

Stem Cells and Cancer: An Overview

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Published online: 23 October 2007
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Abstract Definite evidence of the importance of cancer stem cells in the progression of cancer has now come to light. Key markers of these cells have been identified in many solid tumours as well as leukaemias. Specific studies modelling the tumour induction of specific cells isolated by surface antigens such as CD44 have demonstrated that these cells are not only present in tumours but that they are the key units in their tumourigenicity. These findings provide useful insight for disease progression, treatment and metastasis. The wide variety of proposed markers, and their similarity to endothelial progenitor cells found in angiogenesis, complicates these studies. Definite proof falls only in the induction of tumours *in vivo*. Here we review the developments in cancer stem cells and the markers that have been found for these cells.

Keywords Cancer · Stem cells · Tumour

Introduction

Stem cells differ from the majority of adult cells as they possess the ability for self-renewal utilising microRNA

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expression to bypass G1/S checkpoint [1]. Most adult stem cells are thought to possess insufficient telomerase activity to prevent telomerase loss. Unlike embryonic stem cells that maintain their telomere length between 8 and 12 kb adult mesenchymal stem cells with a higher telomerase activity have been linked to higher risks of malignancy. Repeated cell division allows the accumulation of mutations, non stem cells do not possess the ability to divide making stem cells not only the repair mechanisms for tissues but most likely source of malignancy.

Bone marrow contains two types of stem cells: haematopoietic stem cells (HSC) and other primitive progenitor cells, including mesenchymal stem cells (MSC) and multipotent adult progenitor cells (MAPCs) [2, 3]. MSC are rare pluripotent cells that support HSCs and have the ability to differentiate both *in vivo* and *in vitro* into a variety of adult mesenchymal cells including bone, cartilage, fat, muscle, tendon and marrow stroma [4]. Adult stem cells vary in morphology and characteristics from one tissue to another and tend to concentrate in one site within the tissue and are supported by surrounding cells and microenvironment. These form a group of cells known as tissue stem cells. In addition another cell type are also commonly recognised, the endothelial progenitor cells. These arise from the bone marrow and are believed to be partially differentiated. They circulate in the blood supply and then migrate to areas of damage such as wounds and tumours where they are involved with angiogenesis.

The discovery that tumours contain cancer stem cells that seem responsible for tumorigenicity has revolutionised the way tumour progression is viewed. This sub-population of cells is responsible for the ability of tumours to replicate and has been implicated in resistance to chemotherapy. A substantial body of evidence has appeared in the literature in recent years that demonstrates the presence of such stem cells in both leukemias and solid tumours. Here we look at

the identification of these cells by various groups using surface markers and differential enzyme expression.

To balance the role of stem cells in tumour genesis we have also reviewed how stem cells provide promise for novel therapies for tumours. A better understanding of the tumour stem cell and targeted cell delivery using stem cells that may be recruited to the area of tumour growth provides exciting opportunities for future cancer therapies.

Cancer Stem Cells

A fundamental problem in cancer research is identifying the cell type that is capable of sustaining neoplastic growth. There is evidence that the majority of cancers are clones and that cancer cells represent the progeny of a single cell, what is less clear is which cells possess the tumour-initiating cell function and therefore maintain tumour growth [5]. In order to induce a tumour in an animal model, hundreds of thousands of cancer cells need to be injected. Some researchers explained this as a limitation in the assay to support tumour growth, or by a tumour formation deficiency [6]. Cancer cell biology text books listing up to twelve steps involved in cells leaving the blood vessels until tumour formation have provided a convenient argument for the high failure rate. More recent findings suggest that this is due to the low proportion of cancer stem cells in the tumour population.

Only specific cancer cells (cancer stem cells) possess the required regenerative properties that lead to tumour formation. The injection of many cells, therefore, is needed to maximise the probability that these specific cancer stem cells are present. The first evidence of the existence of cancer stem cells came in studies on leukaemia and multiple myeloma. A small subset of cancer cells were capable of extensive proliferation [7]. These cell subsets were named leukaemic stem cells. Two possibilities were proposed: either all leukaemia cells had a low probability of proliferating and therefore all leukaemia cells behave as leukaemic stem cells, or only a small subset was clonogenic. The later theory was proved by Bonnet and colleagues [8] who were able to separate the $CD34^+CD38^-$ leukaemic stem cells from patient samples. Despite being small in numbers (0.2%), these are the only cells capable to transfer acute myeloid leukaemia from patients to Non obese diabetic sever combined immunogenicity (NOD-SCID).

