Regulation of breast cancer stem cell features

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Cancer stem cells (CSCs) are rare, tumour-initiating cells that exhibit stem cell properties: capacity of self-renewal, pluripotency, highly tumorigenic potential, and resistance to therapy. Cancer stem cells have been characterised and isolated from many cancers, including breast cancer. Developmental pathways, such as the Wnt/β-catenin, Notch/γ-secretase/Jagged, Shh (sonic hedgehog), and BMP signalling pathways, which direct proliferation and differentiation of normal stem cells, have emerged as major signalling pathways that contribute to the self-renewal of stem and/or progenitor cells in a variety of organs and cancers. Deregulation of these signalling pathways is frequently linked to an epithelial-mesenchymal transition (EMT), and breast CSCs often possess properties of cells that have undergone the EMT process. Signalling networks mediated by microRNAs and EMT-inducing transcription factors tie the EMT process to regulatory networks that maintain "stemness". Recent studies have elucidated epigenetic mechanisms that control pluripotency and stemness, which allows an assessment on how embryonic and normal tissue stem cells are deregulated during cancerogenesis to give rise to CSCs. Epigenetic-based mechanisms are reversible, and the possibility of "resetting" the abnormal cancer epigenome by applying pharmacological compounds targeting epigenetic enzymes is a promising new therapeutic strategy. Chemoresistance of CSCs is frequently driven by various mechanisms, including aberrant expression/activity of ABC transporters, aldehyde dehydrogenase and anti-oncogenic proteins (i.e. BCL2, B-cell lymphoma-2), enhanced DNA damage response, activation of pro-survival signalling pathways, and epigenetic deregulations. Despite controversy surrounding the CSC hypothesis, there is substantial evidence for their role in cancer, and a number of drugs intended to specifically target CSCs have entered clinical trials.

Key words: cancer stem cells, breast cancer, signalling pathways.

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Introduction

The "cancer stem cell theory" has attracted a great deal of attention following the identification of a rare population of leukaemia-initiating cells exhibiting stem-like features [1]. It has been further strengthened by the isolation and characterisation of cancer stem cells (CSCs, named also tumour-initiating cells or stem-like cells) from solid tumours such as breast [2], glioblastoma [3], colon [4], lung [5], ovary [6], and thyroid [7]. Besides the properties shared with adult, tissue-specific stem cells, such as self-renewal and the ability to differentiate into other cells, a subpopulation of candidate CSCs must meet certain criteria: 1) the strong ability to engraft; 2) the ability to recapitulate the tumour of origin both morphologically and immunophenotypically in xenografts; and 3) the ability to be serially transplanted.

To distinguish CSCs from other tumour cells and normal stem cells, a search for specific surface and intracellular biomarker phenotypes has been ongoing in recent years. The most common method used to identify CSCs is fluorescence-activated cell sorting (FACS). Breast cancer stem cells (BCSC) have been identified as $CD44^+$, $CD24^{-/low}$, and ESA^+ (epithelial specific antigen) and lacking expression of specific lineage markers (ESA^+ $CD44^+CD24^{-/low}$ Lin^-) [2]. Amongst primary breast tumours, there is an association between the metastatic status and a high prevalence of markers such as $CD44^+/CD24^{-/low}$, ESA^+, $CD133^+$, $CXCR4^+$, and $PROCR^+$ in tumour cells.

Wnt (wingless), Shh (sonic hedgehog), Notch, and BMP/TGF-β (bone morphogenetic proteins/transforming growth factor β) signalling pathways contribute to the self-renewal of stem and/or progenitor cells in a variety of organs. When deregulated, these pathways can contribute to oncogenesis.

The Wnt/β-catenin signalling pathway modulates a balance between stemness and differentiation in several adult stem cell niches, such as the hair follicles in the skin, the mammary gland, and the intestinal crypt. Constitutive Wnt signalling activation resulting from mutations in genes encoding its downstream components underlies tumorigenesis in these tissues. Loss of adenomatous polyposis coli (APC) tumour suppressor function or oncogenic β-catenin mutations occur in the majority of sporadic colorectal cancers and melanoma [8]. Monoclonal antibodies against the Wnt cascade and several inhibitors of Wnt signalling compounds are under investigation in several cancers [9].

