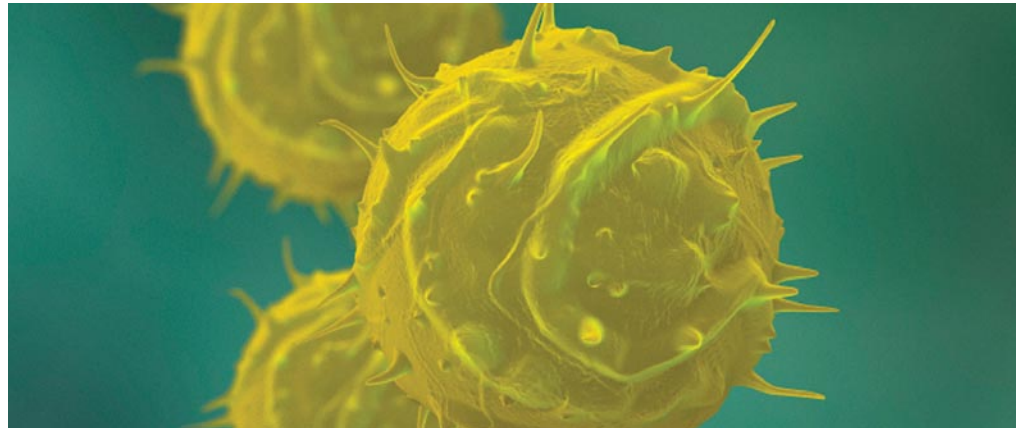


Cancer stem cell theory and emerging clinical application



Abstract

New understanding of the basic nature of malignancy is emerging due to novel studies drawing from stem cell biology. Cancer stem cell (CSC) theory proposes that a small subset of cells within a heterogeneous tumour have the capacity to form tumours, and that these cells demonstrate the abilities of stem cells, namely self-renewal and production of a varied repertoire of daughter cells. Evidence points to dysregulated tissue stem cells as the origin of CSCs. This stem cell nature may confer an inherent resistance to conventional cancer treatment, and be responsible for disease recurrence even after a dramatic clinical response. CSCs have been predominantly demonstrated in mouse xenotransplantation studies, where isolated putative CSC cell populations are able to form and recapitulate parent tumours in the host. Several genes related to stem cells and early embryonic development have been found to be active in CSCs and are thought to give CSCs their tumourigenic abilities. These genes also represent potential therapeutic targets, along with the microenvironment that supports the stem cell. Therapies against these targets are in development and include monoclonal antibodies, RNA interference and small molecules. A small number of CSC-specific therapeutics are in early clinical trials. If CSC theory is clinically validated, it stands to dramatically change the way we think of and treat cancer.

Keywords: Cancer, stem cells, tumour-initiating cells, tumourigenesis, translational medicine, oncology.

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Introduction

Cancer remains the second leading cause of mortality in Ireland and other developed nations despite decades of intensive preventive and novel treatment efforts.¹ However, new understanding of the fundamental mechanisms of tumour initiation and propagation may lead to a dramatic paradigm shift in cancer treatment. It may also explain the existing difficulty in treating cancer with conventional means. This new understanding is at the intersection of cancer biology and stem cell biology, and is positioned to have profound clinical significance in the coming years.

Stem cells

Stem cells have two important abilities. First, they are able to produce a varying range of progenitors or differentiated cells, and second, they are also able to self-renew.² Pluripotent stem cells can produce cells of all three germ layers, i.e., endoderm, mesoderm and ectoderm. Unipotent stem cells in tissue produce one type of downstream progenitor in addition to renewing themselves in asymmetric division. Stem cells between these two are termed multipotent, meaning that they can produce several distinct types of daughter cells but not every cell type in the body.³

