal junction (GEJ) area did not demonstrate characteristics of transitional zone cells [97]. Moreover, the biphenotypic cell population of MLE is supported by the fact that esophageal basal epithelial cells of MLE express a combination of cytokeratin subtypes that are found in both squamous (CK4) and columnar (CK19) cells, and the stimulus of these MLE basal cells, after the intestinal transcription factor Cdx2 overexpression using CK14 promoter, causes the acquirement of both squamous and secretory features by MLE cells [8, 47]. However, the failure of complete transdifferentiation of a squamous epithelial cell into a columnar cell in vitro so far has raised some concerns against the theory of transdifferentiation. On the other hand, production of differentiated squamous epithelial cells that present several features of intestinal mucus producing cells resembling BE cells has been achieved, after the overexpression of the transcriptional factors HET-1A, EPC2, NES-B3T, and NES-B10T [35, 114]. Consequently, a pluripotent basal cell with preserved features of stemness seems to be required in order this formation of an intestinal goblet cell from a differentiated squamous cell to be achieved.

Transcommitment, which describes the genetic reprogramming of stem or progenitor cells in order to proliferate and differentiate into different cell types than they were initially programmed to do, seems to be a basic condition for developing BE intestinal metaplasia regardless the progenitor cells orientation. For example, BE epithelium could include Paneth cells, enteroendocrine cells, and goblet cells, which are usually diagnostic for BE [52]. BE development in patients that had undergone partial esophagectomy including GEJ and gastric cardia outlines that proximal shifting of progenitor cells from GEJ or gastric cardia to the main esophagus does not explain BE formation in every situation; however it seems that a reprogramming of the residual esophageal squamous or glandular progenitor cells takes place [60]. Furthermore, recurrence of BE development after ablation due to BE lesions demonstrates that esophageal epithelium is susceptible to environmental conditions that is exposed to, and as a result differentiation depends on them, as

normal reepithelialization with squamous epithelial cells happens after BE ablation when gastric acid levels are low in esophageal area, while development of BE lesions rises after previous ablation when gastric acid levels in the esophagus are high due to several reasons, such as non-compliance to acid-diminishing pharmaceutic therapy after ablation [9]. Finally, progenitor cells located in esophageal glands could give rise to multiple phenotypes, either squamous or columnar, due to transcommitment, as it has been observed that neo-squamous epithelium after ablation due to BE lesions shares the same mitochondrial DNA mutation with the underlying metaplastic epithelium of the submucosal esophageal glands that led to BE formation [74].

Passing from squamous fate to intestinal fate for an esophageal progenitor cell requires activation of transcription factors that would give the progenitor cell a columnar phenotype such us Sox9, as well as downregulation of the transcription factors that determined the squamous phenotype such as Sox2 and p63. In addition, the final differentiation into a goblet cell requires the expression of intestinal (Cdx1 and Cdx2) and mucus-related (Foxa2) transcription factors.

Sox9 is a member of Sox genes family and is expressed in intestinal crypts of GI tract as well as Paneth cells. In addition, Sox9 expression has been reported in esophageal embryogenesis together with CK8 and CK18, but when the epithelium matures to squamous, Sox9 expression gets lost. Sox9 has been described to express in 100% of BE specimens and in 85% of EAC specimens; however there was no expression in adjacent normal esophageal tissue [112]. Environmental conditions in the esophagus have been proven to play a crucial role in esophageal epithelium fate. As a result, Sox9 activation is caused by bile- and acid-stimulated Hedgehog ligand secretion by epithelial cells, which in turn induce BMP4 secretion by adjacent stromal cells. This stromal BMP4 acts back by increasing Sox9 expression [6]. In addition, the retroviral transduction of Sox9 in a mouse transplant culture system upregulated the expression of columnar CK8 and intestinal glycoprotein A33, as well as altered the esophageal epithelium architecture with inducement of one to two layers of cuboid or columnar-shaped epithelial cells. On the other hand, no alteration was observed neither in squamous epithelium architecture nor in gene expression of the transplant culture mouse model, after retroviral transduction of Cdx2, indicating the crucial role of Sox2 in BE development by altering the esophageal progenitor cells fate directing them toward intestinal phenotype [13].

Sox2 is another member of the Sox gene family that is expressed during esophageal embryogenesis and is responsible for maturing esophageal epithelium into its squamous phenotype [87]. Downregulation of Sox2 in mice leads to a thinner esophageal epithelium, characterized by mucus-secreting columnar cell, as well as decreased expression of p63 and CK14. Nevertheless, overexpression of Sox2 in mouse intestine caused loss of villi; appearance of p63 expressing basal cells, which are characteristic for esophagus and forestomach; and decreased attachment of Cdx2 to the promoters of its target genes [88]. Except from the role of squamous differentiation, Sox2 is responsible for the maintenance of stem cells, as its overexpression in several mouse models leads to esophageal basal cells hyperplasia [55]. Consequently, in the normal adult esophagus, Sox2 is expressed in the progenitor

basal cells of the stratified epithelium, while it is not expressed in MLE or in intestinal metaplasia of BE; thus its downregulation could be an important condition for reprogramming esophageal progenitor cells from which BE arises [11].

P63 is a member of the P53 transcription factors family and presents six isoforms. The key role of downregulated p63 in BE formation has been proven by studies in which mice null for p63 completely lack squamous esophageal epithelium and presents esophagi with simple columnar epithelium [17]. P63 presents escalated expression in esophageal epithelium depending on the different stages of esophageal dysplasia. More specifically, it presents absent to moderate expression in Barrett's esophagus and high expression in Barrett's esophagus with high-grade dysplasia and esophageal adenocarcinoma [34]. High expression of p63 has been observed in normal esophageal epithelium and esophageal squamous cell carcinomas too [27]. Despite the conflicting results of the studies mentioned above, it has been clarified that p63 is required for squamous differentiation, and BE without dysplasia is not likely to express P63, while adenocarcinomas may weakly express P63. In addition, combined exposure of esophageal squamous epithelium to bile salts and acid, like happening in patients suffering from GERD, diminishes the p63 expression in squamous cells leading to transcommitment of esophageal progenitor cells and consequent BE development [92].

However, esophageal intestinal metaplasia does not stop with the acquisition of columnar phenotype by esophageal epithelial cells. Cdx1 and Cdx2 are members of the caudal-related homeobox gene family and are expressed in the intestine, with Cdx1 expressing in the proliferative crypt compartment while Cdx2 in differentiated villus compartment [32]. The role of Cdx1 in BE development had been outlined after the observation that transgenic Cdx1 mice presented intestinal metaplasia of the gastric epithelium including all four cell types of the adult colon such as enterocytes, Paneth cells, goblet cells, and enteroendocrine cells [72]. Moreover, CDX1 mRNA has been found in Barrett's metaplastic tissue, but not in normal esophageal squamous tissue highlighting the ability of Cdx1 in reprogramming columnar progenitors into intestinal columnar cells [116]. Furthermore, increased expression of Cdx1 was observed in the metaplastic epithelium of a rat BE model, which was further induced after bile acid exposure of the esophageal epithelial cells, and promoted upregulation of Cdx2 expression as well, indicating the crucial role of Cdx1 in pathogenesis of BE after condition similar to GERD and its regulatory effect to the Cdx2 expression, establishing a positive feed-forward intestinalization loop [44].

Cdx2 has been involved in transcommitment of columnar progenitor cells in patients with GERD that present BE due to the observation that CDX2 expression has been found in 100% of biopsy specimens from nondysplastic and dysplastic Barrett's metaplasia and esophageal adenocarcinoma [31]. In addition, CDX2 expression has been found in inflamed esophageal squamous epithelium of GERD patients, but not in normal non-inflamed esophageal epithelium [83]. Moreover, human esophageal squamous epithelial cells from GERD patients with Barrett's esophagus differentially respond to acid and bile salt exposure by upregulating CDX2 when compared to human esophageal squamous epithelial cells from GERD

patients without Barrett's esophagus [71]. Finally, Cdx2 seems to be insufficient of stimulate a squamous cell transformation into intestinal cell, unless epigenetic alterations happen, while Cdx2 is able to promote intestinal metaplasia in columnar cells. However, Cdx2 remains a major transcriptional activator within the intestine; thus loss of its expression results in intestinal progenitor cell reprogramming into squamous cells [25, 66, 96].