More recently cancer stem cells have been demonstrated in solid tumours. A group lead by Clarke identified cancer stem cells in breast tissue in 2003. A sub population of human breast cancer cells were shown to express surface antigens associated with stem cells stem cell and that these induced tumour growth in a mouse model. Furthermore, when those tumourigenic cells were injected into mice, the tumours that formed contained multiple cell types, similar to the original tumour. This indicates that the injected cells

were not only capable of self-renewal but also of generating a wide spectrum of progeny, like stem cells [9]. Since this discovery research into cancer stem cells has been prolific. Cancer stems or side population cells (a semi purified groups of cancer cells shown to contain but not be solely made up of cancer stem cells) have been discovered by many groups in almost all tumours tested. The most recent findings are listed in Table 1. Furthermore, studies looking at hepatocellular carcinoma cells lines used side population analysis and cell sorting to confirm that even commercially available cell lines possess a mixed cell population. Of the three lines all possessed less than 1% side population cells. When the tumour genesis of the un-purified population and the side population was tested 1×10^6 cells were required compared to 1×10^3 to reproducibly induce tumours in mice. Thus demonstrating why so many cells are required before tumours are seen [10] when un-purified cells have been used for tumour studies in the past. In NOD-SCID mice as few as 200 $CD24^-CD44^+$ cells have been shown to be sufficient to produce tumours.

Many of the studies looking for cancer stem cells in tumours needed to be carefully evaluated. The identification of stem cell markers in solid tumours may not be definitive. The presence of adult stem cells for the process of angiogenesis may complicate these studies. A more definitive model is to prove the tumourigenicity of these cells in a NOD-SCID, or similar, in vivo model.

Origins of Cancer Stem Cells

The side population of the cancer cells are a small subpopulation of the cells of a tumour. They are defined as excluding Hoescht stain and may be separated by flow cytometry of the dissociated tumour. They are considered to contain the cancer stem cells. They express various markers thought to confer drug resistance such as the ABC transporter family. To date only poor characterisation has been achieved. They remain a potential source of enriched cancer stem cells [11]. Side population cells defined only as Hoescht staining positive have been shown to be tumourigenic in immunocompetent mice [12]. The side population is probably a mixture of cancer stem cells and their daughter cells that possess some but not all cancer stem cell properties. Further purification by surface antigens is required to isolate a pure population of cancer stem cells.

For a cell to become neoplastic a series of changes are needed to overcome the stringent controls on cell division. The origin of cancer stem cells is still not fully understood: they may or may not be derivatives of tissue stem cells. Two main factors need to be considered: (a) a number of mutations are needed for a cell to become cancerous [13] and therefore it is unlikely that all mutations could occur in the lifespan of a progenitor/mature cell, (b) a cancer stem

Table 1 Indicators of stem cell identification with different cancer types

Cancer/tumour type	Markers identified	Methodology	Ref
Bladder	OCT-4	RT-PCR, WB, IHC	[43]
Bone Sarcomas	Activated STAT3, OCT3/4, NANOG	PCR	[44]
Breast	La7	CSC isolation and NOD/SCID	[45]
Breast	CD44 [CD24]		[46]
Breast	CD44	NOD-SCID tumour induction	[47]
Breast	CD44 ⁺ CD24 ⁻ /low, CK ⁺	IHC, flow cytometry	[11]
Breast	Hedgehog components	Gene expression studies and NOD-SCID	[48]
Colorectal	EpCAM, CD44, CD166	Flow cytometry and NOD/SCID	[49]
GI	SP and Hoescht	Flow cytometry Hoechst	[50]
Glioblastoma	N-CoR location	Comparative proteomic analysis	[51]
Glioma	–	Molecular mechanisms studied	[52]
Glioma	HH-GLI, CD133	Gene silencing	[37]
Head and neck*	CD44 ⁺ , cytokeratin 5/14, BMI1	NOD/SCID	[53]
Hepatic	Alpa-fetoprotein, CK14	Serum markers (human), immunohistochemistry	[54]
Hepatocellular	Hoechst	Cell line SP detection, NOD-SCID mice	[10]
Leukaemia	Bmi-1	Nock-in mice	[55]
Leukaemia	BCR-ABL signalling network	Gene regulation	[56]
Leukemia	Hoechst	Hoechst subpopulation immunocompetent mice	[12]
Leukemia	MLL-AF9	Fusion protein isolation	[57]
Leukemia	LMO2	Gene therapy	[58]
Lung (NSCLC)	CD90, CD117, CD133	Cell sorting	[59]
Melanomas	CD20 ⁺	Flow cytometry	[60]
Multiple myeloma	Hedgehog	PCR	[61]
Oral**	hTERT	Enzyme marker expression	[62]
Prostate	c-myc, beta-catenin, CD44(+), CD133(+)	Cell sorting	[63]
Prostate	ABCG2	DNA array and PCR	[64]
Prostate	Androgen receptor	Comparison to normal tissue	[65]
Prostate	CD44 ⁺ /alpha2beta1hi/CD133 ⁺		[66]
Prostate	CD133	Flow cytometry	[67]
Retinoblastoma	ABCG2, MCM2	immunohistochemistry	[68]
Retinoblastoma	ABCG2, Hoescht, ALDH1, MCM2, SCA1	Flow cytometry	[69]
SCLC	Urokinase plasminogen activator CD44 ⁺	FACS, microscopy, clonogenic activity	[70]
Testicular	Decorin	Comparative PCR between cancer cells and ESC	[71]
Thyroid	–	Review	[72]
Uterus	hTERT	Xenograft	[73]

