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Re-evaluating stem cell roles in homeostasis and cancer of the oesophagus

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Abstract

The localization and identity of oesophageal stem cells remain highly debated, prompting the emergence of several hypotheses. While early models proposed a homogeneous basal progenitor layer, recent evidence suggests a heterogeneous pool of stem/progenitor cells with diverse phenotypic and functional characteristics. We present a perspective on this heterogeneity and its implications for both normal tissue maintenance and disease susceptibility. Oesophageal cancers can emerge as a result of random mutations or the activity of cancer stem cells (CSCs). The multifaceted interactions between genetic predisposition and environmental influences demonstrate that cancer onset can be driven not only by cell phenotype but also by various external pressures. Advances in *in vivo* and *in vitro* models now offer valuable tools to explore these stem cell dynamics and may pave the way for novel therapeutic approaches in oesophageal cancer treatment.

Introduction

The oesophagus is a simple muscular tube extending from the oral cavity to the stomach, anatomically divided into cervical, thoracic, and abdominal regions and comprised of four main layers: epithelium (*mucosa*), sub-epithelial layer (*submucosa*), external muscle (*muscularis propria*), and connective tissue layer (*adventitia*)[1] (Fig. 1a). The organ's primary function is to propel ingested content downward through peristalsis, and while the oesophageal mucosa has no digestive function or resident glands, the submucosal layers actively secrete mucus that lubricates the luminal surface to limit mechanical abrasions [1,2]. Oesophageal development is initiated with the foregut tube separation, when the dorsal section splits from the ventral respiratory primordium around embryonic day 9.5 in mice[3] and day 26 in humans [4]. Following this separation, stem cells from different germ layers coordinate the formation of its distinct oesophageal tissue layers. Epithelial stem cells derived from the endoderm drive the differentiation and maturation of the mucosa, transitioning from an early cuboidal cell layer to stratified squamous epithelium[5]. Simultaneously, muscle stem cells originating from the mesoderm contribute to the development of the external muscle[5,6]. The coordinated interplay between different germ layers, mediated by intricate signalling pathways, shapes the structure and function of the oesophagus.

Recent technological advances including single-cell RNA sequencing (scRNAseq) and spatial transcriptomics have facilitated rapid improvements in our understanding of oesophageal biology and the underlying mechanisms driving embryonic development or disease onset in adults. However, despite these advances, many key aspects of stem cell biology during tissue homeostasis and repair, including the existence/identity of the resident epithelial stem cell pool, as well as the mechanisms underlying cancer development and other chronic diseases

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in the adult oesophagus remain contentious[7-10]. The simple architecture of the mucosal layer and the accompanying absence of a morphologically distinct niche housing resident stem cell populations in the adult oesophagus has sparked persistent debates in the field. Unlike other regions of the gastrointestinal (GI) tract, where dedicated epithelial stem cells residing in discrete niches are well established as being responsible for daily tissue maintenance and repair[11-14], there is a lack of consensus on whether the proliferating cells of the adult oesophageal epithelium conform to a classical stem cell definition or should instead be referred to as fast-cycling progenitors. Accumulating evidence suggests that it is unreasonable to entirely reject the existence of dedicated, multipotent stem cells within the oesophageal epithelium, even if they do not fully correspond to the traditional criteria of stem cells[8]. In support of this claim, emerging evidence increasingly highlights the heterogeneity of the basal layer, previously considered to comprise a uniform layer of dividing cells. Some subsets of basal cells have properties of fast-cycling transit-amplifying cells, while others are identified as non-quiescent stem cells[15]. Reported different features of basal cells, including cycling rate, differentiation status or specific gene expression patterns, reflects the outcome of long evolutionary processes that have driven the diversification, redundancy, and plasticity of organ systems, providing tissues with the ability to adapt and respond effectively to an ever-changing environment. The observed heterogeneity of the basal layer stem/progenitor cells in the oesophagus may also be associated with the likelihood of disease development in an individual [16,17]. The connections between the presence of certain subtypes of cells and an individual's susceptibility to cancer remain an active area of research. While certain mutations have been linked to the development of specific cancer types, the identity of cells initiating cancer, driving disease progression, or triggering relapse are less well understood.

This article explores the evidence in support of heterogeneous cell pools with stem cell properties within the basal layer of the epithelium and their critical importance in maintaining the structural integrity and facilitating repair of the mucosa. Such epithelial heterogeneity likely plays a critical role in tissue homeostasis and repair by providing a dynamic cellular repertoire capable of responding to diverse challenges. While this heterogeneity supports normal tissue maintenance, it may also be co-opted during processes such as tumourigenesis and chemotherapy resistance, underscoring its potential importance in cancer initiation and progression. Rather than suggesting the absence of "true" stem cells or the presence of a singular, homogeneous progenitor pool, we highlight that the basal layer contains cells with distinct functional capacities, which together contribute to epithelial resilience and adaptability. By exploring their diverse functions, spatial distribution, and interactions with the epithelial microenvironment, this review sheds light on their biological role in the tissue. Additionally, it underscores their clinical implications, offering new perspectives that could guide future research and shape innovative therapeutic approaches for oesophageal pathologies.