The Hedgehog (Hh) family of secreted proteins includes Sonic (Shh), Indian (Ihh), and Desert (Dhh). The Hh proteins exert their activity by binding to a transmembrane protein, Patched (PTCH), which constitutively represses Hh pathway activity through its interaction with a transmembrane protein Smoothened (SMO). Several groups have exploited cyclopamine (SMO signaling inhibitor), to inhibit the Hh cascade, and thereby inhibiting the growth, invasion, and metastasis of breast, prostatic, pancreatic, and brain malignancies both *in vitro* and *in vivo* [10, 11].

Notch signalling is initiated through the interaction of a receptor on the signal-receiving cell and a ligand on the neighbouring cell. Upon binding to

Delta-Serrate LAG2 (DSL) ligand, the Notch receptor is activated by an ordered proteolytic cleavage. Release of the Notch intracellular domain from the cell membrane mediated by γ-secretase results in its translocation to the nucleus where it interacts with DNA-binding proteins of the CSL family (CBF1 or RBPJ in humans) and induces target gene transcription. The best-characterised Notch target genes are the basic helix-loop-helix (bHLH) transcriptional repressors of the Hairy enhancer of split (Hes) and Hairy-related (Hrt) protein families [12]. Inhibition of Notch1 with specific antibodies significantly reduced the $CD44^{+}CD24^{-/low}$ subpopulation (BCSC) and diminished the incidence of brain metastases from breast cancer cells [13].

Bone morphogenetic proteins (BMPs), TGF-β and GDFs (growth and differentiation factors), belong to the TGF-β superfamily and are pluripotent factors involved in the regulation of embryonic development and postnatal homeostasis of various organs and tissues by controlling cellular differentiation, proliferation, and apoptosis [14]. TGF-β and BMP/GDF form homo- and hetero-dimers that interact with heterodimers of type I and type II receptor to produce signalling complexes, leading to the activation of SMAD transcription factors [15]. Stimulation of an epithelial-to-mesenchymal transition (EMT) by TGF-β is accompanied by the generation of breast CSCs [16]. Many of the genes actively transcribed by $CD44^{+}/CD24^{-/low}$ BCSCs are classical TGF-β targets, associated with a mesenchymal, migratory phenotype. In a breast cancer model of MDA-MB-231 cells injected to athymic mice, BMP7 or BMP2/7 heterodimer antagonised the pro-tumorigenic and pro-metastatic actions of TGF-β, and reduced TGF-β-driven Smad signalling and cancer cell invasiveness. The maintenance of a subpopulation of $ALDH^{hi}/CD44^{hi}/CD24^{-/low}$ BCSCs and formation of bone metastases by MDA-MB-231 cells growing in nude mice was strongly reduced by heterodimeric BMP2/7 [17].

In addition, pro-survival and anti-apoptotic pathways are frequently overactivated in cancer stem cells. STAT (signal transducers and activators of transcription) proteins are activated in response to extracellular ligands that bind to appropriate receptors and activate receptor-associated tyrosine kinases (i.e. as Janus kinase – JAK) and non-receptor tyrosine kinases (i.e. as Src kinase). Phosphorylated STAT proteins form dimers and translocate to the nucleus where they activate target genes [18]. Increased levels of STAT3 were found in CSCs comparing to bulk cells in brain, breast, colon, and liver cancers. Blocking STAT3 function in BCSC correlated with lower proliferation and viability of stem-like cells, suggesting the involvement of this factor in the maintenance of CSCs [19].

Nuclear factor-κB (NF-κB) transcription factors are constitutively active in many solid tumours, including breast, colon, and liver cancers [20]. Nuclear factor-κB activation is regulated by the IκB kinase (IKK) complex composed of IKKα and IKKβ catalytic subunits. IKKα activity is required for self-renewal of ErbB2/Her2-transformed mammary tumour-initiating cells [21]. IKKα phosphorylates p27/Kip1, the cyclin-dependent kinase inhibitor, and stimulates its nuclear export or exclusion. Reduced p27 expression restored mammary tumorigenesis in IKKα knockout mice and self-renewal of mammary tumour-initiating cells.

