

Tumour stem cell-targeted treatment: elimination or differentiation

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A wide range of studies suggest that most cancers are clonal and may represent the progeny of a single cell, a cancer stem cell (CSC) endowed with the capacity to maintain tumour growth. The concept of a cancer stem cell emerged decades ago, and the haematopoietic system is where it has mostly gained ground. More recently, CSC have been described in breast cancer and brain tumours. Growing evidence suggests that pathways regulating normal stem cell self-renewal and differentiation are also present in cancer cells and CSC. Malignant tumours can be viewed as an abnormal organ in which a small population of tumourigenic cancer stem cells have escaped the normal limits of self-renewal giving rise to abnormally differentiated cancer cells that contribute to tumour progression and growth. This new model has important implications for the study and treatment of cancer. Understanding the molecular circuitry which contributes to the maintenance of stem cells may provide an insight into the molecular mechanisms of cancer and thus new approaches for elimination or differentiation therapy. Therapies targeting CSC should focus on pathways such as Wnt, Shh and Notch which are required for the maintenance of cancer stem cells, but also on the ABC transporter family and other specific properties of cancer stem cells.

Key words: cancer stem cell, differentiation, leukaemia, self-renewal, solid tumours, targeted therapy

Introduction

Cancer is a genetic disease that alters three types of genes which are responsible for tumour progression, namely oncogenes, tumour suppressor genes and stability genes [1]. According to the multi-step carcinogenesis concept, cancer develops in a series of steps through the accumulation of molecular changes progressing from pre-invasive to invasive disease [2]. Most current research on human tumours is focused on the molecular and cellular analysis of the bulk tumour mass.

Recent evidence has demonstrated that only a small subpopulation of cancer cells has the capacity to form new tumours in haematological malignancies [3]. Despite the clonal origin of many cancers, evidence in leukaemia and more recently in solid cancers suggests that the tumour cell population is heterogeneous with respect to proliferation and differentiation. This feature could be explained by the cancer stem cell hypothesis. So-called 'cancer stem cells' have been identified and characterised in myeloid leukaemia, breast, brain, and lung cancers [4].

The concept of a cancer stem cell arose from the similarities observed between the self-renewal mechanism of normal organs and continuous proliferation in cancers [5]. The cancer stem cell hypothesis suggests that not all the cells in the tumour have the ability to proliferate and maintain growth of the tumour,

but only a small subpopulation of cells in the tumour, called cancer stem cells, are able to proliferate and self-renew. These rare cells with a self-renewal potential and the capacity to form a tumour and maintain its growth were isolated in haematological cancers such as leukaemia [6], multiple myeloma and a few solid tumours such as breast cancer [7] or brain tumours [8]. The cancer stem cell hypothesis may exert a profound impact on our current perception of cancer diagnosis, management and treatment options [5, 9, 10].

stem cells and cancer stem cells

normal stem cells

Stem cells are undifferentiated and unspecialised cells that can renew themselves and also give rise to one or more specialised cell types with specific functions. Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature tissue through differentiation [11]. Key features of normal stem cells are quiescence, asymmetrical division and multipotency.

There are three kinds of stem cells: embryonal, germinal and somatic or adult stem cells [12]. Embryonal stem cells are derived from the first divisions of the fertilised egg. These stem cells give rise to all the cells in the adult organs. During the embryogenic process, the progeny of embryonal stem cells lose this potential and gain differentiated properties in a process called determination. Germinal stem cells in the adult produce eggs and sperm and are responsible for reproduction. Somatic

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stem cells have a more limited differentiation potential and produce cells that differentiate into mature cells. The various types of stem cells have different potentials and proliferate differently. By definition, an adult stem cell is a cell that comes from a given organ, has a long-term replicative potential and the ability to both self-renew and differentiate into the cellular components of the organ.