Intestinal phenotype is characterized by mucus secretion, which in BE is provided by FOXA2 expression by intestinal columnar cells through presumed transcriptional regulation of MUC2 itself and of AGR2, which is required for proper processing of the MUC2 protein. Despite the fact that FOXA2 expression led to MUC2 protein expression, the cells did not acquire a full goblet cell phenotype [110]. It is possible that other factors may be required additionally to FOXA2 to induce a goblet cell phenotype. These other factors could include downregulation of SOX2 and P63, and similar to Noggin null mice, in which Bmp4 signaling is unopposed, Sox2 null or p63 null mouse embryos have esophagi with columnar epithelium containing goblet-like cells. In addition, Notch pathway modulation may also be required for the formation of goblet cells, as loss of Notch signaling in a surgical model of reflux esophagitis and Barrett's metaplasia led to almost a complete conversion of metaplastic epithelial cells to differentiated goblet cells [68].

Stem Cells in Novel Esophageal Therapeutic Attempts

The unique feature of the esophagus to include a functional population of pluripotent stem cells which regulate its homeostasis and are responsible for repairing possible injuries is usually the reason for several esophageal diseases that are related to stem cells pathophysiology; however stem cell molecular pathways could be possible therapeutic targets for such modalities. Recently, esophageal cancer has been correlated with CSCs, which seem to be responsible for resistance to chemotherapy and radiation; thus they are very attractive pharmacologic targets. CSCs express a variety of molecular markers such as CD44, CD133, and ALDH that contribute to drug resistance and give them features like quiescence, evasion of apoptosis, resistance to DNA damage, and expression of drug transporter pumps. In vitro clonogenic assays with sphere formation and in vivo studies in xenograft models demonstrate the stem-like self-renewal and differentiation capacities of CSCs. Consequently, future therapeutic trials should aim in the direction of exactly clarifying the mechanisms by which CSCs contribute to drug resistance in order to reveal specific molecular targets against esophageal CSCs [20].

Since irradiation has been inducted in the therapeutic protocols of the majority of the thoracic tumors, it has been demonstrated as one of the main factors that cause esophageal injury, commonly complicated with esophagitis. Nevertheless, the presence of subpopulations of esophageal progenitor cells that are characterized by in vitro ability of differentiation to multiple adherent lineages of cells gives the opportunity of using the isolated pluripotent esophageal cells in gene therapy

technology. There are ionizing irradiation mouse models, in which esophageal progenitor cells have been isolated either by the side population method or the serial preplate technique, and demonstrated repopulation in the irradiated esophagus of the recipient mouse [19]. More specifically, the side population cells differentiated to endothelin or vimentin positive colonies, while preplate cells formed colonies that were uni-lineage, bi-lineage, or tri-lineage for macrophage, endothelin, or vimentin positive colonies in vitro. On the other hand, there was no difference in the type of colonies that the two cell types formed in methylcellulose culture. As a result, the utilization of transgenes for the creation of soluble growth factors that would enhance repair process may facilitate innovating transplantation techniques for tissue regeneration after irradiation. Gene therapy with manganese superoxide dismutase plasmid liposome (MnSOD-PL) seems to be protective for esophageal side population cells against irradiation damage both in vivo and in vitro [77]. Another application of the stem cells technology that seems to contribute in healing after radioactive esophageal injury is dental pulp stem cell (DPSC) transplantation. DPSCs were cultured and transplanted into rats in which radioactive esophageal injury had been induced using radioactive I^{125}. In the injured esophagus, the labeled DPSCs were observed to co-localize with the SCs markers PCNA, CK14, CD71, and integrin α_6, which presented increased levels of expression too. After DPSCs transplantation, esophageal tissue presented an increase in epithelial thickness in combination with recovered esophageal functionality and diminished inflammation in the esophageal area [120].

Gastroesophageal reflux (GERD) is another common cause of chronic esophageal injury, which is managed pharmacologically in the majority of cases. However, pharmacologic management of GERD is restricted to the cure of symptoms and the complications, instead of facing the cause, which is the relaxation of the lower esophageal sphincter. As a result, there have been held a lot of studies on alternative invasive therapeutic options for GERD. Endoscopic injections of inert materials or cells have been attempted through the years with controversial results. Nevertheless, the injection of muscle precursor cells (MPCs) that were derived from expanded satellite cells isolated from skeletal muscle fibers, in the gastroesophageal junction presented promising results, offering both regenerative and functional action [22]. In addition, a full-thickness esophageal damage could be caused after swallowing of corrosive substances, with stricture formation presenting as a late complication, due to esophageal SCs destruction. The transplantation of MSCs in rats that had undergone caustic esophageal injury presented increased accumulation of MSCs at the site of injury, but there was no difference in healing between the transplanted and the control group histopathologically. However, new epithelial and muscle cells oriented by the transplanted MSCs were revealed. Consequently, transplantation of MSCs after caustic esophageal injury seems to be effective, but often injections seem to be required [42]. Moreover, esophageal damage has been studied in animal models of esophagogastric myotomy, in which autologous bone marrow mesenchymal stem cells (BM-MSCs) have been tested for their effectiveness to repair the lower esophageal sphincter (LES) after surgery. The results were interestingly promising as the autologous BM-MSCs improved muscle regeneration and

increased the contractile function of the damaged LES, without losing their position at the site of injury and without any phenotype alteration toward smooth or striated muscle cell [67].

Barrett's esophagus (BE) is the most common dysplasia that happens to the esophageal epithelium and the most common precursor lesion for esophageal cancer. During the last years, several efforts have been conducted for the investigation of new therapeutic alternatives instead of the radical surgical methods that have been applied over the years. Endoscopic ablation of BE foci with radiofrequency (RF) technology seems an effective alternative; however the system of ablation should achieve a balance between the radical excision of all the dysplastic cells from the esophageal epithelium, but not deeply enough to cause esophageal perforation or stricture formation. The HALO system seems to achieve the perfect balance using a balloon-based array of closely spaced electrodes to deliver radiofrequency energy to the esophageal mucosa, providing efficacy and safety at the same time [101]. Nevertheless, stem cells pathophysiology gave the chance for testing both the effectiveness of the RF ablation of BE lesions and the safety of the procedure. Enhanced AKT-mediated β-catenin phosphorylation, which is present in activated progenitor cells, is a characteristic of BE-associated carcinogenesis. Three months after RF ablation of BE lesions, an increased expression of AKT-mediated phosphorylated β-catenin was observed, while this increase was followed by a deep quiescence 6 months after RF ablation [49]. These findings reveal that 3 months after RF ablation, a repair process takes place in the neo-squamous esophageal epithelium.

Except from complicated gene therapies and molecular treatments, oral administration of agents that are based on the SCs principles could be proven effective in several esophageal diseases even cancer. An excellent example of this situation is the orally administered conditioned medium derived from mesenchymal stem cells after endoscopic submucosal dissection in the esophagus. The conditioned medium gel prevents esophageal stricture after the endoscopic procedure, diminishes the number of activated myofibroblasts, downsizes the fiber sickness, and restricts the inflammatory infiltration of neutrophils and macrophages at the site of excision [69]. For that reason, it could be used as a preventive agent of esophageal stricture after endoscopic procedures in the esophagus. However, orally administered agents are not used only for the prevention of mechanical injuries of the esophagus or benign diseases but even for cancer prevention. Several studies refer to the preventive role of aspirin against cancer. But, why aspirin could be so beneficial against a so complicated disease? The esophagus, and other tissues with increased concentration of SCs, gives the answer. Inflammatory stress which may be caused due to several reasons, different for each organ, provokes the SCs of each tissue to proliferate through prostaglandins and especially PGE2. Increased proliferative rates for a SC population lead to raised chance for mutations and so for CSCs creation. The anti-inflammatory capacity of aspirin against PGE2 is the key feature that makes it so useful against cancer, especially in tissues that contain squamous cell epithelium and high numbers of SCs, like the esophagus [58].