Mechanisms that regulate self-renewal of breast cancer stem cells

The best characterised signalling pathways controlling self-renewal and differentiation in normal stem cells, such as Wnt/β-catenin, Notch, Hedgehog, and TGF-β/BMP pathways, are frequently deregulated in breast cancer cells, which leads to acquisition of the stem-cell phenotype [22, 23] (Fig. 1). Furthermore, networks of co-ordinately working proto-oncogenes and tumour suppressors have evolved to control self-renewal of stem cells throughout their life. For example, the Polycomb group (PcG) protein, Bmi-1, a proto-oncogene consistently required for the self-renewal of diverse adult stem cells, is also essential for the proliferation of cancer stem cells in the same tissues [24–26]. Moreover, EMT also may impart a self-renewal capability to cancer cells [16]. This process enables reprogramming of polarised epithelial cells towards a mesenchymal motile phenotype, and a growing body of evidence links EMT to the acquisition of stem cell properties by breast cancer cells [27, 28]. While overexpression of *OCT3/4, SOX2, KLF4*, and *c-MYC* genes in somatic cells leads to dedifferentiation into induced pluripotent stem cells (iPSCs) [29, 30], the activation of the molecular targets of these pluripotency-associated genes is frequently observed in poorly differentiated breast tumours and other cancers [31–33]. Of interest, accumulating evidence indicates that the expression of Oct3/4, Nanog, and Sox2 transcription factors have a strong correlation with CSCs; knockdown of these genes decreased tumour sphere formation and inhibited tumour formation in xenograft tumour models [34–38].

Epigenetic regulation of the expression of pluripotency markers in breast cancer stem cells

Recent studies have elucidated epigenetic mechanisms that control pluripotency and stemness, thus allowing an assessment of how embryonic and normal tissue stem cells are deregulated in cancer to give rise to CSCs. Levels of transcription factors acting in embryonic stem cells (ESCs), such as Oct3/4, Nanog, or Sox2, strongly correlate with acquisition and maintenance of CSC phenotype. The expression of these factors in cancer stem cells is regulated by epigenetic mechanisms [37, 38]. Wang *et al*. demonstrated that DNA methylation acts synergistically with histone modifications in regulation of *NANOG, OCT3/4*, and *c-MYC* gene expression and contribute to the metastatic potential of CSCs. Results from this work suggest that the reactivation of pluripotency circuits by aberrant epigenetic alterations is one of the key events of CSC initiation [39]. Furthermore, Rivenbark *et al*. have shown that active expression of the *SOX2* gene in breast cancer cells is critically controlled by its promoter demethylation. Direct methylation of this region by zinc-finger (ZF)-based artificial transcription factors (ATFs) having a methyltransferase activity led to repression of the promoter activity and down-regulation of *SOX2* expression [40]. Further examples of epigenetic alterations affecting the expression of pluripotency markers are presented in Table 1.