Re-evaluating Stem Cell Localization and Identity in the Oesophagus

The mucosa in adult oesophagus is a fast-cycling, stratified squamous epithelium with distinct layers of proliferating and differentiating cells, each contributing to the overall structure and function of the tissue. At the bottom of the tissue is the basal layer, which is positioned adjacent to the basement membrane and contains cells that are actively proliferating to support the continuous renewal and repair of the epithelium. In mice, the proliferating zone is a single layer of cells, while the human oesophagus harbours several layers of actively proliferating cells, arranged into connective-tissue papillae that project toward the lumen, indicating a more complex organization that may reflect differences in homeostatic or regenerative processes between species[18] (Fig. 1b). These basal cells are widely accepted as being critical for the maintenance and repair of the oesophageal epithelium. Through continuous division, they generate progeny that differentiate into more mature cell types as they migrate toward the

luminal surface During this upward migration, the cells undergo maturation, acquiring specialized features that enable them to protect the oesophagus as food is propelled toward the stomach. In mice, maturation culminates in formation of a cornified surface layer, an adaptation to their more abrasive diet, while the human surface remains non-cornified but is renewed at a similar pace. The most superficial layer is regularly shed and replenished by new cells originating from the basal layer (Fig 1b).

Several hypotheses were formed to define the presence and localization of putative stem cell populations in the epithelium of oesophagus[7-9]. Conceptual models of basal layer organization and division dynamics, including stochastic, hierarchical, and regionally compartmentalized frameworks, are summarized in Box 1 and illustrated in Fig. 1c.

The increasingly appreciated regional diversity of the adult oesophagus may account for some of the reported differences in basal cell properties and function amongst studies incorporating distinct sampling methods. This regionally influenced phenotypic diversity may preserve epithelial integrity under different environmental stresses and could provide a new perspective on the precise mechanisms of oesophageal maintenance and repair along the oesophagus. Furthermore, these findings position the postulated quiescent or slow-cycling, long-term retained basal layer cells as potential candidates for cancer initiation, underscoring the need for further studies into the relationship between regional heterogeneity, stem cell distribution, and oesophageal diseases. Based on the observed regional specialization and functional diversity of basal cells, the presence of specialized stem cells seems more plausible than the equivalent progenitor model in explaining the maintenance and repair of the oesophageal epithelium.

Oesophageal Cancer Origins & Evolution

While our understanding of cancer biology and genetics has vastly improved in recent years, epithelial cancers remain a leading cause of death globally. Translating this knowledge into clinical practice requires addressing the cellular complexity of the disease and its dynamic, rapidly-evolving nature. Oesophageal cancers in particular pose a significant challenge due to their inherent phenotypic and functional heterogeneity, the absence of reliable biomarkers, and the tendency for late detection. Two predominant histological subtypes of oesophageal cancers are oesophageal squamous cell carcinoma (ESCC) and oesophageal adenocarcinoma (EAC), with distinct incidence rates, locations, and histological characteristics. ESCC accounts for more than 80% of cases globally and typically develops in upper to mid oesophagus close to the trachea and major blood vessels. EAC, with a rising incidence, arises in the lower third of the oesophagus or gastrooesophageal junction and is characterized by glandular or mucinous differentiation[25].

In 1976, Peter Nowell published a groundbreaking perspective on cancer as an evolutionary process driven by stepwise somatic-cell mutations and sequential subclonal selection[26]. *The clonal evolution model*, provides one of the frameworks for understanding tumour heterogeneity and adaptability (Fig. 2). This model posits that cancers originate from a single mutated cell, which acquires successive genetic mutations over time. These mutations confer a selective advantage to certain clones, irrespective of their cell of origin, within the tumour, allowing them to expand under the selective pressures of their microenvironment. Factors such as nutrient availability, immune responses, and therapy-induced stress further shape this evolutionary landscape, driving the emergence of increasingly aggressive and treatment-resistant clones. This dynamic process results in tumours composed of genetically distinct subpopulations, creating a mosaic of phenotypically diverse cells that hinders treatment.

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Stem/progenitor cells within the entire GI tract, including those of the oesophagus, play a critical role in maintaining epithelial homeostasis but are also considered key players in tumourigenesis[27-29]. Their long-term retention and self-renewal capacity make them particularly vulnerable to the accumulation of multiple genetic alterations, positioning them as ideal candidates for initiating cancer. Genomic studies of human oesophageal cancers have provided significant evidence in support of cancer clonal evolution, revealing intrinsic heterogeneity of tumours and the temporal evolution of resistance mechanisms[30,31]. As the epithelial lining of oesophagus harbours a continuously proliferating basal layer, it is particularly susceptible to acquiring and accumulating genetic mutations[32]. These mutations can be passed on to daughter cells, promoting the expansion of mutant populations over time. This process is intensified by the natural aging process of tissues, during which time mutant clones may gradually outcompete non-mutant counterparts[32]. Epithelial cells also possess a remarkable ability, known as plasticity, to adapt and transition between different phenotypes, often as a response to injury, inflammation, or environmental stressors or under pathological conditions[33], which further complicates efforts to identify and target cells responsible for disease onset. Growing evidence shows that processes that do not involve mutations, but rather plasticity of tumour cells are responsible for drug resistance, as they render tumour cells insusceptible to drug targets, thereby facilitating tumour survival and growth [34]. Plasticity mechanisms under pathological conditions are not limited to the reacquisition of adult stem cell identities but can also involve the reactivation of embryonic programs. These programs, originally active during development, may be reinitiated in specific contexts such as tissue repair and malignancies, where they play pivotal roles in driving metastasis and therapy resistance [35]. In recent years, there has been significant interest in exploring alternative stem cell programs that resemble those observed during embryonic stages and are reactivated in adult tissues during tissue repair or cancers[36]. Morphological changes associated with transition from embryonic to adult tissues are well known, however molecular mechanisms underlying this transition are not sufficiently explored. Some of the factors described to play a role in this process are interferon gamma (INFγ)[37], Wnt3a[38], tumour necrosis factor α (TNF-α)[39], and YAP/TAZ[40] which is also one of the key regulators of epithelial maturation[41]. Reversal to foetal states was also reported in oesophageal tumours[42,43], and with the recent discovery of an Lgr5+ cell subset in embryonic oesophagus[24], exploring the markers present during development may reveal novel, clinically relevant cancer biomarkers or therapeutic targets. Additionally, studies have shown that in some tissues, subsets of short-lived progenitor cells or fully differentiated cells can acquire stem cell-like properties through de-differentiation, subsequently driving tumour development[44,45] and similar concepts might apply to oesophageal tumours. Given the continuous proliferation of the basal layer and the proposed equivalency of all cells within the stem/progenitor compartment, as well as the recurrent mutations detected in patients, such as p53 mutations and alterations in the Wnt and Notch signalling pathways, a clonal evolution model might offer some explanations related to susceptibility of cells to develop into tumour[46-49]. However, it does not address whether these cells originate from stem/progenitor compartments or differentiated layers, and provides limited insight into tumour relapse, a common occurrence in oesophageal cancers, or the poor survival rates and drug resistance associated with these tumours.