Including principal methodology used for identification of these markers.

NSCLC non small cell lung carcinoma

NOD/SCID Non-obese diabetic/ severe combined immunodeficiency

SCLC small cell lung carcinoma

N-CoR nuclear receptor corepressor

RT-PCR

WB western Blot

IHC immunohistochemistry

Ck cytokeratin

ABC ATP-binding cassette transporter

ALDH1 aldehyde dehydrogenase 1

MCM2 minichromosome maintenance marker 2

SCA1 stem cell antigen 1

*Head and neck squamous cell carcinoma

**Oral squamous cell carcinoma

cell needs to overcome any genetic constraint on both self-renewal and proliferation [14]. It follows that cancer stem cells must be derived from either self-renewing normal stem cells or from progenitor cells that have acquired the ability of self-renewal due to a mutation [15].

These questions have been addressed primarily in the origin of leukaemias such as acute myelogenous leukemia (AML). Work by Dick and others has revealed that stem-like leukaemia initiating cells are obtained from various subtypes of AML showing different stages of differentia-

tion share the same cell-surface markers with the normal long-term haematopoietic stem cells. This supports the hypothesis that cancer stem cells are derived from stem cells and not from more committed progenitors [8, 16].

Studies by Weissman et al. have suggested that cancer stem cells can be derived from stem cells, as well as the committed short-lived progenitors, giving rise to tumours with comparable latencies, phenotypes and gene expression profiles [17–19]. In solid tumours the lack of markers to characterise tumour cells has made it difficult to study the origins of cancer stem cells. This situation may change in the future due to the identification of cell surface markers in the lung [3], brain [20–22] and prostate [23]. These will allow the separation of stem or progenitor cells that have the tumour initiating function.

Implications for Treatment

The idea that growth of tumours is driven by cancer stem cells may lead to a radical change in treatment by identifying new diagnostic markers and therapeutic targets expressed by the stem cells. Clearing the tumour of such cells should cure the disease as the remaining cells have limited proliferative capabilities. Cytotoxic cancer treatment targets tumour cell proliferation potential and ability to metastasize. Findings implicating cancer stem cells in these key areas of cancer suggest that current treatments will be even less likely to be curative than was previously thought. The more similar the cancer cell to stem cells is the smaller the window of opportunity for treatment with non-targeted cytotoxic drugs. Current treatments may shrink the size of the tumour, but these effects are often transient and often do not improve patient survival [24, 25]. If agents spare cancer stem cells then the disease is more likely to relapse. Targeting cancer stem cells is problematic as they do not appear to have the hyper proliferation signals activated such as tyrosine kinase. In addition, the tumour suppressor gene *PTEN* [26], polycomb gene *Bmi1* [27] and the molecular signalling pathways (such as Wnt, Hh and Notch) that play a role in normal stem cell self-renewal, all participate in cancer development. Thus targeting such modulators of cancer stem cells is likely to increase side effects resulting from stem cell loss.