Stem cells in adult tissues are mainly quiescent, and have a capacity for self-renewal and differentiation in all tissue cell types. By asymmetrical division, one stem cell produces one daughter cell that remains a stem cell and another daughter cell that begins the process of determination and produces rapidly proliferating progenitor cells, committed to differentiation. Progenitors have a limited number of cell divisions and then differentiate or die [13]. Thus, in normal tissue, three different compartments can be described: a self-renewal compartment with quiescent stem cells, a proliferating compartment with proliferating progenitors with a limited potential for self-renewal and a terminal compartment with differentiated cells or apoptotic cells.

properties of normal stem cells

One of the most important functions in stem cell biology is the regulation of self-renewal [13]. Self-renewal is required by stem cells so that they may last a lifetime, and all stem cells must self-renew and regulate the balance between self-renewal and differentiation. Many pathways that were primarily described in cancer may also regulate normal stem cell development. For example, signalling pathways associated with oncogenesis such as Oct-4, BMP (bone morphogenic protein), Janus family kinase, Notch, Sonic hedgehog (Shh) and Wnt signalling pathways, also regulate stem cell self-renewal [14].

Notch proteins are located in the cell membrane and are activated by membrane activated ligands (Delta family protein). Upon activation such proteins release their intracellular domains by proteolysis, and bind to HLH transcription factors. The Notch pathway is implicated in the regulation of neuronal stem cell self-renewal and differentiation.

Hedgehog genes encode for secreted proteins that activate a membrane receptor complex, which activates the transcription factor of the GLI family [15]. There are three hedgehog family members (Desert, Indian and Sonic), Sonic being the most widely expressed among them. Recent studies have demonstrated that the Shh signalling pathway regulates adult neuronal progenitor and self-renewal, and could play a role in brain tumour biology.

The Wnt proteins are extracellular molecules that bind to the receptor of the Frizzled family, and activate a pathway that inhibits proteolysis of β -catenin, which is able to promote gene transcription via interaction with TCF/LEF proteins. This pathway is involved in self-renewal and differentiation [16].

Polycomb family members repress transcription through chromatin remodelling. The Bmi1 gene is an important factor for the self-renewal of stem cells, and Bmi1 expression has been associated with several cancers. A recent study demonstrated that inhibiting cancer stem cell self-renewal after deletion of the polycomb gene Bmi1 could prevent leukaemic relapse [1].

cancer stem cells

As previously stated, the concept of the cancer stem cell arose from the observation of similarities between the self-renewal mechanisms of stem cells and those of cancer cells [5]. Both types of cells self-renew and differentiate into other cells. Moreover, tumours are heterogeneous in terms of cell phenotype and proliferative potential, and ongoing mutations can only partially explain this heterogeneity. Cancer stem cells are a small population of tumour cells capable of self-renewal that give rise to all the components of a heterogeneous tumour. In previous models of cancer, the unregulated growth of tumours was attributed to the serial acquisition of genetic events that resulted in the activation of genes promoting proliferation, silencing of genes involved in inhibiting proliferation, and circumventing genes involved in programmed cell death [13]. In the cancer stem cell hypothesis, another key event in tumour progression is the alterations of genes involved in the regulation of stem cell renewal. Thus, it is not surprising that several genes initially identified due to their role in tumour progression were later implicated in normal stem cell self-renewal [17]. Cancer stem cells and normal stem cells may share similar self-renewal and proliferation mechanisms. Mutations that dysregulate the pathways controlling normal stem cell self-renewal have also been observed in different cancers, suggesting that dysregulation of self-renewal pathways could be required for cancer development.

When cancer cells of different origins were analysed for their proliferative potential in various *in vitro* and *in vivo* assays, only a small minority of cells were able to proliferate extensively. The cellular origin of cancer stem cells has not been clearly determined [6]. Cancer stem cells are derived either from transformed normal stem cells or from more differentiated progenitor cells that have acquired the ability to self-renew as a result of oncogenic mutations.