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Understanding of basic stem cell biology, including the replicative repertoire of different stem cells, is crucial to the development of cancer stem cell (CSC) theory. This emerging and controversial theory states that both solid tumours and leukaemias are composed of a heterogeneous population of cells, of which a certain fraction are cells that have stem cell-like properties and are able to initiate and recapitulate a new heterogeneous tumour.⁴ This theory also implicates CSCs as the cause of metastasis because of their tumourigenic capability.⁵ Evidence of CSCs, also called tumour-initiating cells, was first reported in leukaemia in 1997, and has since been shown in many solid tumours including brain, breast, pancreatic, lung, colon, ovarian, and endometrial.⁶⁻¹⁴ The primary assay used in demonstrating CSCs is serial xenotransplantation of a putative CSC-enriched population in immunodeficient mice. Specifically, a definition of what CSCs are in the given solid tumour is established by an expression pattern of cell-surface markers, which is then used to isolate cells via fluorescence-activated cell sorting (FACS). This cell population is transplanted into immunodeficient mice, most often NOD/SCID (non-obese diabetic/severe combined immunodeficiency) and a tumour containing many different cells, including those expressing the CSC definition markers, is found. CSCs of the same definition can then be enriched from the secondary tumour and again transplanted into another mouse. This serial transplantation can be repeated for several generations, the number of which is associated with the aggressiveness of the tumour.¹⁴ Tumourigenic cells with stem cell abilities can also be characterised based on expression of intracellular enzymes. An example of such is aldehyde dehydrogenase (ALDH), which has been found to confer resistance to taxane and platinum-based chemotherapies in ovarian cancer, and is associated with poor prognosis in pancreatic adenocarcinoma.^{15,16}

There is controversy regarding the origin of CSCs and whether they actually are as rare as previous reports have demonstrated.¹⁷ It is important to understand if CSCs are normal stem cells that have become dysregulated, or if they are cancerous progenitors that have acquired stem cell abilities. Studies have shown evidence for both but neoplastic transformation of tissue stem cells is often more efficient and phenotypic similarities between normal stem cells and CSCs are very strong.^{5,18}

Research

Recent research has elucidated the mechanisms that CSCs use to exert their effects. Many of them are related to signalling pathways and gene expression profiles seen in early embryonic development. For example, Notch, Wnt/ β -catenin, Hedgehog, and Epithelial-to-mesenchymal transition (EMT) are key developmental processes that have been implicated in CSCs.¹⁹⁻²³ In addition, Oct4, one of the primary genes responsible for maintenance of pluripotency in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPS) is also involved in CSCs.^{18,24} Fitting emerging CSC findings into models of cancer recurrence

after treatment offers some hypotheses. The CSC population within a given tumour may be small, far less than 1%, but if this population has intrinsic resistance to the applied therapy, an apparently dramatic clinical response could still leave behind those cells responsible for repopulating a tumour. This hypothesis is supported by studies showing that the expression of CSC markers is associated with poor prognosis in colorectal carcinoma and other cancers.^{16,25,26} CSCs are thought to be resistant to conventional cancer therapies in a number of ways. First, they can be quiescent, much like normal stem cells, with a very low mitotic rate, making them insensitive to agents that preferentially affect rapidly dividing cells. Downstream progenitor cells are much more mitotically active than CSCs and are responsible for populating the bulk of a solid tumour.²⁷ Second, CSCs may express a higher level of multidrug resistance ATP-binding cassette pumps, which allow these cells to pump out chemotherapeutic compounds, greatly reducing their effectiveness.²⁸ CSCs have also been found to upregulate DNA damage response checkpoint genes, making them more resistant to radiation therapy.²⁹

Implications for treatment

The first step in the treatment of malignancies from a CSC perspective is to target them. One targeting strategy is based on their definition of extracellular surface markers. A unique cell surface epitope separating CSCs from normal tissue stem cells would make an ideal target. Initial investigations have nominated CD133 and EpCAM as potential candidates for monoclonal antibodies.³⁰ In reality, true CSCs may have a subtle pattern of extracellular marker expression that is a combination of surface proteins expressed by a variety of normal cells. This probably makes them poor targets, and even the 'selective' markers like CD133 have been found on normal stem cells.^{31,32} Another strategy is to disrupt the supportive microenvironment that maintains the CSC.³³ It is established that factors in the extracellular milieu of each tissue are crucial to the function of normal tissue stem cells.³⁴ This knowledge can be translated to CSCs, especially as the link between normal tissue stem cells and CSCs grows stronger. The importance of microenvironment is dampened by the use of non-HLA-matched immunodeficient mice for xenotransplantation studies that have become the standard for demonstrating CSCs. In a clinical situation, the malignant cells are the patient's own, so an inherent immunocompatibility allows tumourigenic cells to more easily engraft within a tissue and form a new tumour. Because HLA compatibility is not part of the criteria of the xenotransplantation model, human cancer cells might have a more difficult time adapting to the foreign mouse microenvironment, lowering the tumourigenic cell frequency. When Kelly and colleagues transplanted leukaemic cells into histocompatible mice, the percentage of cells able to initiate new tumours increased to at least 10%. This was far above the figure reported in the prototype CSC report of 1997, as well as most of the solid tumour CSC reports, which found tumourigenic