Apparently from human studies on SCs therapies or animal models based on human patterns, original animal studies have contributed to new therapeutic approaches against esophageal diseases. First of all, porcine 3D culture models have been developed that reproduce esophageal gland proliferation in vivo and provide laboratory technology with two different phenotypes of spheroids: one that expresses markers of squamous epithelium and one that expresses markers of columnar epithelium [111]. These models could allow the evaluation of the molecular factors that drive epithelialization toward the squamous or the columnar direction, as well as the generation of technically manufactured scaffold for different applications related to esophageal diseases. Furthermore, the unique feature of echinoderms to reconstruct both external appendages and internal organs has been studied during the last years and has uncovered plenty of secrets about SCs biology such as Notch signaling and expression of SCs markers (Piwi and Vasa) that are expressed in human tissues like the esophagus [91]. All this data will be the basis for the future therapies in organs (esophagus) that are molecularly similar to these organisms.

The complicated congenital diseases of the esophagus such as esophageal atresia or tracheoesophageal fistula and several benign esophageal diseases like caustic ingestion of toxic substances, esophageal cancer, and radical surgical operations of the adjacent thoracic or abdominal organs that involve the esophagus due to its anatomical complexity require replacement of whole or segment of the esophagus. So far, surgical connection of the two remaining esophageal segments and replacement of the missing segment with a transplant from an adjacent organ, like the stomach or large bowel, were the only therapeutic options after esophageal dissection [12]. Nevertheless, the raised morbidity rates after the surgical repairs in combination with the simple function of food and water transport from the pharynx to the stomach that the esophagus is responsible for raised the efforts of constructing a functional substitute based on the principles of tissue engineering, which includes tissue scaffolds, cell sources, and bioreactors (Fig. 2) [85]. Tissue engineering for the esophagus, as well as the rest of the tubular organs of the intestinal tube, utilizes somatic cells from human fibroblasts that are reprogrammed into induced pluripotent cells (iPS), which are able to differentiate into any type of cell of the three germ layers [107]. Autologous muscle cells, epithelial cells, or mesenchymal stem cells (MSCs) that are provided by this process undergo reproduction and then are implanted into artificial scaffolds that are constructed using biological materials. The enriched scaffolds are left to mature in a bioreactor toward the direction of the willing organ [85].

In the case of gastrointestinal organs, like the esophagus, scaffolds have to support proliferation, differentiation, and attachment of the iPS; thus both artificial materials and biological substances have been investigated for their properties to provide the ideal scaffold for esophageal tissue engineering [7]. A variety of materials such as polylactic acid (PLA), polyvinylidene fluoride (PVDF), polyglycolic acid (PGA), poly-dl-lactic acid (PLGA), poly-l-lactide-co-caprolactone (PLLC), polyvinylidene fluoride (PVDF), poly-caprolactone (PCL), and poly-l-lactic acid (PLLA) have been used for the construction of artificial scaffolds [18, 30, 63, 125]. Nevertheless, the use of these materials in scaffold constructing for tissue engineering

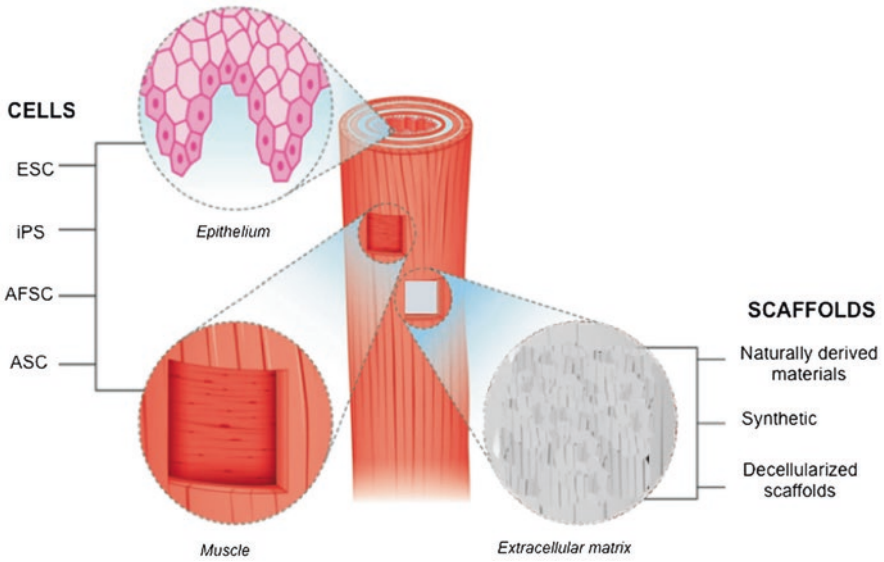

Fig. 2 Esophageal tissue engineering requires the combination of appropriate scaffolds and cells. Cells used for repopulation of the epithelial and muscular layers can be derived from ESC, iPS, AFSC, and ASC. ESC embryonic stem cells, iPS induced pluripotent stem cells, AFSC amniotic fluid stem cells, ASC adult stem cells [64]

has been correlated with anastomotic leakage and stricture formation after surgical operation. On the other hand, these limitations seem to be overtaken with the induction of acellular biological tissue scaffold in tissue-engineering technology. Acellular tissue scaffolds maintain the extracellular matrix (ECM) of the original tissue that they come from, presenting the advantage of improved cellular attachment on the scaffold [45, 46, 51]. In addition, they usually include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF), which enhance the vascularization of an implanted transplant [61]. Acellular scaffolds include the esophageal submucosa, small intestinal submucosa, aortic acellular matrix, and aortic acellular matrix [21].

Except from a substrate where the "artificial esophagus" will develop, esophageal tissue-engineering models require a source of cells that would provide the developing esophagus with the appropriate number of functional cells in order to proliferate and differentiate into the specific esophageal epithelium. A possible source of cells for the developing esophagus could be provided by the adjacent esophageal epithelium, from which epithelial cells would migrate toward the developing organ [64, 82]. However, this process would be uncontrolled in terms of cell orientation that would be sparse and time intervals that would be undefined. Another possible technique that has been described is the transplantation of autologous buccal epithelial cells, which offer the advantage of the immunological similarity with the recipient tissue minimizing the risk for immune-mediated rejection, as well as

the capacity of inducing muscular regeneration at the site of implantation, with the latter being a controversial observation [86, 100]. Therefore, a specified cell source that would provide both epithelial esophageal cells that would protect the scaffold from caustic injuries and infections and specified muscle cells that would support the construction and help peristaltic function of the "artificial organ" is required. This cell source is provided by patient-derived iPS, for instance, bone marrow mesenchymal stem cells or adipose-derived stem cells, which differentiate into mesenchymal stem cells (MSCs), which maintain the ability of differentiating both in the direction of epithelial cells and in the direction of smooth muscle cells [76, 78, 81, 115]. Finally, the production of esophageal organoid units (EOU) after transplantation of murine-derived tissue-specific stem/progenitor cells in vitro in a degradable biological scaffold and after a few days, re-transplantation in vivo at the site of esophageal defect, with the formation of expanding spheres of proliferative basal cells on a neuromuscular network that demonstrated spontaneous peristalsis, gave another prospective in esophageal tissue engineering [95, 102].