Another valuable perspective on tumour development is provided by the cancer stem cell (CSC) hypothesis. Whilst this model, which originated from studies on acute myeloid leukaemia (AML)[50], arose much later, it has garnered significant attention and has been extensively studied and reviewed in the field[51-54]. This model postulates that instead of being initiated by random mutations in proliferating cells, tumours develop from a distinct subset of cells with stem-like properties, including self-renewal and a capacity for both symmetric and asymmetric division that is preserved during tumour development. While the hypothesis

suggests a different view on cancer initiation and progression, the model itself is not incompatible with the stochastic model, as the origin of cancer stem cells (CSCs) remains debated, and these cells do not necessarily have to be the ones initiating tumour formation but may instead maintain the tumour and facilitate its progression. Currently, there are two theories about the origin of CSCs. CSCs may arise from mutations or epigenetic changes occurring in normal stem cells, transforming them into malignant cells. Alternatively, mutation of non-stem cells may drive acquisition of stem cell identities through plasticity mechanisms to facilitate cancer initiation[55]. CSCs are considered instrumental in driving tumour initiation, development, and resistance to treatment, making them critical targets for cancer therapies. As CSCs are believed to be the driving force behind continuous tumour growth, relapse and metastasis, they are often associated with poor patient outcomes which makes them a focal point for research aimed at understanding and disrupting cancer progression. Detailed putative markers of SC/CSCs are presented in Box 2. Sequencing analyses have provided a more comprehensive understanding of oesophageal tumour heterogeneity, revealing an astonishing 42 distinct cell types within human ESCC.[60]. The same approach also facilitated the identification of additional markers in oesophageal squamous precancerous lesions that could serve as molecular indicators to predict which lesions are likely to progress to ESCC. Notably, it was found that expression of TAGLN2 significantly increases, while CRNN expression levels decrease throughout the progression of ESCC.[61]. These findings represent a significant advance in early detection and treatment, as oesophageal tumours are often diagnosed at advanced stages, and no well-established early biomarkers or preconditions are closely associated with the development of malignancies.

A range of animal models has been instrumental in elucidating oesophageal cancer mechanisms and evaluating therapeutic strategies (see Box 3 for a comparative overview of mouse and rat models). Hypotheses on cancer mechanisms can be rigorously tested using a combination of murine models and organoid systems. Murine models provide in vivo contexts to assess tumour-immune interactions, stromal influences, and systemic responses, while organoids offer a complementary, reductionist ex vivo system for detailed cellular and molecular studies. Together, these near-physiological tools allow researchers to dissect signalling pathways, identify key drivers of cancer progression, and evaluate drug responses[64]. In addition to relying on cell surface markers for the isolation of CSCs, organoid assays provide a reliable method for the functional validation of the stemness of isolated cells. Another valuable approach involves testing radiation resistance, which could potentially identify cells responsible for therapy resistance or relapse. The integration of patient-derived organoids with genetically engineered or chemically induced mouse models facilitates a comprehensive approach to validate findings and refine therapeutic strategies, ensuring translational relevance and mechanistic precision[64,65] (Fig. 3).

In the case of oesophageal cancer, where survival rates remain low and treatment options relatively limited, effective strategies for detection of cancer-initiating cells and cells responsible for maintenance/progression of tumours could represent a paradigm shift in patient management. However, significant barriers persist, including the lack of reliable biomarkers for early detection and therapeutic targeting of CSC populations, which currently limit the translation of these models into clinical practice. Despite these challenges, understanding the mechanisms that drive the transformation of oesophageal epithelial cells and processes that fuel cancer development and growth, remains critical for developing targeted therapies. Viewing oesophageal SCs and cancer outside established paradigms and definitions might prove to be a more productive approach towards filling in knowledge gaps and gaining insights into disease development, whilst also helping to deliver novel therapeutic approaches for ultimately improving patient outcomes.