Surprisingly, few studies have looked at the differences in drug sensitivity between cancer stem cells and other cancer cells. A study by Costello et al. [28] showed that $CD34^+CD38^-$ leukemic cells were less sensitive to daunorubicin than the more committed $CD34^-CD38^+$. Similar results were reported on the antimetabolite cytarabine which was shown to be less effective in killing leukaemia-initiating cells compared to the remaining cells [29]. Better characterisation of specific cancer stem cell markers are required for more accurate targeting of new therapies. An

example is the $CD34^+CD38^-$ subpopulation in haematopoietic stem cells expressed different cellular markers; haematopoietic stem cells expressed Thy-1 and c-kit [30, 31] whereas leukemic stem cells expressed IL-3 (interleukin-3) receptor α -chain. Such markers may be the key to antibody targeted therapies.

Much research is focused on targeting essential genes or pathways crucial for cancer development, with any therapies against targets expressed by tumour initiating cells more likely to be successful. For example, in chronic myelogenous leukemia, Gleevec® targets ATP-binding domain of the Abl kinase; most patients treated experienced complete cytogenetic responses [32, 33]. The therapy may not be curative, though, because of reported presence of the fusion transcript [34].

A comparison of the pathways that regulate stem cell homing with those responsible for metastasis may prove useful too. Treatment of mice with a Hedgehog pathway inhibitor such as cyclopamine [35] inhibits the growth of medulloblastomas in mouse models, without any apparent toxicity. Thus, the Hh pathway may be inactive in most normal adult tissues, hence minimising the toxicity effects of these inhibitors [36]. The use of specific gene silencing has been used by some to study the importance of some of the pathways identified in cancer stem cells. Interference with the hedgehog-GLI signalling using a viral vector has been demonstrated to remove resistance to temozolomide in glioblastoma multiforme [37].

Stem Cells as Delivery Vehicles for Gene Therapy

This group has previously reviewed the potential usefulness of adult stem cells as delivery vehicles for gene therapy [38]. This subject will be briefly highlighted again here as the importance of this area of research remains a potential future therapy.

The solid tumour acts as a wound recruiting circulating endothelial progenitor cells. This is achieved via signalling such as VEGF [38]. In this case the tumour utilises recruited cells for the process of angiogenesis. This differs from ongoing vessel repair that is thought to rely upon in situ cell division rather than recruiting circulating cells [39, 40]. Reports that MSC, genetically modified with viral vectors to over express IFN- β , suggest that such cells may produce sufficient gene product to raise local IFN levels in mouse gliomas [41]. Systemic administration of IFN have demonstrated that insufficient levels can be achieved in tissues to promote cellular response [42]. Numbers of such studies are limited so far and difficulties of gene expression as a clinical tool will remain for long to come. In general the success of such techniques relies on the assumption that circulating endothelial progenitor cells, or the chosen cell type, are primarily involved in angiogenesis but have a

limited role in normal vessel repair. Historic evidence suggests that this is the case but to date this is not conclusive.

Conclusion and Future Prospects

It is increasingly becoming clear that cancer is a stem cell disorder, and further research is needed to look at the similarities and differences between normal and cancer stem cells self-renewal processes. Cancer therapy has been the subject of intense research for many years and yet in many areas the cytotoxic drugs of choice remain the older drugs such as cisplatin and 5flourouracil. The similarities of the cancer stem cell to the adult tissue stem cell seriously hinder systemic cytotoxic therapies.

Cancer stem cells have been shown in several tumour types is the key to tumour promotion in NOD-SCID mice models. Purification of the cancer stem cells using key markers such as CD44⁺ has demonstrated that the cancer stem cells may be isolated by surface antigens. Using such purified cells has demonstrated that the large numbers of cells needed to induce tumours experimentally are most likely due to the small percentage of cancer stem cells in the initial population. To date little consensus is present as to which markers are key to cancer stem cell status. Further caution is needed as both CD34 and CD44 have been identified on circulating progenitor cells. Confusion with studies using surface antigens alone may occur. The key at present seems to be in vivo evidence that these cells are tumourgenic.

Cancer therapy has entered an exciting new era with stem cells and related therapies emerging as a novel approach to therapy especially in inoperable tumours. Identifying differences between cancer stem cells and adult tissue stem cells will prove as bigger challenge as gene delivery to these sites. However, the addition of genes that may control the cell cycle and apoptosis to cultured MSC or EPC look set to be the next big area of research in the field of cancer therapy.

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