After implantation of the cell source in the artificial or biological esophageal scaffold, two very important issues need to be overcome. Firstly, the new developing esophagus requires blood supply in order to maintain nutritional exchange for the proliferating iPS and differentiating epithelial cells. A possible approach is the implantation of the graft into the omentum or latissimus dorsi muscle before connecting it with the esophagus. Another option is to deliver angiogenic growth factors, such as fibroblast and platelet-derived growth factors, to the transplanted segment after implantation in the esophagus. Retention of VEGF in the protocol of an acellular scaffold after decellularization in combination with the proangiogenic properties of the scaffold enhances angiogenesis in a rodent tissue-engineering model [99]. In addition, gastrointestinal organs like the esophagus require peristaltic movement in order to be fully functional; thus a local neural network to initiate and maintain esophageal peristalsis is required. Intestinal organoids recombined with iPS-derived neural crest cells, which differentiated into neurons and glial cells, provided a neural network that was successfully integrated into intestinal smooth muscle and achieved a rhythmic wave movement [117].

Several animal models that apply the esophageal tissue-engineering expertise in the field have been developed. A full-thickness circumferential replacement of the esophagus of pigs has been attempted using synthetic polyurethane electrospun grafts seeded with autologous adipose-derived mesenchymal stem cells and a disposable bioreactor. After adipose tissue biopsy in order to provide adipose-derived mesenchymal stem cells, pigs underwent endoscopic circumferential resection of the mid-lower segment of the esophagus and replacement with the engineered scaffold. This model demonstrated gradual structural regrowth of endogenous esophageal tissue, including squamous esophageal mucosa, submucosa, and smooth muscle layers with blood vessel formation [50]. Furthermore, there has been a comparison between an acellular scaffold seeded with MSCs and an acellular scaffold alone after a 3 cm circumferential resection of the abdominal esophagus in a pig model. The comparative histological analysis presented a mature squamous epithelium covering the scaffold at 45th postoperative day for the MSCs group, while in

the control group, the mature esophageal epithelium was observed at 95th postoperative day. Moreover, desmin-positive cells were observed in the graft area in the MSCs group at 45th postoperative day, indicating muscle cell colonization, while in the control group, desmin-positive cells were never observed [10]. In addition, a dog model of 5 cm half circumference replacement of the esophagus with a small intestine scaffold seeded with BM-MSCs highlighted an increase in reepithelialization, revascularization, and muscular regeneration compared to the control group that included transplant of a small intestine scaffold alone [104].

Despite the fact that the findings of the in vitro experimental and animal models of esophageal tissue engineering are impressive, very few applications of tissue-engineered scaffolds have been conducted to humans. Esophageal endoscopic procedures that are the basic therapeutic option for early esophageal cancer in the stage of BE very often cause scar ulcers that lead to stricture formation, which is accompanied by annoying symptoms and requires often dilatations. Circumferential sleeve resection of the mucosa and placement of an ECM scaffold over the site of the resected tissue in five patients with high-grade esophageal dysplasia or BE demonstrated a successful prevention of intractable stricture, as well as complete maturation of squamous esophageal epithelium over the placed ECM scaffold 4 months after the operation [3]. Moreover, patch esophagoplasty with urinary bladder-ECM scaffolds in four patients presenting strictures due to surgery or past ingestion of a caustic substance outlined stricture avoidance and recovery of the oral intake with an obvious improvement of the patient's quality of life [75]. Finally, the application of cell sheets composed of the patients' oral mucosa (using a temperature-responsive culture dish) over post-ESD esophageal ulcers after endoscopic procedures due to esophageal carcinoma presented reduction of the reepithelialization period and stricture prevention of post-ESD stricture [79].

Conclusion

The principles of embryonic stem cells (ESCs) interaction have been utilized to explain the secrets of the development of the esophagus postnatally. In addition, the investigation of the metabolic pathways that regulate ESCs' differentiation into esophageal progenitor cells and finally into differentiated esophageal squamous epithelial cells established a matching between specific SCs markers and different steps of esophageal development. Consequently, specific molecules, such us mediators or receptors, have been correlated to specific cell features. Furthermore, SCs fundamentals have been applied in the explanation of the pathophysiology of several benign esophageal diseases such as eosinophilic esophagitis or esophageal achalasia.

Scientific progress about SCs has been widely used in the field of esophageal tumorigenesis and clinical features of esophageal malignancies. The raised morbidity and mortality rates of esophageal adenocarcinoma (EAC) and esophageal squamous cell cancer (ESCC) formed the necessity to reveal the behavioral patterns of

these tumors as well as the molecular factors that affect their clinical outcome. SCs played an important role to this as it is considered that esophageal cancer arises from a population of cancer stem cells (CSCs), a theory called the CSCs hypothesis. Moreover, the main precursor lesion of esophageal cancer, Barrett's esophagus (BE), causes a columnar metaplasia in the esophageal epithelium with an intestinal pattern instead of the ordinary squamous epithelium, due to progenitor cells' mutations and altered molecular profile.

A large amount of pediatric and adult esophageal diseases such as esophageal atresia, tracheoesophageal fistula, and post-endoscopic esophageal stricture require radical surgical treatment with high morbidity rates. Nevertheless, the development of SCs technology has opened new ways in the management of such modalities with the invention of tissue-engineered esophageal scaffolds that could replace circumferentially the damaged part of the natural esophagus. In addition, molecular therapies based on esophageal CSCs and progenitor cells markers have been developed, which target exclusively esophageal cancer cells without affecting other organs, ensuring greater quality of life for the patient with the highest effectiveness against the tumor.

References

1. Alcolea MP. Oesophageal stem cells and cancer. Adv Exp Med Biol. 2017;1041:187–206.
2. Alcolea MP, Greulich P, Wabik A, Frede J, Simons BD, Jones PH. Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change. Nat Cell Biol. 2014;16:615–22.
3. Badylak SF, Hoppo T, Nieponice A, Gilbert TW, Davison JM, Jobe BA. Esophageal preservation in five male patients after endoscopic inner-layer circumferential resection in the setting of superficial cancer: a regenerative medicine approach with a biologic scaffold. Tissue Eng Part A. 2011;17:1643–50.
4. Barbera M, di Pietro M, Walker E, Brierley C, MacRae S, Simons BD, Jones PH, Stingl J, Fitzgerald RC. The human squamous oesophagus has widespread capacity for clonal expansion from cells at diverse stages of differentiation. Gut. 2015;64:11–9.
5. Barker N. Epithelial stem cells in the esophagus: who needs them? Cell Stem Cell. 2012;11:284–6.
6. Bien-Willner GA, Stankiewicz P, Lupski JR. SOX9cre1, a cis-acting regulatory element located 1.1 Mb upstream of SOX9, mediates its enhancement through the SHH pathway. Hum Mol Genet. 2007;16:1143–56.
7. Bitar KN, Raghavan S, Zakhem E. Tissue engineering in the gut: developments in neuromusculature. Gastroenterology. 2014;146:1614–24.
8. Boch JA, Shields HM, Antonioli DA, Zwas F, Sawhney RA, Trier JS. Distribution of cytokeratin markers in Barrett's specialized columnar epithelium. Gastroenterology. 1997;112:760–5.
9. Brandt LJ, Blansky RL, Kauvar DR. Repeat laser therapy of recurrent Barrett's epithelium: success with anacidity. Gastrointest Endosc. 1995;41:267.
10. Catry J, Luong-Nguyen M, Arakelian L, Poghosyan T, Bruneval P, Domet T, Michaud L, Sfeir R, Gottrand F, Larghero J, Vanneaux V, Cattan P. Circumferential esophageal replacement by a tissue-engineered substitute using mesenchymal stem cells: an experimental study in mini pigs. Cell Transplant. 2017;26:1831–9.