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Tumour Microenvironment and Oesophageal Cancer Development

While often studied in isolation, tumours exist within a dynamic and specialized microenvironment in vivo. This environment is known as the tumour microenvironment (TME) and it encompasses a diverse array of cellular and non-cellular components. Cellular elements of the TME may include stromal cells, located just underneath the basal layer (fibroblasts, macrophages, endothelial cells, and immune cells) and extracellular components, such as extracellular matrix (ECM) and a variety of growth factors, hormones, cytokines, all sustained by an intricate vascular network[66]. Apart from providing a favourable environment for cancer growth, the TME also plays a role in evasion of immune surveillance, resistance to therapeutic interventions as well as providing a unique niche for CSCs[66]. The TME is not static but is instead an actively remodelled compartment, often responsible for tumour responses to clinical therapies[67-69].

Highly complex, heterogeneous oesophageal cancers arise from a multifaceted interaction between genetic predisposition and environmental influences. In oesophageal cancers, the link between environmental exposures and disease onset is particularly pronounced, with chronic injury or inflammation being a major driver[70-72]. Diverse risk factors, including gastrooesophageal reflux or infections generate chronic inflammatory responses that disrupt the normal epithelial architecture. This disruption leads to the transformation of epithelial cells into pre-cancerous lesions, eventually culminating in malignant tumours[70]. Inflammation can also re-programme epithelial lineage regulators. In mice, basal Sox2 over-expression alone is benign, yet when combined with IL-6/Stat3 signalling it drives invasive squamous tumours, revealing that extrinsic cytokine cues unlock Sox2's oncogenic potential[73]. Mechanistically, inflammation enhances Stat3 activity, which amplifies Sox2's transcriptional programs and drives epigenetic shifts associated with cellular plasticity and therapeutic resistance [74,75]. Thus, inflammatory cytokines do more than stimulate proliferation, they also convert lineage regulators such as Sox2 into potent oncogenic drivers, exemplifying how the tumour microenvironment can dictate cell fate and initiate oesophageal cancer. This interplay between intrinsic factors and chronic environmental stress may be a key step in the early transformation of basal progenitor cells, particularly within inflamed or damaged oesophageal regions.

It remains an open question as to how epithelial cells from different regions of the oesophagus are influenced by these factors, and whether their susceptibility to mutations or factors secreted by the surrounding cells plays a role in tumour development. As previously mentioned, it is well established that adenocarcinoma and squamous cell carcinoma typically occur in different regions of the oesophagus in most patients and harbour distinct genetic alterations[76]. While differences in local insults and resulting injury responses between the proximal and distal oesophagus may partly explain this, the innate heterogeneity within the basal layer, combined with variations in the local microenvironment, could also account for the development of distinct tumour types in these regions. If that is the case, understanding the distribution of distinct subsets of epithelial cells and identifying specific markers associated with them could potentially provide a path for early risk assessment.

Another approach could be to consider that within a heterogeneous pool of basal layer cells some subpopulations might play a pivotal role in the tissue as components of the stem cell niche. While the notion that specialized epithelial cells in the oesophagus may act as niche components for neighbouring epithelial cells is not sufficiently explored, these subpopulations could exist and assist other stem/progenitor epithelial cells, thereby contributing to the maintenance and regeneration of the oesophageal mucosa. In parallel, this niche can also be utilized by CSCs, enabling their survival, self-renewal, and contribution to tumour

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heterogeneity and progression. Research suggests that such hijacking of the epithelial stem cell niche by CSCs disrupts normal regenerative processes, creating a permissive environment for malignancy[77,78]. Exploring epithelial cells as a potential stem cell niche component is vital for deciphering the mechanisms driving oesophageal cancer and for developing targeted therapeutic strategies.

As a consequence of injury responses driven by local inflammation, fibroblasts are recruited to the damage site[79]. Substantial evidence shows that a specific fibroblast subset, known as cancer-associated fibroblasts (CAFs)[80] is detected at all stages of cancer development in both ESCC and EAC[81], and has the ability to modulate the TME by interacting with other cells in stromal layers, modifying ECM or by disrupting normal cell signalling via different secreted factors [82,83]. One of the key pathways implicated in oesophageal cancer development is canonical Wnt/β-catenin signalling[84] with more compelling data showing the involvement of non-canonical Wnt[85,86]. This pathway serves as a central regulator of essential cellular processes, including proliferation, differentiation, apoptosis, migration, invasion, and tissue homeostasis[87]. Lgr5 functions as a facultative component of the Wnt signalling pathway, with Lgr5-expressing cells shown to play a crucial role in the development and progression of various GI tract tumours[28,29,88]. Although the presence of Lgr5-expressing cells in healthy oesophageal tissues was not reported until recently[24], studies focusing on human oesophageal squamous cell carcinoma (hESCC) have revealed a link between the expression of Lgr5 and its homolog, Lgr6, with poor patient prognosis[89,90]. Dysregulation of Wnt/β-catenin signalling promotes tumourigenesis and facilitates cross-talk with other essential pathways, including PI3K/Akt, Notch, Sonic Hedgehog, Hippo/YAP, NF-κB, and EGFR[91]. These interconnected signalling networks synergistically drive the molecular mechanisms of cancer initiation and progression. In conclusion, the current understanding of oesophageal CSCs has advanced significantly, revealing their pivotal role in tumour initiation, progression, and therapeutic resistance. However, further functional characterization is needed to fully elucidate their behaviour and therapeutic potential. Future studies should focus on applying innovative techniques such as lineage tracing, transplantation, targeted ablation, and organoid cultures to dissect CSC properties and their interactions within the tumour microenvironment across different regions of the oesophagus. Additionally, mouse models, including chemically induced or genetically modified variants, offer valuable platforms for assessing CSC-driven tumourigenesis and testing novel therapeutic strategies. These approaches will be crucial for identifying targeted interventions that can effectively eradicate CSCs and improve treatment outcomes in oesophageal cancers.