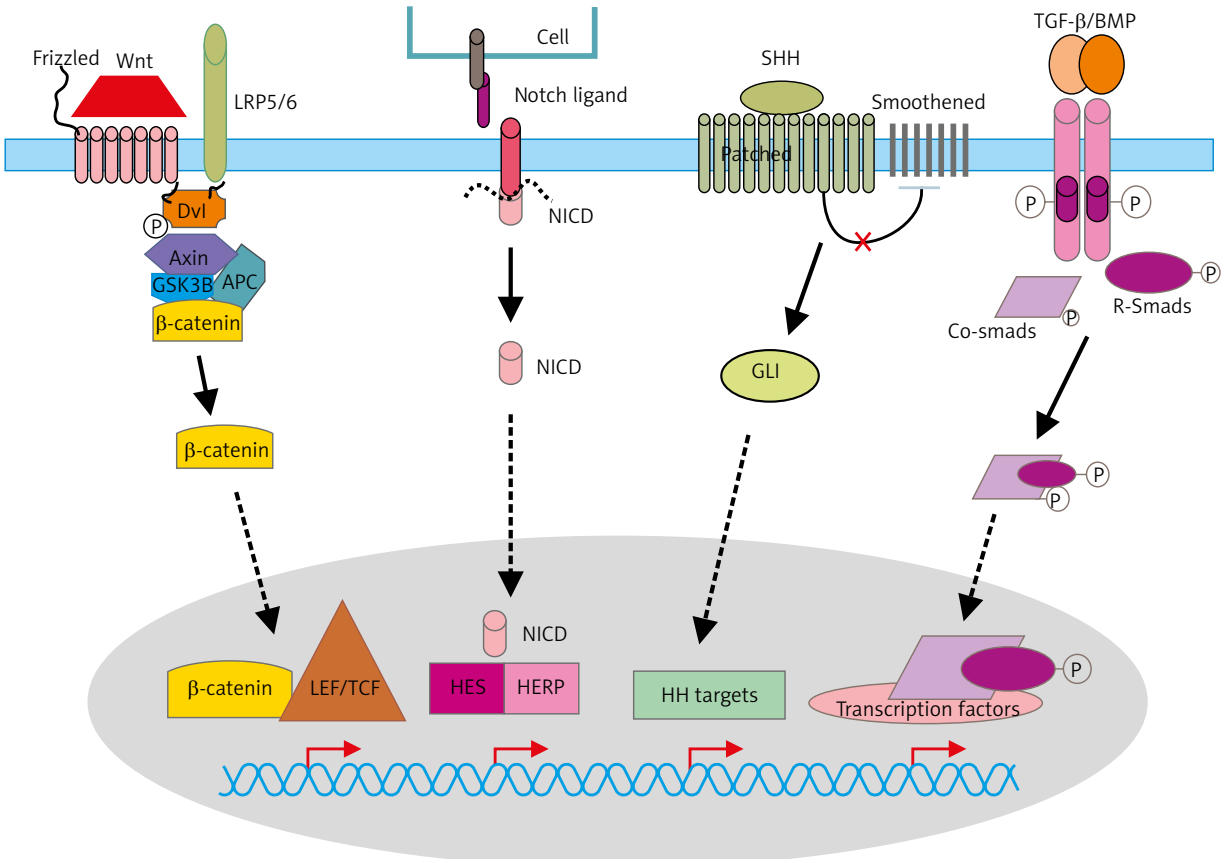

Fig. 1. Schematic representation of signalling pathways that control maintenance of BCSCs

Wnt binds its co-receptors: Frizzled (Fz) and LRP5/LRP6. Various molecules that interact with the receptors and GSK-3 and CK1 (casein kinase 1). In the presence of Wnt, β-catenin is stabilised and induces gene expression by complexing with various transcription factors such as TCF/LET. The Delta-like and the Jagged proteins produced by signal-sending cells serve as ligands for Notch receptors. Upon ligand binding, the receptor fragment is cleaved by TACE (TNF-α ADAM metalloprotease converting enzyme) then γ-secretase to create NICD (Notch intracellular domain), which translocates to the nucleus, forms a complex with transcription factors RBPJ and CSL (CBF1/Suppressor of Hairless/LAG-1), and activates the expression of target genes (f.e. Hes1 *and* Herp). *Sonic Hedgehog (SHH) binds to a transmembrane protein Patched (PTCH), which constitutively represses Hh pathway activity through its interaction with a transmembrane protein Smoothened (SMO). Shh-bound PTCH activates SMO, and activated SMO releases GLI1 (Glioma-Associated Oncogene Homolog 1) from cytoplasmic sequestration, and, in turn, GLI1 translocates into the cell nucleus to regulate gene expression. Activation of TGF-β type I and type II receptors leads to activation of receptor kinases and phosphorylation of the R-Smads, forming a complex with co-Smad 4, which translocates to the nucleus*

Deregulation of Wnt/β-catenin pathway by altered methylation and aberrant histone modifications

