Challenges and limitations of targeting cancer stem cells and/or the tumor microenvironment

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Abstract

The existence of cancer cells with stem cell properties (Cancer Stem Cells, CSCs) and their association with tumor resistance and relapse has led to the search for active compounds to eliminate these cells or modulate their stemness in the hope of curing cancer. So far, three classes of drugs that target cancer stemness (Stemness Modulator Drugs) have been identified: i) drugs that selectively eliminate CSCs (stem cell targeting drugs); ii) drugs that decrease stemness (stemness inhibitor drugs); and iii) drugs that promote stemness (stemness promoting drugs). In addition, microenvironment modulating drugs aimed at selectively targeting the stem cell niche are being investigated and may represent an important class of drug for cancer therapy. This article will briefly review the current use of these substances and discuss the potential outcomes, challenges and limitations of treatment modalities using these classes of drugs for cancer treatment. Finally, a modular tumor model will be proposed as a guide to integrate our knowledge on the biology of cancer stem cell with that of the tumor microenvironment to promote a more rational development of anticancer therapy.

Introduction

In most types of cancers, different subpopulations of cancer stem cell (CSCs) or cancer stem-like cells (CS-LCs) have been described. There is a still considerable debate on the existence of CSCs and on the exact nature of these cells.[1-4] Due to technical limitations and the lack of reliable markers for CSCs, the data from some scientific articles that reported the isolation of CSCs and their characterization in term of their biological properties and chemosensitivity should be taken with caution. To illustrate this, the initial identification of cancer stem cells in human brain tumors was made by assessing the expression of the neural stem cell surface marker CD133.[5] In that study, $CD133^+$ cells, but not $CD133^-$ cells, showed marked stem cell activity. Later, several authors used CD133 as a glioma stem cell marker.[6,7] In one study, $CD133^+$ cells were significantly resistant to temozolomide, carboplatin, paclitaxel and etoposide compared to autologous $CD133^-$ cells.[7] In another study, temozolomide was shown to preferentially deplete $CD133^+$ cells.[8] It was later found that CD133 is not a reliable marker for glioma stem cells since $CD133^-$ glioblastoma cells (for the anti-CD133 antibody) actually express a truncated variant of the CD133 protein and that $CD133^-$ cells were also tumorigenic and able to repopulate the $CD133^+$ fraction.[9,10] The limitations of CD133 as stem cell marker are not restricted to brain tumors[11] and have also been documented for lung cancer.[12] For some types of immunotherapy, CD133 status seems to be irrelevant since both $CD133^+$ and $CD133^-$ are susceptible to NK-mediated cytotoxicity.[13] In consideration of these data, the real impact of the research performed on glioma stem cells using CD133 as a surface marker has to be carefully evaluated. Other methods to isolate cancer stem cells (*e.g.* side population (SP) fraction, neurospheres) also have severe limitations and, once again, the results should be analyzed with care. Due to space limitations, this issue will remain beyond the scope of this manuscript. In this manuscript, CSCs and CS-LCs will be used indistinctly and referred to as CSCs/CS-LCs.

The classical division between CSCs/CS-LCs and non-CSCs is not the best model for cancer biology. Instead, there is a growing consensus that tumors are more heterogeneous and contain several subpopulations of cells with different degrees of stemness (CSCs/CS-LCs) that are usually associated with increased resistance to chemotherapy.[14,15] It is not a surprise then that CSCs/CS-LCs can be selected *in vitro* following treatment with anticancer drugs[16,17] and standard chemotherapy enrich for CSCs/CS-LCs in both xenografted mice and treated patients. The obvious implication is that to cure cancer all subpopulations should be the target and this leads us to ask: how many subpopulations actually exist in a given tumor and how different are they from each other? How many drugs do we need to target all of them?

This article will emphasize the general effect and outcomes we can expect from CSCs/CS-LCs targeting drugs and/or the tumor microenvironment according to our present knowledge of cancer biology. Providing an entire update of the list of CSCs/CS-LCs targeting drugs is beyond the scope of this review. However, reviews covering these specific topics have been recently published and are referred to through this article.