Oesophageal Organoids as Avatars of Human Disease

Organoids have emerged as transformative research tools offering a robust, physiologically relevant platform for stem cell and cancer biology. Derived from IPS cells or tissue-specific stem cells, organoids can replicate the three-dimensional architecture and cellular heterogeneity of the tissue of origin, making them an invaluable model system for understanding various aspects of tissue biology and disease onset. Oesophageal organoids have shown great promise, not only for elucidating the homeostatic patterns of stem/progenitor cells, but also the molecular underpinnings of oesophageal cancer. They can be generated from both healthy and malignant oesophageal tissues, delivering a controlled environment to investigate how genetic and environmental factors drive carcinogenesis[92,93]. Importantly, oesophageal organoids have been used to model key processes such as the transition from normal epithelium to dysplasia and cancer, as well as the role of specific genetic mutations in tumour development[93,94].

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Organoid models offer a platform that faithfully recapitulates the complexity of oesophageal tissues while allowing for experimental manipulation. One of the significant advantages of organoids is their ability to largely maintain the molecular and histological characteristics of the tissue they were derived from. Using CRISPR/Cas9-mediated introduction of genetic alterations, researchers were able to establish and validate the roles of multiple frequently mutated genes in ESCC, such as EP300, NOTCH2, and TGFBR2[95]. Using a similar approach, the role of transcription factor Sox2 in oesophageal formation and function was confirmed[92], as well as the interplay between different signalling cascades to induce epithelial changes[93]. More recent work using organoids and single-cell sequencing showed that chronic inflammation skews the fate of $Sox2^+$ progenitors, disrupting balanced renewal and promoting aberrant differentiation[96]. This approach can be further advanced by culturing epithelial organoids with other cell types in co-culture systems to explore functional cell interactions and to study tumour cell and TME interactions by including stromal, vascular or immune cells[97,98]. This feature is particularly relevant in oesophageal cancer, where the TME plays a critical role in disease development, progression and therapy resistance.

As the field continues to evolve, the integration of oesophageal organoids with cutting-edge technologies, holds great promise for further unravelling the complexities of oesophageal cancer and improving patient outcomes. By using patient-derived samples, researchers can establish organoid biobanks that capture the genetic diversity and heterogeneity of oesophageal cancers. This approach could additionally enable the testing of validated therapies on a personalized basis, paving the way for precision medicine approaches in oesophageal cancer treatment.

Concluding Remarks

The relative lack of structural complexity of the oesophageal epithelium and its potential for cellular heterogeneity along its length continues to pose challenges to advancing our understanding of epithelial tissue maintenance, disease progression, and therapeutic resistance. While significant advances have been made in deciphering oesophageal stem cell and cancer biology, the absence of a morphologically distinct stem cell niche in the mucosa raises fundamental questions about the nature of stem or progenitor cells in this tissue. Emerging evidence supporting the phenotypic heterogeneity of basal cells suggests that these cells may not conform to classical models of stemness but instead reflect a dynamic and adaptive pool of cells capable of responding to environmental stressors. This adaptability may be an evolutionary advantage, enabling the oesophagus to endure the diverse challenges it faces along its length, from mechanical damage near the oral cavity to acid exposure closer to the stomach. Such regional variation provides an intriguing avenue for future studies aimed at dissecting the molecular and functional differences between cell populations in proximal and distal regions of the oesophagus.

The implications of basal cell heterogeneity extend beyond homeostasis and repair to the realm of disease susceptibility and progression. A key challenge lies in unravelling the link between specific basal cell subtypes and their potential role in oesophageal tumourigenesis. The stochastic model of cell division, which posits functional equivalency among basal cells, contrasts sharply with hierarchical models suggesting the existence of long-lived, self-renewing stem cells. It is possible that the reality lies somewhere between these two extremes, with distinct subpopulations exhibiting varying degrees of proliferative potential and plasticity. The influence of microenvironmental factors, such as mechanical stress, inflammation, and exposure to carcinogens, may further drive the emergence of aggressive clones, highlighting the need for an integrated approach that considers both intrinsic cellular properties and extrinsic environmental cues.

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From a therapeutic perspective, the cancer stem cell model offers a compelling framework for understanding treatment resistance and relapse in oesophageal cancer. The ability of CSCs to evade conventional therapies, coupled with their capacity for self-renewal and differentiation, underscores the need for strategies targeting these elusive populations. However, the lack of definitive markers for CSCs in the oesophagus poses a significant barrier to their study and clinical targeting. Advances in single-cell sequencing and spatial transcriptomics/proteomics hold promise for identifying novel biomarkers and elucidating the molecular pathways driving CSC behaviour. These approaches could pave the way for therapies that selectively target CSCs while sparing healthy tissue, a critical consideration given the high turnover and regenerative capacity of the oesophageal epithelium.

Physiologically-relevant mouse cancer models and organoid systems are essential for advancing our understanding of cancer stem cell biology and for facilitating functional assays that accurately assess stemness, mechanistic insights, and pre-clinical screening. Mouse models, particularly those that recapitulate human tumour microenvironments, such as humanised variants, enable in vivo analysis of cancer stem cell dynamics, tumour progression, and response to treatments. Meanwhile, organoid models provide a reproducible, patient-specific platform for studying tumour heterogeneity and drug responses in a 3D context. Together, these models offer invaluable tools for exploring the molecular mechanisms underlying cancer stem cell maintenance and resistance, as well as for testing potential therapeutic agents in a more clinically relevant setting. Their integration into cancer research promises to accelerate the development of targeted therapies and improve the translational potential of pre-clinical findings.