Wnt/β-catenin is an important pathway involved in the regulation of stem cells as well as cancer stem cells. In breast cancer, many genes coding for components of Wnt/β-catenin signalling, including the Wnt inhibitor – *WIF1* (Wnt inhibitory factor1), *SFRP1-5* (Secreted Frizzled-Related Protein 1), and *DKK1* (Dickkopf-related protein 1), are methylated and silenced, which leads to aberrant activation of the Wnt-pathway and acquisition of stem-cell phenotype [52–54, 57]. Among various antagonists of the Wnt pathway, DACT1 (Dapper/Frodo) has been identified as a protein interacting with Dishevelled (Dvl), a central mediator of Wnt signalling. As demonstrated by Yin *et al*., *DACT1* expression was silenced in breast tumour through promoter methylation leading to overactivation of Wnt signalling and enhanced migratory behaviour of breast cancer cells [58]. Upregulated Wnt signalling was also observed when the *APC* gene promoter was found methylated. Loss of APC favours β-catenin accumulation and stimulates TCF/LEF-induced transcription [52, 86]. Further studies demonstrated aberrant histone modifications such as enrichment of EZH2-mediated H3K27me3 histone mark on Wnt genes in parathyroid tumour cells, which leads to deregulated Wnt signalling [87]. Furthermore, miRNAs have also been implicated in the regulation of different players of Wnt/β-catenin in different types of cancer modulating the acquisition of stem cell properties [43, 45, 48], as recently reviewed elsewhere [22, 23, 88].

Posttranscriptional modifications impair Notch signalling in BCSCs

The Notch pathway is associated with the regulation of cell fate at several distinct developmental stages of the mammary gland and has been implicated in cancer initiation and progression. Recent reports have shown that Notch signalling is essential for maintaining the CSC population in breast cancer cell lines [89, 90]. In a large proportion of breast carcinomas, epigenetic mechanisms that activate Notch signalling were related to the role of miR-146a, which targets NUMB, a negative regulator of Notch. Numb is known to down-regulate Notch signalling through direct interaction and the subsequent ubiquitin-mediated

protein degradation. Recent reports demonstrate a role of miR-146a in mediating the induction and maintenance of breast CSCs during EMT and provide new insights into the mechanisms for breast cancer progression [65, 91, 92]. Furthermore, an epigenetic enzyme EZH2, which belongs to the Polycomb group, was demonstrated to regulate NOTCH signalling in breast carcinoma. Gonzalez *et al*. have identified EZH2 as a direct regulator of NOTCH1, inducing activation of Notch1 expression and signalling, leading to stem cell expansion in triple-negative breast cancer [93].

Epigenetic regulation of epithelial-to-mesenchymal transition

Epigenetic changes may influence acquisition of stem cell-like phenotype by cancer cells via methylation of genes implicated in EMT. Taube *et al*. identified micro-

Table 1. Epigenetic regulation of selected pathways and mechanisms in cancer stem cells

Mechanism/Pathway	Target	Epigenetic modification	Epigenetic modulator	Reference
Wnt/β-catenin	APC	Promoter DNA hypermethylation	DNMTs	41
		miRNA targeting	miR-135a, miR-135b	42
	β-catenin	miRNA targeting	miR-200a	43
			miR-203	44
			miR-214	45
			miR-1826	46, 47
			miR-320	48, 49
	WNT3A	miRNA targeting	miR-15a cluster	50
			miR-16-1	50
	AXIN2	H3K27me3 repressive mark	EZH2	51
	WIF1	Promoter DNA hypermethylation	DNMT1, DNMT3b	52–54
	SFRP1-5	Promoter DNA hypermethylation	DNMTs	52
	DKK1	Promoter DNA hypermethylation	DNMTs	52
		Decreased H4K16Ac and increased H3K27me3	Polycomb group proteins	55
		miRNA targeting	miR-371-373 cluster	56
	DKK3	Promoter DNA hypermethylation	DNMTs	54, 57
	DACT1	Promoter DNA hypermethylation	DNMTs	58
	DACT3	Bivalent H3K27me3 and H3K4me3 histone modifications	Polycomb group proteins	59
NOTCH	NOTCH1	miRNA targeting	miR-34a	60
	NOTCH2	miRNA targeting	miR-34a	60
	NOTCH4	miRNA targeting	miR-34c	61
	JAGGED 1	miRNA targeting	miR-200c	62
			miR-141	62
			miR-34a	63, 64
	JAGGED 2	Acetylated histone	Down-regulated HDACs activity	65
		miRNA targeting	miR-34a	63, 64
	NUMB	miRNA targeting	miR-146a	66
Hedgehog	Gli1	miRNA targeting	miR-324-5p	67
TGF-β	TGF-β	miRNA targeting	miR-106b-25 cluster; miR-179-92 cluster	68

Table 1. Cont.