11. Chen X, Qin R, Liu B, Ma Y, Su Y, Yang CS, Glickman JN, Odze RD, Shaheen NJ. Multilayered epithelium in a rat model and human Barrett's esophagus: similar expression patterns of transcription factors and differentiation markers. BMC Gastroenterol. 2008;8:1.
12. Chirica M, Veyrie N, Munoz-Bongrand N, Zohar S, Halimi B, Celerier M, Cattan P, Sarfati E. Late morbidity after colon interposition for corrosive esophageal injury: risk factors, management, and outcome. A 20-years' experience. Ann Surg. 2010;252:271–80.
13. Clemons NJ, Wang DH, Croagh D, Tikoo A, Fennell CM, Murone C, Scott AM, Watkins DN, Phillips WA. Sox9 drives columnar differentiation of esophageal squamous epithelium: a possible role in the pathogenesis of Barrett's esophagus. Am J Physiol Gastrointest Liver Physiol. 2012;303:G1335–46.
14. Croagh D, Frede J, Jones PH, Kaur P, Partensky C, Phillips WA. Esophageal stem cells and genetics/epigenetics in esophageal cancer. Ann N Y Acad Sci. 2014;1325:8–14.
15. Croagh D, Phillips WA, Redvers R, Thomas RJ, Kaur P. Identification of candidate murine esophageal stem cells using a combination of cell kinetic studies and cell surface markers. Stem Cells. 2007;25:313–8.
16. Croagh D, Thomas RJ, Phillips WA, Kaur P. Esophageal stem cells – a review of their identification and characterization. Stem Cell Rev. 2008;4:261–8.
17. Daniely Y, Liao G, Dixon D, Linnoila RI, Lori A, Randell SH, Oren M, Jetten AM. Critical role of p63 in the development of a normal esophageal and tracheobronchial epithelium. Am J Physiol Cell Physiol. 2004;287:C171–81.
18. Diemer P, Markoew S, Le DQ, Qvist N. Poly-epsilon-caprolactone mesh as a scaffold for in vivo tissue engineering in rabbit esophagus. Dis Esophagus. 2015;28:240–5.
19. Epperly MW, Shen H, Jefferson M, Greenberger JS. In vitro differentiation capacity of esophageal progenitor cells with capacity for homing and repopulation of the ionizing irradiation-damaged esophagus. In Vivo. 2004;18:675–85.
20. Facompre N, Nakagawa H, Herlyn M, Basu D. Stem-like cells and therapy resistance in squamous cell carcinomas. Adv Pharmacol. 2012;65:235–65.
21. Fan MR, Gong M, Da LC, Bai L, Li XQ, Chen KF, Li-Ling J, Yang ZM, Xie HQ. Tissue engineered esophagus scaffold constructed with porcine small intestinal submucosa and synthetic polymers. Biomed Mater. 2014;9:015012.
22. Fascetti-Leon F, Malerba A, Boldrin L, Leone E, Betalli P, Pasut A, Zanon GF, Gamba PG, Vitiello L, De Coppi P. Murine muscle precursor cells survived and integrated in a cryoinjured gastroesophageal junction. J Surg Res. 2007;143:253–9.
23. Forghanifard MM, Taleb S, Abbaszadegan MR. Notch signaling target genes are directly correlated to esophageal squamous cell carcinoma tumorigenesis. Pathol Oncol Res. 2015;21:463–7.
24. Frede J, Greulich P, Nagy T, Simons BD, Jones PH. A single dividing cell population with imbalanced fate drives oesophageal tumour growth. Nat Cell Biol. 2016;18:967–78.
25. Gao N, White P, Kaestner KH. Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. Dev Cell. 2009;16:588–99.
26. Ge C, Wu S, Wang W, Liu Z, Zhang J, Wang Z, Li R, Zhang Z, Li Z, Dong S, Wang Y, Xue Y, Yang J, Tan Q, Wang Z, Song X. miR-942 promotes cancer stem cell-like traits in esophageal squamous cell carcinoma through activation of Wnt/beta-catenin signalling pathway. Oncotarget. 2015;6:10964–77.
27. Geddert H, Kiel S, Heep HJ, Gabbert HE, Sarbia M. The role of p63 and deltaNp63 (p40) protein expression and gene amplification in esophageal carcinogenesis. Hum Pathol. 2003;34:850–6.
28. Gen Y, Yasui K, Nishikawa T, Yoshikawa T. SOX2 promotes tumor growth of esophageal squamous cell carcinoma through the AKT/mammalian target of rapamycin complex 1 signaling pathway. Cancer Sci. 2013;104:810–6.
29. Goscinski MA, Larsen SG, Giercksky KE, Nesland JM, Suo Z. PDGFR-alpha and CD117 expression pattern in esophageal carcinomas. Anticancer Res. 2015;35:3793–9.

30. Grikscheit T, Ochoa ER, Srinivasan A, Gaissert H, Vacanti JP. Tissue-engineered esophagus: experimental substitution by onlay patch or interposition. J Thorac Cardiovasc Surg. 2003;126:537–44.
31. Groisman GM, Amar M, Meir A. Expression of the intestinal marker Cdx2 in the columnar-lined esophagus with and without intestinal (Barrett's) metaplasia. Mod Pathol. 2004;17:1282–8.
32. Guo RJ, Suh ER, Lynch JP. The role of Cdx proteins in intestinal development and cancer. Cancer Biol Ther. 2004;3:593–601.
33. Hagey DW, Klum S, Kurtsdotter I, Zaouter C, Topcic D, Andersson O, Bergsland M, Muhr J. SOX2 regulates common and specific stem cell features in the CNS and endoderm derived organs. PLoS Genet. 2018;14:e1007224.
34. Hall PA, Woodman AC, Campbell SJ, Shepherd NA. Expression of the p53 homologue p63alpha and DeltaNp63alpha in the neoplastic sequence of Barrett's oesophagus: correlation with morphology and p53 protein. Gut. 2001;49:618–23.
35. Huo X, Zhang HY, Zhang XI, Lynch JP, Strauch ED, Wang JY, Melton SD, Genta RM, Wang DH, Spechler SJ, Souza RF. Acid and bile salt-induced CDX2 expression differs in esophageal squamous cells from patients with and without Barrett's esophagus. Gastroenterology. 2010;139:194–203 e191.
36. Ishida H, Kasajima A, Kamei T, Miura T, Oka N, Yazdani S, Ozawa Y, Fujishima F, Sakurada A, Nakamura Y, Tanaka Y, Kurosumi M, Ishikawa Y, Okada Y, Ohuchi N, Sasano H. SOX2 and Rb1 in esophageal small-cell carcinoma: their possible involvement in pathogenesis. Mod Pathol. 2017;30:660–71.
37. Jiang M, Ku WY, Zhou Z, Dellon ES, Falk GW, Nakagawa H, Wang ML, Liu K, Wang J, Katzka DA, Peters JH, Lan X, Que J. BMP-driven NRF2 activation in esophageal basal cell differentiation and eosinophilic esophagitis. J Clin Invest. 2015;125:1557–68.
38. Kalabis J, Oyama K, Okawa T, Nakagawa H, Michaylira CZ, Stairs DB, Figueiredo JL, Mahmood U, Diehl JA, Herlyn M, Rustgi AK. A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. J Clin Invest. 2008;118:3860–9.
39. Kano Y, Ishii H, Konno M, Yamasaki M, Miyata H, Nishikawa S, Hamabe A, Ogawa H, Takahashi H, Ohta K, Hasegawa S, Tanaka K, Fukusumi T, Otsuka M, Kawamoto K, Haraguchi N, Fujimoto R, Isobe M, Tomita Y, Matsuura N, Takiguchi S, Mori M, Doki Y. Cells of origin of squamous epithelium, dysplasia and cancer in the head and neck region after bone marrow transplantation. Int J Oncol. 2014a;44:443–50.
40. Kano Y, Konno M, Kawamoto K, Tamari K, Hayashi K, Fukusumi T, Satoh T, Tanaka S, Ogawa K, Mori M, Doki Y, Ishii H. Novel drug discovery system for cancer stem cells in human squamous cell carcinoma of the esophagus. Oncol Rep. 2014b;31:1133–8.
41. Kano Y, Konno M, Ohta K, Haraguchi N, Nishikawa S, Kagawa Y, Hamabe A, Hasegawa S, Ogawa H, Fukusumi T, Noguchi Y, Ozaki M, Kudo T, Sakai D, Satoh T, Ishii M, Mizohata E, Inoue T, Mori M, Doki Y, Ishii H. Jumonji/Arid1b (Jarid1b) protein modulates human esophageal cancer cell growth. Mol Clin Oncol. 2013;1:753–7.
42. Kantarcioglu M, Caliskan B, Demirci H, Karacalioglu O, Kekilli M, Polat Z, Gunal A, Akinci M, Uysal C, Eksert S, Gurel H, Celebi G, Avcu F, Ural AU, Bagci S. The efficacy of mesenchymal stem cell transplantation in caustic esophagus injury: an experimental study. Stem Cells Int. 2014;2014:939674.
43. Katoh M, Katoh M. Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. Int J Mol Med. 2006;17:681–5.
44. Kazumori H, Ishihara S, Kinoshita Y. Roles of caudal-related homeobox gene Cdx1 in oesophageal epithelial cells in Barrett's epithelium development. Gut. 2009;58:620–8.
45. Keane TJ, DeWard A, Londono R, Saldin LT, Castleton AA, Carey L, Nieponice A, Lagasse E, Badylak SF. Tissue-specific effects of esophageal extracellular matrix. Tissue Eng Part A. 2015;21:2293–300.