Beyond intrinsic tumour biology, the tumour microenvironment plays a crucial role in shaping disease progression and therapeutic outcomes. The dynamic interplay between tumour cells, stromal components, immune cells and peripheral neurons creates a complex ecosystem that supports tumour growth and metastasis. For oesophageal cancer, understanding how the TME contributes to therapy resistance and immune evasion is an area ripe for exploration. For instance, the role of cancer-associated fibroblasts in remodelling the extracellular matrix or the influence of immune checkpoint signalling on T-cell activity could offer insights into novel therapeutic avenues. Additionally, the TME may provide clues about the origins of CSCs, particularly in cases where epithelial cells undergo dedifferentiation in response to environmental stimuli.

Looking ahead, it is essential to move beyond static models of oesophageal biology and adopt a systems-level perspective that integrates cellular, molecular, and microenvironmental data. The application of advanced imaging techniques, combined with functional assays and computational modelling, could offer unprecedented insights into the dynamic processes underlying oesophageal maintenance and disease. Moreover, leveraging organoid and in vivo models that mimic the unique challenges of the oesophageal environment could help bridge the gap between experimental findings and clinical application. In this context, understanding how regional variations within the oesophagus influence stem cell behaviour and disease susceptibility could inform the development of tailored therapeutic strategies.

Ultimately, addressing the challenges posed by oesophageal diseases requires a multidisciplinary approach that unites basic research with translational and clinical efforts. By focusing on the interplay between cellular heterogeneity, the TME, and therapeutic resistance, future studies have the potential to transform our understanding of the oesophagus and improve outcomes for patients with oesophageal cancer. Whether through the identification of novel biomarkers, the refinement of targeted therapies, or the exploration of adaptive

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mechanisms in tissue homeostasis, the next wave of research promises to shed light on one of the most enigmatic and clinically significant tissues in the human body.

Author Contributions

Lana Kostic: Conceptualization, visualisation, writing - original draft. Nick Barker: Writing - review and editing. Both authors reviewed and approved the final version of the manuscript.

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Conflict of interest statement

The authors declare no conflict of interest.

Data Availability Statement

No new data were created or analysed in this study. Data sharing is not applicable to this article.

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Figure Legends:

Figure 1. Structural Organization and Stem-Cell Models of the Adult Oesophagus. **a)** Anatomical regions and histological structure of the adult oesophagus (transverse section). **b)** Epithelial cell layers in the adult mouse and human oesophagus. **c)** Proposed oesophageal stem-cell models.

Figure 2: Cancer origin models: clonal evolution and cancer stem-cell model.

Figure 3: In vivo and in vitro approaches in oesophageal disease modelling.

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Box 1: Stem Cell Organization and Division Dynamics in the Oesophageal Epithelium

The basal layer of the oesophageal epithelium plays a critical role in maintaining tissue integrity and regenerative capacity. Several conceptual models have been proposed to explain its organization and division behaviour.

Equivalency (Stochastic) Model

Early autoradiographic studies[7] observed continuous proliferation within the basal layer, suggesting that all basal cells contribute to epithelial maintenance. These findings formed the basis for a theoretical model in which all basal cells possess equal proliferative potential and divide stochastically, giving rise to either two basal cells (**symmetric self-renewal**), two differentiating daughters (**symmetric differentiation**), or one basal and one differentiating suprabasal cell (**asymmetric division**), depending on local cues and tissue needs. This model was subsequently supported by modern lineage tracing in mice[8] and reinforced by clonal competition data showing neutral drift dynamics [32].

Hierarchical Model

Contrasting with stochastic equivalency, an alternative theoretical model suggests the presence of long-lived, self-renewing stem cells within the basal layer that divide asymmetrically to generate transit-amplifying (TA) progenitors. These TA cells undergo transient proliferation before migrating into the suprabasal layer and differentiating. This model is supported by experimental evidence from label-retention studies and Hoechst dye efflux assays, which provide evidence of a slow-cycling subset of basal cells with stem cell potential[9].

Spatial Compartmentalization: The PBL/IBL Model

Other evidence suggests these models may not be mutually exclusive. Focussing on human oesophageal epithelium Seery and Watt (2000) proposed a spatial model based on histological observations, in which the basal layer is structurally divided into two functionally distinct zones in humans: the **papillary basal layer (PBL)** overlying connective tissue papillae, and the **interpapillary basal layer (IBL)** between them[99]. The IBL showed infrequent but **vertical asymmetric divisions**, producing a basal stem cell and a suprabasal differentiating daughter, suggesting that it harbours long-lived stem cells. In contrast, the PBL exhibited more frequent **symmetric divisions**, likely supporting rapid cell turnover. This spatial framework adds nuance to the broader stochastic and hierarchical frameworks and implies that division orientation (horizontal versus vertical) is not random but regionally biased and functionally significant.