Mechanism/Pathway	Target	Epigenetic modification	Epigenetic modulator	Reference
Pluripotency factors	Sox2	miRNA targeting	miR-200c	69, 70
		Promoter DNA hypomethylation		39
	Oct3/4	Promoter DNA hypomethylation		39
		H3K4me3 active mark	Trithorax group proteins	71
	Klf4	miRNA targeting	miR-200c	69, 70
			miR-7	72
		Promoter DNA hypomethylation		39
	Nanog	H3K27me3 repressive mark	EZH2	73
		Promoter DNA hypomethylation		39
		H3K4me3 active mark	Trithorax group proteins	71
EMT	CDH1 (E-cadherin)	Promoter DNA hypermethylation	DNMTs	74
		H3K27me3 repressive mark	EZH2/PRC2	75, 76
		Histone H3 and H4 deacetylation	HDAC1/Snai1; HDAC2/Snai1	77
		miRNA targeting	miR-495	78
		Decreased H3K4me2	LSD1/Snai1	79, 80
	CDH2 (*N*-cadherin)	H4K20me1 active mark	SET8/Twist	81
	ZEB1	miRNA targeting	miR-200 family	82–84
	ZEB2	miRNA targeting	miR-200 family	82–84
miRNA	miR-34c	DNA methylation	DNMTs	61
	miR-200c	DNA methylation	DNMTs	85
	miR-203	DNA methylation	DNMTs	44

RNAs, which are regulated by DNA methylation and which regulate EMT-derived stemness properties [44]. The promoter of microRNA-203 (miR-203) – a known regulator of skin cell differentiation – was methylated significantly in cells that had undergone EMT due to Twist overexpression, and its down-regulation facilitated the gain of mesenchymal/stemness properties. Thus, activating miR-203 – either epigenetically or by other means – may inhibit invasion and metastasis [44]. Furthermore, miR-200 family members are also down-regulated due to epigenetic alteration in breast CSCs in comparison to non-tumorigenic cancer cells [69, 94]. Demethylation of the miR-200 promoter was strongly inhibited by another group of miRNAs, miR-22, expression of which correlates with tumour invasiveness and metastatic properties. Down-regulation of miR-200 expression expanded the stem cell compartment and promoted breast cancer progression. Therefore, miR-22 is a crucial epigenetic modifier and promoter of EMT and cancer stemness toward metastasis [69, 94].

A rapidly growing body of research demonstrates that EMT is also epigenetically regulated by chromatin remodelling, DNA methylation, and changes to histone modification levels. Yang *et al*. demonstrated that TWIST, a master modulator of EMT process, is physically associated with SET8, a methyltransferase specifically targeting H4K20 for monomethylation in breast cancer cells. SET8 was recruited by TWIST to the *CDH2* (N-cadherin) gene promoter and its H4K20 monomethylation activity contributed to activation of the *CDH2* expression. On the other hand, TWIST protein functions as a transcriptional repressor and cooperates with SET8 to repress *CDH1* (E-cadherin) expression, during which SET8 acts as a co-repressor by establishing H4K20me1 mark on the *CDH1* gene promoter [81]. The expression of *CDH1* could be also regulated by Snai1 transcription factor. It was previously shown that Snai1 induces repressive histone modifications at the *CDH1* gene promoter through recruitment of histone deacetylases (HDACs) and H3K27 methyltransferase EZH2 [75, 76, 95]. Furthermore, Lin *et al*. demonstrated that Snai1 directly interact with LSD1, a histone demethylase, recruiting LSD1 complex to the *CDH1* and other epithelial gene promoters, resulting in down-regulation of the active H3K4me2 mark and promoter activity. Down-regulation of epithelial gene promoters correlates with acquisition of cancer stem-cell properties, so targeting the enzymatic components of the LSD1 complex with therapeutic agents may offer a new