The cancer stem cell hypothesis was first documented in acute myelogenous leukaemia (AML) by John Dick et al. These authors demonstrated that only a minority of the leukaemic cells were able to proliferate extensively, self-renew and form a new tumour [6]. More recently this concept has been extended to breast cancers [7] and brain tumours [18, 19]. This concept is also applicable to other forms of leukaemias, lymphomas and multiple myeloma. This concept implies that leukaemias arising in the stem cell compartment can only be eliminated by total bone marrow ablation. Until recently, data to support a cancer stem cell basis for solid tumours were sparse [13]. Recent experiments using methodologies first developed for the study of haematopoietic stem cells, suggest however that such cancer stem cells may exist in breast cancers, and glioblastomas. In 2003, Al-Haji et al. [7] reported that only a small population of tumour cells were able to induce new tumour formation in mice. These cells were found to be CD44+ (an adhesion molecule that binds to hyaluronate) and CD24- (an adhesion molecule that binds to P-selectin). Primary tumour cells expressing a CD44+/CD24 low cell surface phenotype were shown to initiate tumours when they were transplanted into immune-deficient NOD/SCID mice, whereas other cell types failed to form tumours. These data demonstrate the presence of a hierarchy of cells within a breast tumour in which

only a fraction of these cells have the ability to generate a new tumour. Recent evidence also suggests that brain tumours contain small numbers of cells with normal stem cell properties. Studies by Singh et al. [20] have shown that the neural stem cell antigen CD133 is expressed in a subset of cells from brain tumours. The CD133 subpopulation from brain tumours is able to initiate clonally-derived neurospheres *in vitro*, with self-renewal, differentiation and proliferation features analogous to normal brain stem cells. Moreover, CD133+ cells (but not CD133- cells) transplanted into NOD/SCID mice are able to form a tumour *in vivo*. Other groups have cultured cells with characteristics of CNS stem cells from various human brain tumours, and described the existence of a cancer stem cell population in human brain tumours of different phenotypes [20]. Both prostate cancer and benign prostatic hyperplasia are believed to arise as a modification of cell proliferation and differentiation. There are three types of epithelial cells in the prostate (basal, luminal and neuroendocrine), and these different cells are derived from a common stem cell. It has been shown that 2–3% of basal epithelial cells have stem cell characteristics, but more work is required to better understand prostate biology. A recent study suggests that signalling pathways such as hedgehog and wntless are implicated in prostate carcinogenesis [21, 22].

Another recent study suggests that cancer stem cells are also directly regulated by more frequent cancer mutations such as that affecting the PTEN gene. Deletion of *Pten* in a murine model of prostate cancer recapitulates the disease progression seen in humans. PTEN negatively regulates p63-positive prostatic basal cell proliferation without blocking differentiation. Concomitant with basal cell proliferation is the expansion of a prostate stem/progenitor-like subpopulation expressing the stem cell antigen-1 (Sca-1) and BCL-2-positive cells. This suggests that the stem cell compartment in prostate cancer is under the dependence of the PTEN tumour suppressor. Whether these prostate cancer stem cells directly harbour genetic mutations or give rise to daughter cells that will secondarily accumulate oncogenic mutations remains to be studied [23]. Tumour stem cells have also recently been identified in a melanoma model [24].

cancer stem cell therapy

general concepts

The cancer stem cell hypothesis could have fundamental and profound implications for cancer therapy. Cancer stem cells like normal stem cells are more resistant to conventional chemotherapy than other more differentiated cancer cells. Therefore, it is also important to use therapies that target not only proliferating cells but also stem cells in order to cure cancer [25]. Currently, anti-cancer therapy can shrink primary and metastatic tumours, but such effects are usually transient and tumour relapses of most metastatic cancers frequently occur. One potential reason for failure is that current therapies fail to kill cancer stem cells. Therapy that kills non-tumourigenic cancer cells can shrink tumours, but will not cure patients, whereas therapies that kill, or induce differentiation of cancer stem cells could better contribute to curing patients. An

understanding of the cellular signalling that controls stem cell proliferation and differentiation could lead to the development of new anticancer strategies. These therapies could be designed to target cancer stem cells in order to induce the differentiation of cancer stem cells [26], or to eliminate cancer stem cells by inhibiting the maintenance of the stem-cell state. Targeting pathways that maintain stem cell properties might be useful to transform malignancies into benign tumours. That the dormancy and stem cells concepts strictly overlap has yet to be clearly defined.

The conventional treatments indiscriminately kill proliferating cells. However, therapy can fail due to the survival of quiescent tumour stem cells.