46. Keane TJ, Londono R, Carey RM, Carruthers CA, Reing JE, Dearth CL, D'Amore A, Medberry CJ, Badylak SF. Preparation and characterization of a biologic scaffold from esophageal mucosa. Biomaterials. 2013;34:6729–37.
47. Kong J, Crissey MA, Funakoshi S, Kreindler JL, Lynch JP. Ectopic Cdx2 expression in murine esophagus models an intermediate stage in the emergence of Barrett's esophagus. PLoS One. 2011;6:e18280.
48. Koseki J, Matsui H, Konno M, Nishida N, Kawamoto K, Kano Y, Mori M, Doki Y, Ishii H. A trans-omics mathematical analysis reveals novel functions of the ornithine metabolic pathway in cancer stem cells. Sci Rep. 2016;6:20726.
49. Krishnan K, Komanduri S, Cluley J, Dirisina R, Sinh P, Ko JZ, Li L, Katzman RB, Barrett TA. Radiofrequency ablation for dysplasia in Barrett's esophagus restores beta-catenin activation within esophageal progenitor cells. Dig Dis Sci. 2012;57:294–302.
50. La Francesca S, Aho JM, Barron MR, Blanco EW, Soliman S, Kalenjian L, Hanson AD, Todorova E, Marsh M, Burnette K, DerSimonian H, Odze RD, Wigle DA. Long-term regeneration and remodeling of the pig esophagus after circumferential resection using a retrievable synthetic scaffold carrying autologous cells. Sci Rep. 2018;8:4123.
51. Lee E, Milan A, Urbani L, De Coppi P, Lowdell MW. Decellularized material as scaffolds for tissue engineering studies in long gap esophageal atresia. Expert Opin Biol Ther. 2017;17:573–84.
52. Levine DS, Rubin CE, Reid BJ, Haggitt RC. Specialized metaplastic columnar epithelium in Barrett's esophagus. A comparative transmission electron microscopic study. Lab Investig. 1989;60:418–32.
53. Li Y, Wo JM, Ellis S, Ray MB, Jones W, Martin RC. Morphological transformation in esophageal submucosa by bone marrow cells: esophageal implantation under external esophageal perfusion. Stem Cells Dev. 2006;15:697–705.
54. Liu J, Fan H, Ma Y, Liang D, Huang R, Wang J, Zhou F, Kan Q, Ming L, Li H, Giercksky KE, Nesland JM, Suo Z. Notch1 is a 5-fluorouracil resistant and poor survival marker in human esophagus squamous cell carcinomas. PLoS One. 2013a;8:e56141.
55. Liu K, Jiang M, Lu Y, Chen H, Sun J, Wu S, Ku WY, Nakagawa H, Kita Y, Natsugoe S, Peters JH, Rustgi A, Onaitis MW, Kiernan A, Chen X, Que J. Sox2 cooperates with inflammation-mediated Stat3 activation in the malignant transformation of foregut basal progenitor cells. Cell Stem Cell. 2013b;12:304–15.
56. Liu K, Lin B, Zhao M, Yang X, Chen M, Gao A, Liu F, Que J, Lan X. The multiple roles for Sox2 in stem cell maintenance and tumorigenesis. Cell Signal. 2013c;25:1264–71.
57. Long A, Giroux V, Whelan KA, Hamilton KE, Tetreault MP, Tanaka K, Lee JS, Klein-Szanto AJ, Nakagawa H, Rustgi AK. WNT10A promotes an invasive and self-renewing phenotype in esophageal squamous cell carcinoma. Carcinogenesis. 2015;36:598–606.
58. Lopez-Lazaro M. Understanding why aspirin prevents cancer and why consuming very hot beverages and foods increases esophageal cancer risk. Controlling the division rates of stem cells is an important strategy to prevent cancer. Oncoscience. 2015;2:849–56.
59. Lopez-Lazaro M. A local mechanism by which alcohol consumption causes cancer. Oral Oncol. 2016;62:149–52.
60. Lord RV, Wickramasinghe K, Johansson JJ, Demeester SR, Brabender J, Demeester TR. Cardiac mucosa in the remnant esophagus after esophagectomy is an acquired epithelium with Barrett's-like features. Surgery. 2004;136:633–40.
61. Luc G, Charles G, Gronnier C, Cabau M, Kalisky C, Meulle M, Bareille R, Roques S, Couraud L, Rannou J, Bordenave L, Collet D, Durand M. Decellularized and matured esophageal scaffold for circumferential esophagus replacement: proof of concept in a pig model. Biomaterials. 2018;175:1–18.
62. Lukaszewicz-Zajac M, Mroczko B, Kozlowski M, Szmitkowski M. Stem cell factor in the serum of patients with esophageal cancer in relation to its histological types. Arch Med Sci. 2017;13:1357–64.