way to halt tumour progression and dissemination [79]. The protein KAP1 (KRAB-associated protein 1), an interaction partner of members of the family of KRAB (Krüppel-associated box) domain-containing zinc finger transcription factors, may play a role in regulation of EMT. Venkov *et al*. demonstrated formation of a ternary complex composed of the CArG box-binding factor-A (CBF-A) and the KAP-1 protein at the fibroblast transcription site-1 (FTS-1) within the promoter of *FSP1* gene (also known as *S100A4*), which can activate other known transcriptional regulators of EMT, including Snai1 and Twist. However, the exact role of KAP1 protein in acquisition mesenchymal-properties by EMT is unknown [96–98].

Mechanisms of CSC resistance to anti-cancer therapy

The numerous mechanisms of chemoresistance have been identified in CSCs of different origin, and these mechanisms include the following: aberrant ABC transporter expression/activity, augmented aldehyde dehydrogenase (ALDH) activity, enhanced DNA damage response, activation of self-renewal signalling pathways, and epigenetic deregulations (for a review see [99]). Cancer stem cells also have a slow rate of cell turnover and therefore can escape from chemotherapeutic agents that target rapidly proliferating cells. Adenosine triphosphate-binding cassette (ABC) transporters belong to a family of transmembrane transporters, amongst which at least 15 of these genes are implicated in drug resistance. Some of them have specific and narrow substrate recognition (ABCA3 for anthracyclines) while others exhibit resistance to a wide group of chemotherapeutic drugs (ABCC1 for anthracyclines, mitoxantrone, vinca alkaloids, imatinib, epipodophyllotoxins, camptothecins, colchicine, saquinivir, and methotrexate) [100]. Many studies demonstrate that ABCB1 and ABCG2 proteins cooperate at the blood-brain and blood-tumour barriers to restrict penetration of various anti-cancer drugs. Tissue-specific stem cells and cancer stem cells express a higher level of specific ABC pumps in comparison to their differentiated progeny that protect stem cells against toxins, but none of these efflux pumps operate as a regulator of "stemness". The feature of higher activity/expression of ABC transporters is used to isolate a side population (SP), which could be sorted using fluorescent rhodamine 123 (ABCB1 specific) or Hoechst 33342 (ABCG2 specific) dyes and contains cells with high capability for efflux anti-mitotic drugs.

The expression of ABC proteins in cancer stem cells is regulated mostly at the transcriptional level. Transcription factors that directly regulate the expression of the genes coding for ABC pumps include TP53 [101], liver X receptor/retinoid X receptor – LXR/RXR [102], and thyroid hormone receptor [103]. The expression of *ABCG2* gene coding for breast cancer resistance protein is regulated by progesterone and oestrogen receptors, nuclear factor-κB (NF-κB), hypoxia-inducible factors (HIFs), nuclear factor erythroid 2-related factor 2 (Nrf2), aryl hydrocarbon receptor (AhR), peroxisome proliferator-activated receptors (PPARs), and Krüppel-like factor 5 (KLF5) [104]. Oncogenic miR451 and miR-27a up-regulate ABCB1/MDR1/p-glycoprotein expression in multidrug-resistant cancer cell lines [105, 106].

Aldehyde dehydrogenases (ALDH) are a family of-NAD(P)+-dependent enzymes which catalyse irreversible oxidation of endogenous and exogenous aldehydes generated during cellular metabolism processes. The human *ALDH* superfamily consists of 19 known genes grouped in 11 families and 4 subfamilies (www.aldh.org). Aldefluor assay is based on staining living cells with aldefluor substrate – BODIPY aminoacetate (BAAA), which is converted to a negatively charged compound (BAA–) and becomes highly fluorescent, which allows sorting of ALDH bright cells (ALDHbri). Aldehyde dehydrogenases 1 activity is higher in human progenitor cells (there is a lower level of ALDH activity in primitive stem cells) and CSCs [107]. Aldehyde dehydrogenases bright cells of breast cancer are highly tumorigenic in NOD-SCID mice [108]. Although ALDH has been detected in CSCs from various tumour types, it is not a universal CSCs marker and could be used for CSCs derived from tumours that do not express ALDH1 at a high level, such as breast, lung, or colon, but not of CSCs from tumours that normally express a high level of ALDH1 (liver and pancreatic cancers).