In order to be successful, therapy must first kill all proliferating tumour cells, and then differentiate or eliminate cancer stem cells. It has been suggested that occult, disseminated metastatic cells are refractory to chemotherapy due to a lack of proliferation termed 'dormancy'. Tumour dormancy has been hypothesised to be a key limiting event in the treatment of malignant diseases inducing resistance to cytotoxic agents. These cells can survive chemotherapy and then return to a proliferative growth state due to some mechanisms that have been analysed to account for late failures. Tumour cells have been shown to enter dormant metastatic cells either due to a lack of autocrine growth factors or to inhibiting agents secreted by other metastases

differentiation therapy

Although crucial for the understanding of cancer development, anticancer approaches aimed at killing cancer stem cells may not be relevant in all clinical settings. Glioblastoma multiforme contains cancer stem cells. However, there is no evidence that they may be responsible for tumour recurrence following therapy, because glioblastoma multiforme does not respond to therapy. Here, therapy fails because it does not even affect proliferating cells [12]. Thus, targeting proliferating cells is the first step required to control cancer because these progeny cells will have enough divisions to kill a patient. Targeting cancer stem cells must therefore be a secondary objective in some clinical settings such as pancreatic, brain and lung tumours.

One way to handle the CSC problem is to treat cancer by inducing differentiation of cancer stem cells. Differentiation therapies in oncology are broadly defined as those that induce malignant reversion [11]. Differentiation therapy could force cancer stem cells to differentiate and lose their self-renewal property. Whereas a number of agents have been studied, only two anticancer drug categories affect cancer cell differentiation, retinoic acid and drugs that target tumour epigenetic changes.

The only differentiating agent used in clinical practice is retinoic acid (RA, Vitamin A). Vitamin A and its analogues (retinoids) are modulators of differentiation and proliferation of epithelial cells, and they can invert the malignant progression process through transduction signal modulation mediated by nuclear retinoid receptors.

Acute promyelocytic leukaemia has become a curable disease through all-trans retinoic acid (ATRA)-based induction therapy followed by chemotherapy. Currently about 90% of newly diagnosed patients with acute promyelocytic leukaemia

achieve complete remission and over 70% are cured with ATRA therapy [27]. *In vitro*, retinoid acid can induce differentiation in a great number of other cell types such as embryonic cells, teratocarcinomas, and melanomas [11]. However, limited benefit has been achieved with retinoids in the treatment of different solid tumours (squamous cell carcinomas, prostate adenocarcinoma, neuroblastomas, and hepatocarcinoma).

Differentiation therapies could also be used for chemoprevention. Chemoprevention can be defined as the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent progression to invasive cancer [28]. Retinoids can also induce differentiation in precancerous cells and be used as chemopreventive agents for head and neck and lung cancer.

Histone deacetylase (HDACs) regulates histone acetylation by catalyzing the removal of acetyl groups on the NH(2)-terminal lysine residues of core nucleosomal histones. Modulation of the acetylation status of core histones is involved in the regulation of the transcriptional activity of certain genes. Aberrant recruitment of HDAC activity has been associated with the development of certain human cancers [30].

The HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), was initially identified as a result of their ability to induce differentiation of cultured murine erythroleukaemia cells. Additional studies showed that SAHA inhibits the proliferation and accumulation of cells in a dose-dependent manner in G(1) then G(2)-M phase of the cell cycle in breast cancer cell lines. SAHA can induce morphological changes including flattening and enlargement of the cytoplasm, and a decrease in the nuclear-cytoplasmic ratio and induction of milk fat globule protein, milk fat membrane globule protein, and lipid droplets in the breast cancer MCF7 cell line [31], suggesting that HDAC can induce differentiation. SAHA and other HDAC inhibitors are currently in Phase I clinical trials.

elimination therapy

Another way to increase the efficacy of cancer therapy is to eliminate cancer stem cells. This can be achieved using different approaches: transplantation of allogeneic haematopoietic stem cells with graft versus leukaemia/tumour effect, targeted therapies against self-renewal signalling pathways and the cell cycle, modulation of chemoresistance mechanisms or tackling other specific properties of cancer stem cells.