Regional Heterogeneity and Rare Subsets

In recent years the notion of a uniform basal layer has been further challenged by transcriptomic profiling, which has experimentally revealed region-specific basal cell signatures between proximal and distal oesophagus [23]. It is postulated that these differences likely reflect adaptation to local stressors, such as mechanical abrasion or acid reflux. Within this heterogeneous landscape, rare, long-lived subpopulations such as Lgr5-expressing cells, representing less than 0.1% of the basal population, have been identified. These cells are retained long-term in basal layers and exhibit multilineage potential, contributing to tissue maintenance under homeostatic and stress conditions[24].

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Box 2: Stem Cell and Cancer Stem Cell Markers in Oesophagus

Identifying and targeting CSCs holds immense therapeutic potential, as it may address the root cause of tumour recurrence and treatment resistance. Considering the ongoing debate over the existence of a dedicated stem cell pool in the normal oesophageal epithelium, defining a CSC population still presents a challenge. A wide range of markers has been used to identify stem-like populations in the oesophageal epithelium, yet no definitive marker set has been universally established. Below is a summary of key basal layer markers and their functional context:

- **CD44**: A cell-surface glycoprotein often enriched in basal cells and CSC populations; associated with epithelial proliferation and radioresistance in ESCC[56]
- **CK14 (KRT14)**: Intermediate filament protein marking basal keratinocytes, highly expressed in both homeostatic basal and malignant epithelial populations[57].
- **p63**: A transcription factor essential for epithelial stratification, commonly used to demarcate basal progenitor compartments[59].
- **Sox2**: Critical for maintaining epithelial identity. Expression is not limited to stem cells but includes also lineage-committed progenitors[58].
- **CK15 (KRT15)**: Marks a subset of basal cells with higher regenerative potential[100]
- **COL17A1 (BP180)**: An anchoring collagen involved in basal cell adhesion and retention. Label-retaining cells in the esophagus show high COL17A1 expression, supporting its use as a long-lived stem/progenitor marker[100]
- **Integrins ITGα6 (CD49f) and ITGβ4**: Form the α6β4 heterodimer critical for hemidesmosome formation and basal membrane attachment. Their high expression enriches for clonogenic stem-like basal cells in both human and mouse models [15,101].

Putative oesophageal SC markers also appear in cancerous tissues where their co-expression patterns and localization can aid in distinguishing potential stem cells from rapidly cycling progenitors or dedifferentiated tumour cells. Their proper integration into lineage tracing, organoid culture, and functional assays remains essential for CSC identification.

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Box 3: Animal Models for Oesophageal Cancer Research

Preclinical models are essential for dissecting oesophageal cancer mechanisms and evaluating therapeutic interventions. Both **mouse** and **rat** systems offer complementary insights depending on the research focus:

Mouse Models

- **4-Nitroquinoline-1-oxide (4NQO)**-induced carcinogenesis mimics human ESCC progression, generating stepwise epithelial dysplasia, in situ carcinoma, and invasive tumours harbouring genetic alterations seen in patient samples [62]
- **Genetically engineered models (GEMMs)** allow targeted manipulation of oncogenes and tumour suppressors such as Trp53, Notch1, and Wnt pathway components, enabling lineage-specific tumour initiation studies and functional dissection of epithelial, stromal, and immune interactions[63].
- Mouse models are suitable for studying cancer stem cell behaviour, immune modulation, and therapeutic resistance, particularly when combined with organoid and transplantation platforms.

Rat Models

- Rats have a longer oesophagus and more anatomically similar epithelial structure to humans, making them especially valuable for translational work.
- **N-nitrosomethylbenzylamine (NMBA)** is the standard carcinogen used in rat models. Administered subcutaneously over several weeks, NMBA induces high-penetrance squamous dysplasia and ESCC, closely mimicking human disease histopathology and mutation patterns[102,103]
- Because of their uniform tumour incidence and reproducibility, NMBA-induced rat models are widely used in **chemoprevention research**. Agents such as black raspberries[104], NSAIDs[105], and resveratrol[102] have been shown to reduce lesion burden, proliferation, and genomic instability in this system.

Complementary Use

While mice offer genetic manipulability and high-throughput scalability, rat models provide physiologically relevant anatomy and robust phenotypic consistency. Together, they enable a comprehensive understanding of ESCC initiation, progression, and prevention, critical for guiding therapeutic development.