Overexpression of ALDH1 in many drug-resistant cancer cell lines, tissues derived from chemotherapy-resistant patients, and in various CSCs makes it a promising target for anticancer treatment. The best-known inhibitors of ALDH enzymes are ATRA (all trans retinoic acid) and DEAB (diethylaminobenzaldehyde). All trans retinoic acid is a differentiation agent with the ability to indirectly down-regulate ALDH expression, while DEAB is a small molecule which directly inhibits enzymatic activity of ALDH1 [109]. Treatment of breast cancer patients with ATRA in combination with tamoxifen gave a more promising outcome than ATRA alone [110]. Disulfiram (DSF commercially known as Antabuse), which is a potent inhibitor of ALDH1A1 and has cytotoxic effects on glioblastoma stem-like cells inhibiting the growth of temozolamide-resistant tumour cells and blocking self-renewal [111], has entered a phase II clinical trial for newly diagnosed glioblastoma (ClinicalTrials.gov Identifier: NCT01777919).

Tissue-specific stem cells are equipped with multiple protective mechanisms to ensure the lifetime function of tissues. For example, epidermal stem cells show greater resistance to DNA-damaging agents than other cells of the epidermis, due to higher expression of anti-apoptotic molecules, shorter p53 activation, and enhanced non-homologous end-joining (NHEJ) activity (this repair system has lower fidelity of repair than other systems). These mechanisms are shared by other tissue-resident stem cells, suggesting that stem cells have evolved highly efficient repair mechanisms [112]. Current evidence suggests that CSCs have evolved even more efficient repair mechanisms. Comparison of breast tumour biopsies showed an increase in CSCs with mammosphere-forming capacity following chemotherapy with the EGFR/HER2 inhibitors lapatinib [113] and cisplatin [114]. In addition, mammary gland CSCs harboured lower levels of reactive oxygen species (ROS) compared to the rest of the tumour cells. This was due to increased levels of genes regulating free radical scavenging systems, such as the glutathione metabolism,

which may contribute to radioresistance [115]. It has been shown that subsets of CSCs in human, and murine breast tumours, contain lower ROS levels than corresponding non-tumorigenic cells. As ROS are critical mediators of ionising-radiation-induced cell killing; CSCs in these tumours exhibit less DNA damage and are preferentially spared after irradiation compared to normal counterparts. Pharmacological depletion of ROS scavengers in CSCs decreases their clonogenicity and results in radiosensitisation [115]. In general, mechanisms related to DNA repair and repair other than DNA (in particular low proliferation, low ROS levels, and activation of the DNA damage checkpoint response) could be responsible for CSC chemoresistance.

Conclusions

Despite the controversy surrounding the CSC hypothesis, arising due to inconsistencies in phenotypic and functional markers, there is growing evidence for their role in carcinogenesis and cancer progression. Cancer stem cells share many mechanisms described for tissue-specific stem cells, but due to oncogenic deregulations these cells have evolved even more efficient repair mechanisms, antioxidant and drug-intoxicating systems that may contribute to tumour recurrence and enhanced resistance to chemo- and/or radiotherapy. Wnt, BMP/TGF-β, Shh, and Notch signalling pathways contribute to the self-renewal of stem and/or progenitor cells in a variety of organs, but deregulation of these pathways can contribute to oncogenesis and maintenance of CSCs. A number of drugs affecting properties specific to CSCs and intended to specifically target these cells have entered clinical trials. Understanding intrinsic properties of CSCs resulting in their resistance to chemotherapy will help to develop more personalised approaches to treating cancer and improve clinical outcomes for cancer patients.

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