populations as low as 0.1%.¹⁷ Unfortunately, the incredible diversity of tumour architecture, phenotype, and the definition of CSCs themselves, will make it difficult to target the stem cell microenvironment.³⁵ Success hinges on finding common extracellular factors crucial for the maintenance of pluripotency and self-renewal.

However, some progress has been made in specific tissues. In breast cancer, Wicha and colleagues discovered that after administration of chemotherapy, dying tumour cells release inflammatory interleukin-8 (IL-8), which selectively stimulates the CSC population to expand. Therefore, blocking the IL-8 receptor CXCR1 with an antibody or a small molecule inhibits this effect *in vivo*.³⁶

Understanding the active molecular pathways that give CSCs their abilities opens the door to targeted molecular therapy. Turning off or inhibiting genes responsible for driving tumorigenic cells depends on delivery of therapeutic agents, and thus RNA interference (RNAi) delivery technology is a burgeoning field that will impact on all of medicine, not just oncology. RNAi utilises small double-stranded RNA elements targeted to a specific RNA desired to be knocked down.

The cell's own machinery recognises the dsRNA, processes it, and allows the antisense portion to hybridise to the target RNA, causing degradation or translational repression of the target. In this way, the expression of specific genes can be significantly inhibited.³⁷ In the context of CSCs, genes related to the maintenance of stemness and early embryonic development are ideal targets for RNAi knockdown.

By knocking down these genes, it may be possible to differentiate CSCs to a state of reduced aggressiveness, and/or increased sensitivity to traditional chemotherapeutics. Additionally, anti-apoptotic, signal transduction pathway, and growth factor or cytokine elements are all plausible targets. Currently, there are three RNAi-based therapies in early clinical trials for cancer.³⁸ RNAi is also powerfully used for discovery, where high-throughput assays can knock down thousands of different genes in independent experiments, with different measures of output depending on the investigation. Previous cancer studies in

this regard have looked at the viability and proliferation of tumour cells as outputs. However, in the case of CSCs, some measure of stemness and tumorigenicity will need to be used in the *in vitro* setting of these screening assays.^{39,40} Small molecule inhibitors that can block crucial molecular pathways may radically change cancer treatment in the future. Even though the mechanisms by which these pathways affect CSCs are not completely understood, clinical trials aimed at inhibiting them are underway.

Currently, five small molecule Hedgehog antagonists are in phase I and II trials.²² In addition, a Notch pathway inhibitor developed by Merck is being evaluated in four different cancer trials.⁴¹ Preliminary results are not yet available, as this field is in its clinical infancy. Assuming that CSC theory is correct, measurement of residual tumour bulk is not a good indicator of treatment success, since intrinsically resistant CSCs may still be present in very small numbers. Creation of assays that measure the presence and activity of CSCs is crucial for drug development, treatment response and prognostic index.¹⁶

Conclusion

In summary, CSC theory is poised to have a significant impact on cancer treatment because it changes our basic understanding of what cancer is and where it comes from. This perhaps provides an intriguing explanation for current difficulty in treating malignancy.

The rapid progression of the basic science and emerging translational studies regarding tumorigenesis, coupled with the increasing relevance of this disease in our ageing population, makes this field an exciting and important focus for researchers, clinicians and patients.

It warrants much more investigation, and stretches across many disciplines in biology and medicine. Creation of improved experimental and diagnostic assays for early identification and patient-specific characterisation of CSCs, followed by targeted blockade or elimination of their molecular drivers, are the keys to a potentially dramatic increase in therapeutic efficacy in our treatment of cancer.

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