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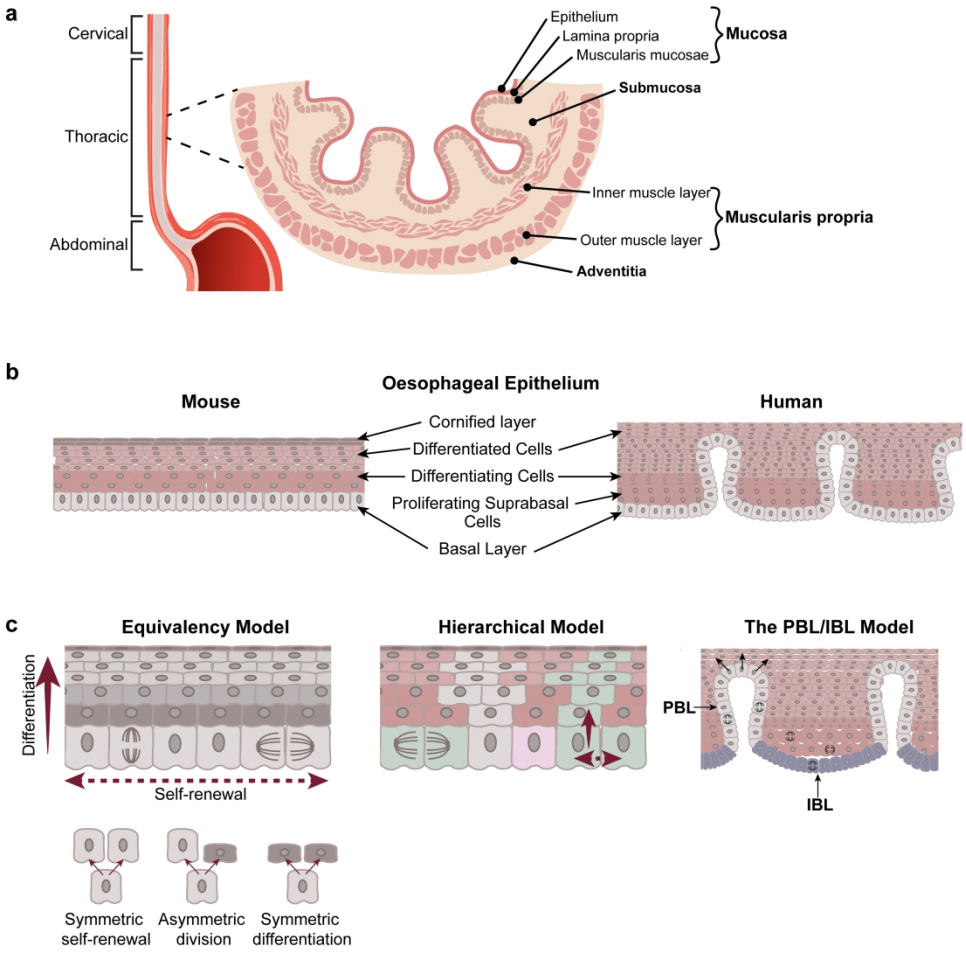

Figure 1. Structural Organization and Stem-Cell Models of the Adult Oesophagus. a) Anatomical regions and histological structure of the adult oesophagus (transverse section). b) Epithelial cell layers in the adult mouse and human oesophagus. c) Proposed oesophageal stem-cell models.

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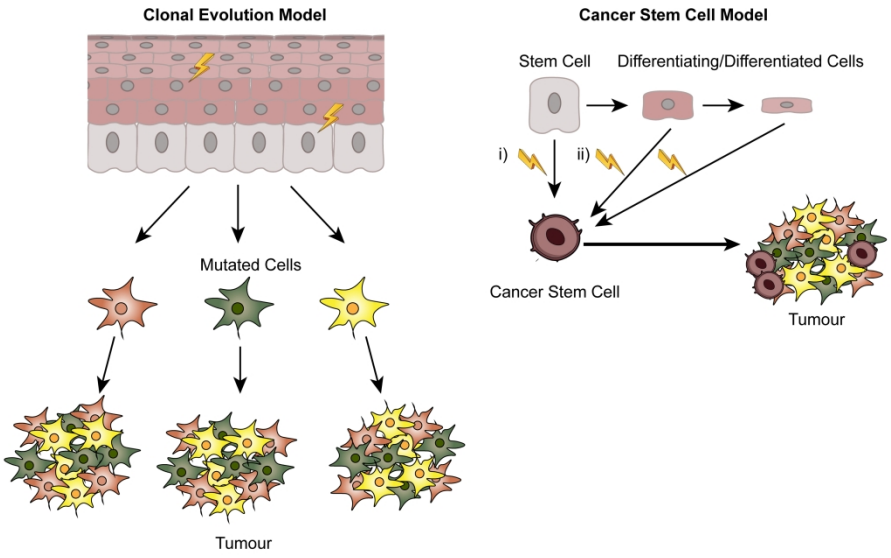

Figure 2: Cancer origin models: clonal evolution and cancer stem-cell model.

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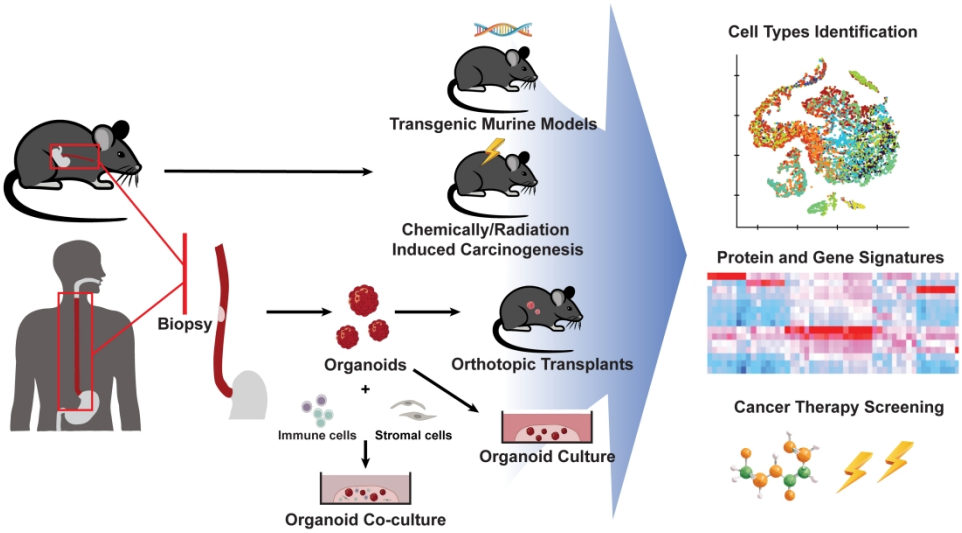

Figure 3: In vivo and in vitro approaches in oesophageal disease modelling.

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