Transplantation of allogeneic haematopoietic stem cells can be curative in leukaemias that have a poor prognosis with conventional chemotherapy. Immunological mechanisms mediated by donor immune cells contained in stem cell grafts are known to be involved in the eradication of malignant cells (and in particular cancer stem cells) after allogeneic haematopoietic stem cell transplantation, and are responsible for the graft versus leukaemia/tumor effect. This approach is seldom used in solid tumours and the survival benefit is small.

Several molecular signalling pathways including PTEN, the polycomb gene [15], Wnt [14, 32], Hh [3] and Notch play a role in normal stem cell self-renewal. It is possible to selectively target these pathways involved in normal and cancer stem cell self-renewal. A recent study showed that treatment of mice with an Hh pathway inhibitor such as cyclopamine inhibits the

growth of medulloblastoma. Moreover, inhibition of the Notch pathway with specific gamma secretase inhibitors could also inhibit cancer stem cell self-renewal and decrease tumour growth [33].

Furthermore, understanding the molecular mechanisms of cell cycle control in stem cells could also provide new strategies for cancer stem cell therapies [15]. There are no data on the roles of CDKs or other cell cycle regulators in cancer stem cells. Recent studies showed that cancer stem cells and normal stem cells share the Bm1 and Wnt pathways, i.e. mechanisms for self-renewal.

Stem cells have many properties that separate them from mature and differentiated cells, and particularly stem cells can acquire resistance to chemotherapy via a range of mechanisms, including the mutation or overexpression of the drug target, drug inactivation, or drug elimination from the cell [34, 35]. Resistance to chemotherapy could also be explained by the fact that cancer stem cells are quiescent and have high levels of ABC transporters, as well as a high capacity for DNA repair and high levels of anti-apoptotic proteins. Stem cells usually exhibit a high expression of members of a self-defence system against xenobiotics: the ATP-binding cassette transporters (breast cancer resistance protein BCRP-ABCG2; multi-drug resistance-1 ABCB1/MDR1). Cancer stem cells are believed to maintain this property. But only few studies have addressed the potential differences in drug sensitivity between cancer stem cells and their non tumorigenic cancer cell counterpart. It has been established that leukaemic progenitors have the ability to expulse mitoxantrone and daunorubicin, two agents used in treatment of AML [36]. Although inhibitors of ABC transporters exist, most clinical trials with these agents were largely negative and none of them is used in daily clinical practice.

The cancer stem cell hypothesis also has an impact on the identification of new and future therapies. Gene expression in normal stem cells has been shown to be significantly different from gene expression in their more differentiated progenitors [37]. It is possible that gene expression analysis of tumourigenic cancer cells could identify novel diagnostic markers and novel therapeutic targets. There are limited examples of agents that are selectively toxic against cancer stem cells. Recent data have shown that chemotherapy with a proteasome inhibitor (bortezomib) killed leukaemia stem cells, but not normal haematopoietic stem cells [38]. Differences between normal and cancer stem cells could also involve post-transcriptional and post-translational modifications. A recent study has shown that different splicing variants of the adhesion receptor CD44 are differentially expressed by cancer stem cells and normal stem cells [39]. This cell surface marker which is differentially expressed between normal and cancer stem cells could be a new target for antibody-based therapy.

conclusions and perspectives

The cancer stem cell hypothesis is a promising new paradigm that could potentially influence cancer diagnosis and management. Current cancer treatments (surgery, chemotherapy, radiotherapy) have been evaluated for their ability to kill proliferating cells. However, these therapies can fail due to the survival of cancer stem cells. Future therapies must

therefore first aim at killing all proliferating tumour cells, and secondarily differentiating or eliminating cancer stem cells [12]. Nevertheless, little information is currently available about the nature of the stem cell niche and the pathways regulating quiescence and self-renewal in both normal and cancer stem cells. The differences in signalling pathways between normal and cancer stem cells need to be elucidated to provide new therapeutic targets that could contribute to eliminating residual disease and recurrence.

Furthermore, the cancer stem hypothesis is only one of the important aspects in the current understanding of cancer biology. An appropriate local micro-environment [40], as well as newly-developed vessels [41], are key elements required for tumour growth, and targeting them is also of paramount importance. Finally, in current practice failure of treatment in the metastatic setting is mostly related to progression of bulky proliferating cancer cells, and not to our inability to block cancer stem cells.

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