63. Lynen Jansen P, Klinge U, Anurov M, Titkova S, Mertens PR, Jansen M. Surgical mesh as a scaffold for tissue regeneration in the esophagus. Eur Surg Res. 2004;36:104–11.
64. Maghsoudlou P, Ditchfield D, Klepacka DH, Shangaris P, Urbani L, Loukogeorgakis SP, Eaton S, De Coppi P. Isolation of esophageal stem cells with potential for therapy. Pediatr Surg Int. 2014;30:1249–56.
65. Malvasio V, Ainoedhofer H, Ackbar R, Hoellwarth ME, Saxena AK. Effects of sodium hydroxide exposure on esophageal epithelial cells in an in vitro ovine model: implications for esophagus tissue engineering. J Pediatr Surg. 2012;47:874–80.
66. Marchetti M, Caliot E, Pringault E. Chronic acid exposure leads to activation of the cdx2 intestinal homeobox gene in a long-term culture of mouse esophageal keratinocytes. J Cell Sci. 2003;116:1429–36.
67. Mazzanti B, Lorenzi B, Lorenzoni P, Borghini A, Boieri M, Lorenzi M, Santosuosso M, Bosi A, Saccardi R, Weber E, Pessina F. Treatment of experimental esophagogastric myotomy with bone marrow mesenchymal stem cells in a rat model. Neurogastroenterol Motil. 2013;25:e669–79.
68. Menke V, van Es JH, de Lau W, van den Born M, Kuipers EJ, Siersema PD, de Bruin RW, Kusters JG, Clevers H. Conversion of metaplastic Barrett's epithelium into post-mitotic goblet cells by gamma-secretase inhibition. Dis Model Mech. 2010;3:104–10.
69. Mizushima T, Ohnishi S, Hosono H, Yamahara K, Tsuda M, Shimizu Y, Kato M, Asaka M, Sakamoto N. Oral administration of conditioned medium obtained from mesenchymal stem cell culture prevents subsequent stricture formation after esophageal submucosal dissection in pigs. Gastrointest Endosc. 2017;86:542–552 e541.
70. Moghbeli M, Moghbeli F, Forghanifard MM, Abbaszadegan MR. Cancer stem cell detection and isolation. Med Oncol. 2014;31:69.
71. Moons LM, Bax DA, Kuipers EJ, Van Dekken H, Haringsma J, Van Vliet AH, Siersema PD, Kusters JG. The homeodomain protein CDX2 is an early marker of Barrett's oesophagus. J Clin Pathol. 2004;57:1063–8.
72. Mutoh H, Sakurai S, Satoh K, Osawa H, Hakamata Y, Takeuchi T, Sugano K. Cdx1 induced intestinal metaplasia in the transgenic mouse stomach: comparative study with Cdx2 transgenic mice. Gut. 2004;53:1416–23.
73. Nayal B, Vasudevan G, Rao AC, Kudva R, Valliathan M, Mathew M, Rao L. Primary small cell carcinoma of the esophagus – an eight year retrospective study. J Clin Diagn Res. 2015;9:EC04–6.
74. Nicholson AM, Graham TA, Simpson A, Humphries A, Burch N, Rodriguez-Justo M, Novelli M, Harrison R, Wright NA, McDonald SA, Jankowski JA. Barrett's metaplasia glands are clonal, contain multiple stem cells and share a common squamous progenitor. Gut. 2012;61:1380–9.
75. Nieponice A, Ciotola FF, Nachman F, Jobe BA, Hoppo T, Londono R, Badylak S, Badaloni AE. Patch esophagoplasty: esophageal reconstruction using biologic scaffolds. Ann Thorac Surg. 2014;97:283–8.
76. Nieponice A, Gilbert TW, Johnson SA, Turner NJ, Badylak SF. Bone marrow-derived cells participate in the long-term remodeling in a mouse model of esophageal reconstruction. J Surg Res. 2013;182:e1–7.
77. Niu Y, Shen H, Epperly M, Zhang X, Nie S, Cao S, Greenberger JS. Protection of esophageal multi-lineage progenitors of squamous epithelium (stem cells) from ionizing irradiation by manganese superoxide dismutase-plasmid/liposome (MnSOD-PL) gene therapy. In Vivo. 2005;19:965–74.
78. Ohki T, Yamato M, Okano T, Yamamoto M. Regenerative medicine: tissue-engineered cell sheet for the prevention of post-esophageal ESD stricture. Gastrointest Endosc Clin N Am. 2014;24:273–81.
79. Ohki T, Yamato M, Ota M, Takagi R, Murakami D, Kondo M, Sasaki R, Namiki H, Okano T, Yamamoto M. Prevention of esophageal stricture after endoscopic submucosal dissection using tissue-engineered cell sheets. Gastroenterology. 2012;143:582–588 e582.

80. Okumura T, Shimada Y, Imamura M, Yasumoto S. Neurotrophin receptor p75(NTR) characterizes human esophageal keratinocyte stem cells in vitro. Oncogene. 2003;22:4017–26.
81. Park SY, Choi JW, Park JK, Song EH, Park SA, Kim YS, Shin YS, Kim CH. Tissue-engineered artificial oesophagus patch using three-dimensionally printed polycaprolactone with mesenchymal stem cells: a preliminary report. Interact Cardiovasc Thorac Surg. 2016;22:712–7.
82. Perin S, McCann CJ, Borrelli O, De Coppi P, Thapar N. Update on foregut molecular embryology and role of regenerative medicine therapies. Front Pediatr. 2017;5:91.
83. Phillips RW, Frierson HF Jr, Moskaluk CA. Cdx2 as a marker of epithelial intestinal differentiation in the esophagus. Am J Surg Pathol. 2003;27:1442–7.
84. Piazzolla D, Palla AR, Pantoja C, Canamero M, de Castro IP, Ortega S, Gomez-Lopez G, Dominguez O, Megias D, Roncador G, Luque-Garcia JL, Fernandez-Tresguerres B, Fernandez AF, Fraga MF, Rodriguez-Justo M, Manzanares M, Sanchez-Carbayo M, Garcia-Pedrero JM, Rodrigo JP, Malumbres M, Serrano M. Lineage-restricted function of the pluripotency factor NANOG in stratified epithelia. Nat Commun. 2014;5:4226.
85. Poghosyan T, Catry J, Luong-Nguyen M, Bruneval P, Domet T, Arakelian L, Sfeir R, Michaud L, Vanneaux V, Gottrand F, Larghero J, Cattan P. Esophageal tissue engineering: current status and perspectives. J Visc Surg. 2016;153:21–9.
86. Poghosyan T, Gaujoux S, Vanneaux V, Bruneval P, Domet T, Lecourt S, Jarraya M, Sfeir R, Larghero J, Cattan P. In vitro development and characterization of a tissue-engineered conduit resembling esophageal wall using human and pig skeletal myoblast, oral epithelial cells, and biologic scaffolds. Tissue Eng Part A. 2013;19:2242–52.
87. Que J, Okubo T, Goldenring JR, Nam KT, Kurotani R, Morrisey EE, Taranova O, Pevny LH, Hogan BL. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development. 2007;134:2521–31.
88. Raghoebir L, Bakker ER, Mills JC, Swagemakers S, Kempen MB, Munck AB, Driegen S, Meijer D, Grosveld F, Tibboel D, Smits R, Rottier RJ. SOX2 redirects the developmental fate of the intestinal epithelium toward a premature gastric phenotype. J Mol Cell Biol. 2012;4:377–85.
89. Rassouli FB, Matin MM, Bahrami AR, Ghaffarzadegan K, Cheshomi H, Lari S, Memar B, Kan MS. Evaluating stem and cancerous biomarkers in CD15+CD44+ KYSE30 cells. Tumour Biol. 2013;34:2909–20.
90. Rassouli FB, Matin MM, Bahrami AR, Ghaffarzadegan K, Sisakhtnezhad S, Cheshomi H, Abbasi F. SOX2 expression in gastrointestinal cancers of Iranian patients. Int J Biol Markers. 2015;30:e315–20.
91. Reinardy HC, Emerson CE, Manley JM, Bodnar AG. Tissue regeneration and biomineralization in sea urchins: role of Notch signaling and presence of stem cell markers. PLoS One. 2015;10:e0133860.
92. Roman S, Petre A, Thepot A, Hautefeuille A, Scoazec JY, Mion F, Hainaut P. Downregulation of p63 upon exposure to bile salts and acid in normal and cancer esophageal cells in culture. Am J Physiol Gastrointest Liver Physiol. 2007;293:G45–53.
93. Rosekrans SL, Baan B, Muncan V, van den Brink GR. Esophageal development and epithelial homeostasis. Am J Physiol Gastrointest Liver Physiol. 2015a;309:G216–28.
94. Rosekrans SL, Heijmans J, Buller NV, Westerlund J, Lee AS, Muncan V, van den Brink GR. ER stress induces epithelial differentiation in the mouse oesophagus. Gut. 2015b;64:195–202.
95. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141:1762–72.
96. Satoh K, Mutoh H, Eda A, Yanaka I, Osawa H, Honda S, Kawata H, Kihira K, Sugano K. Aberrant expression of CDX2 in the gastric mucosa with and without intestinal metaplasia: effect of eradication of Helicobacter pylori. Helicobacter. 2002;7:192–8.

97. Sawhney RA, Shields HM, Allan CH, Boch JA, Trier JS, Antonioli DA. Morphological characterization of the squamocolumnar junction of the esophagus in patients with and without Barrett's epithelium. Dig Dis Sci. 1996;41:1088–98.
98. Shoreibah MG, Jackson CL, Price PW, Meagher R, Godwin AK, Cai Q, Gildersleeve JC. Anti-human embryonic stem cell monoclonal antibody Hesca-2 binds to a glycan epitope commonly found on carcinomas. Stem Cells Dev. 2011;20:515–25.
99. Sjoqvist S, Jungebluth P, Lim ML, Haag JC, Gustafsson Y, Lemon G, Baiguera S, Burguillos MA, Del Gaudio C, Rodriguez AB, Sotnichenko A, Kublickiene K, Ullman H, Kielstein H, Damberg P, Bianco A, Heuchel R, Zhao Y, Ribatti D, Ibarra C, Joseph B, Taylor DA, Macchiarini P. Experimental orthotopic transplantation of a tissue-engineered oesophagus in rats. Nat Commun. 2014;5:3562.
100. Soliman S, Laurent J, Kalenjian L, Burnette K, Hedberg B, La Francesca S. A multilayer scaffold design with spatial arrangement of cells to modulate esophageal tissue growth. J Biomed Mater Res B Appl Biomater. 2018;107:324–31.
101. Spechler SJ, Souza RF. Stem cells in Barrett's esophagus: HALOs or horns? Gastrointest Endosc. 2008;68:41–3.
102. Spurrier RG, Speer AL, Hou X, El-Nachef WN, Grikscheit TC. Murine and human tissue-engineered esophagus form from sufficient stem/progenitor cells and do not require microdesigned biomaterials. Tissue Eng Part A. 2015;21:906–15.
103. Takubo K, Fujita M, Izumiyama N, Nakamura K, Ishikawa N, Poon SS, Fujiwara M, Sawabe M, Matsuura M, Grabsch H, Arai T, Aida J. Q-FISH analysis of telomere and chromosome instability in the oesophagus with and without squamous cell carcinoma in situ. J Pathol. 2010;221:201–9.
104. Tan B, Wei RQ, Tan MY, Luo JC, Deng L, Chen XH, Hou JL, Li XQ, Yang ZM, Xie HQ. Tissue engineered esophagus by mesenchymal stem cell seeding for esophageal repair in a canine model. J Surg Res. 2013;182:40–8.
105. Taniguchi H, Moriya C, Igarashi H, Saitoh A, Yamamoto H, Adachi Y, Imai K. Cancer stem cells in human gastrointestinal cancer. Cancer Sci. 2016;107:1556–62.
106. Taylor C, Loomans HA, Le Bras GF, Koumangoye RB, Romero-Morales AI, Quast LL, Zaika AI, El-Rifai W, Andl T, Andl CD. Activin a signaling regulates cell invasion and proliferation in esophageal adenocarcinoma. Oncotarget. 2015;6:34228–44.
107. Tevlin R, Atashroo D, Duscher D, Mc Ardle A, Gurtner GC, Wan DC, Longaker MT. Impact of surgical innovation on tissue repair in the surgical patient. Br J Surg. 2015;102:e41–55.
108. Tomita H, Tanaka K, Tanaka T, Hara A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget. 2016;7:11018–32.
109. Vaiphei K, Sinha SK, Kochhar R. Comparative analysis of Oct4 in different histological subtypes of esophageal squamous cell carcinomas in different clinical conditions. Asian Pac J Cancer Prev. 2014;15:3519–24.
110. van der Sluis M, Vincent A, Bouma J, Korteland-Van Male A, van Goudoever JB, Renes IB, Van Seuningen I. Forkhead box transcription factors Foxa1 and Foxa2 are important regulators of Muc2 mucin expression in intestinal epithelial cells. Biochem Biophys Res Commun. 2008;369:1108–13.
111. von Furstenberg RJ, Li J, Stolarchuk C, Feder R, Campbell A, Kruger L, Gonzalez LM, Blikslager AT, Cardona DM, McCall SJ, Henning SJ, Garman KS. Porcine esophageal submucosal gland culture model shows capacity for proliferation and differentiation. Cell Mol Gastroenterol Hepatol. 2017;4:385–404.
112. Wang DH, Clemons NJ, Miyashita T, Dupuy AJ, Zhang W, Szczepny A, Corcoran-Schwartz IM, Wilburn DL, Montgomery EA, Wang JS, Jenkins NA, Copeland NA, Harmon JW, Phillips WA, Watkins DN. Aberrant epithelial-mesenchymal Hedgehog signaling characterizes Barrett's metaplasia. Gastroenterology. 2010;138:1810–22.
113. Wang DH, Souza RF. Transcommitment: paving the way to Barrett's metaplasia. Adv Exp Med Biol. 2016;908:183–212.

114. Wang DH, Tiwari A, Kim ME, Clemons NJ, Regmi NL, Hodges WA, Berman DM, Montgomery EA, Watkins DN, Zhang X, Zhang Q, Jie C, Spechler SJ, Souza RF. Hedgehog signaling regulates FOXA2 in esophageal embryogenesis and Barrett's metaplasia. J Clin Invest. 2014;124:3767–80.
115. Wang F, Maeda Y, Zachar V, Ansari T, Emmersen J. Regeneration of the oesophageal muscle layer from oesophagus acellular matrix scaffold using adipose-derived stem cells. Biochem Biophys Res Commun. 2018;503:271–7.
116. Wong NA, Wilding J, Bartlett S, Liu Y, Warren BF, Piris J, Maynard N, Marshall R, Bodmer WF. CDX1 is an important molecular mediator of Barrett's metaplasia. Proc Natl Acad Sci U S A. 2005;102:7565–70.
117. Workman MJ, Mahe MM, Trisno S, Poling HM, Watson CL, Sundaram N, Chang CF, Schiesser J, Aubert P, Stanley EG, Elefanty AG, Miyaoka Y, Mandegar MA, Conklin BR, Neunlist M, Brugmann SA, Helmrath MA, Wells JM. Engineered human pluripotent-stem-cell-derived intestinal tissues with a functional enteric nervous system. Nat Med. 2017;23:49–59.
118. Wroblewski LE, Peek RM Jr, Coburn LA. The role of the microbiome in gastrointestinal cancer. Gastroenterol Clin N Am. 2016;45:543–56.
119. Xu M, Luo J. Alcohol and cancer stem cells. Cancers (Basel). 2017;9:158.
120. Zhang C, Zhang Y, Feng Z, Zhang F, Liu Z, Sun X, Ruan M, Liu M, Jin S. Therapeutic effect of dental pulp stem cell transplantation on a rat model of radioactivity-induced esophageal injury. Cell Death Dis. 2018;9:738.
121. Zhang X, Lu F, Wang J, Yin F, Xu Z, Qi D, Wu X, Cao Y, Liang W, Liu Y, Sun H, Ye T, Zhang H. Pluripotent stem cell protein Sox2 confers sensitivity to LSD1 inhibition in cancer cells. Cell Rep. 2013;5:445–57.
122. Zhang Y, Jiang M, Kim E, Lin S, Liu K, Lan X, Que J. Development and stem cells of the esophagus. Semin Cell Dev Biol. 2017;66:25–35.
123. Zhong B, Wang T, Lun X, Zhang J, Zheng S, Yang W, Li W, Xiang AP, Chen Z. Contribution of nestin positive esophageal squamous cancer cells on malignant proliferation, apoptosis, and poor prognosis. Cancer Cell Int. 2014;14:57.
124. Zhuang ZH, Tsao SW, Deng W, Wang JD, Xia HH, He H, Feng HC, Wang LD, Gu Q, Lam SK, Lin MC, Kung HF, Wong BC. Early upregulation of cyclooxygenase-2 in human papillomavirus type 16 and telomerase-induced immortalization of human esophageal epithelial cells. J Gastroenterol Hepatol. 2008;23:1613–20.
125. Zhuravleva M, Gilazieva Z, Grigoriev TE, Shepelev AD, Kh Tenchurin T, Kamyshinsky R, Krasheninnikov SV, Orlov S, Caralogli G, Archipova S, Holterman MJ, Mavlikeev M, Deev RV, Chvalun SN, Macchiarini P. In vitro assessment of electrospun polyamide-6 scaffolds for esophageal tissue engineering. J Biomed Mater Res B Appl Biomater. 2018;107:253–68.
126. Zinovyeva MV, Monastyrskaya GS, Kopantzev EP, Vinogradova TV, Kostina MB, Sass AV, Filyukova OB, Uspenskaya NY, Sukhikh GT, Sverdlov ED. Identification of some human genes oppositely regulated during esophageal squamous cell carcinoma formation and human embryonic esophagus development. Dis Esophagus. 2010;23:260–70.