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Cancer stem cells/cancer stem-like cells targeting drugs

Selectively targeting CSCs/CS-LCs was initially considered a very promising strategy to cure cancer. In theory, any specific cancer stem cell marker is a potential target and attempts have been made to formulate strategies to exploit them. The limitations of the *cancer stem cell markers* will likely be translated into clinical failure when trying to target these markers. For instance, targeting $CD133^+$ cells with monoclonal antibodies for glioma treatment will serve no purpose since it will spare the $CD133^-$ cells that can repopulate the original tumor. Monoclonal antibodies targeting other surface markers (*e.g.* CD123, CD44, CD33, and CD326) have been developed and are under evaluation. As expected, some of them, such as gemtuzumab ozogamicin (Mylotarg) and lintuzumab[18] and bivatuzumab mertansine[19] have, in the best cases, shown only modest benefit and have been discontinued. Despite the extensive list of potential specific CSCs/CS-LCs targeting drugs, for the moment, few drugs that selectively kill these cells have been identified (Table 1).[20-31] Salinomycin was identified as selective inhibitors of cancer stem cells by high-throughput screening from a collection of 16,000 compounds.[20] It was shown to induce apoptosis in human $CD4^+$ T-cell

leukemia cells, but not in normal CD4+ T cells.[24] However, salinomycin is very toxic to other normal cells at concentrations effective against CSCs.[32] Thus, it is unlikely that salinomycin will be useful as a single agent.[33]

The identification of signaling pathways important for maintaining the stemness phenotype was received with great enthusiasm and several small molecules are currently being investigated. The signaling pathways that can be targeted include Hedgehog, Notch and Wnt, among others.[34,35] For instance, cyclopamine[36] and SANT-1 target the Hedgehog pathway that may contribute to the induction and maintenance of pancreatic[37] and breast[38] tumors. During the period 1999-2009, at least 44 novel substances have been patented as inhibitors of the Hedgehog pathway[39] and many others (including some activators) are being tested.[40] Some novel compounds (GDC-0449 and IPI-926) have entered clinical trials.[41] ZTM000990 and PKF118-310 target the canonical WNT signaling cascade[35] and gamma-secretase inhibitor GSI-18 is active against the Notch signaling pathway.[6,42] Most of these substances are in the very early phase of pre-clinical testing. Similar to salinomycin, they may be of limited use as single agents. Other available substances, while not selective CSCs/CS-LCs inhibitors or selective targeting stemness signaling pathways, are able to modify the stemness properties of cancer cells (Table 2).[43-57] Therefore, at least three classes of stemness modulating drugs (SMDs) have been identified.

i) Stem cell targeting drugs (SCTDs) selectively kill CSC/CS-LCs sparing non-CSCs. Salinomycin may be a prototype of this class but it use may be limited by its high toxicity.[33] Other SCTDs have been reported (Table 1).

ii) Stemness inhibiting drugs (SIDs) do not kill CSCs/CS-LCs but reduce the stemness properties of the cancer cells. Few drugs with these properties have been identified (Table 2). Although there is little distinction between an SCTD and an SID (SID at high doses may eliminate CSCs/CS-LCs), they are conceptually different classes of drugs. While an SCTD eliminates CSCs/CS-LCs at concentrations that are much less toxic to non-CSCs (*e.g.* salinomycin, see below), an SID may eliminate non-CSCs and CSCs/CS-LCs with similar potency but at the same time reduce the stemnnes of CSCs/CS-LCs.

iii) Stemness promoting drugs (SPDs) do not kill CSCs/CS-LCs but increase the stemness properties of the cancer cells. So far, to our knowledge, only metformin has been reported to have this property (Table 2).

Table 1. Examples of selective cancer stem cells targeting drugs (SCTDs).

Agent	Cancer type	Refs.
Salinomycin	Breast	20
	Gastric	21
	Prostate	22
	Osteosarcoma	23
	Acute T-cell leukemia	24
	Chronic lymphocytic leukemia	25
	Human promyeloblastic leukemia	26
	Colorectal	27
	Lung adenocarcinoma	28
3-O-methylfunicone	Breast	29
LSD1 inhibitors	Several pluripotent germ cell tumors (F9, NCCIT, NTERA-2)	30
Cyclopamine	Patient-derived (glioma)	31

Table 2. Examples of stemness modulator drugs (SMDs).

Cell line/cancer	Agent	Effect on stemness / (effect on cytotoxicity)	Refs.
U87MG and U373MG (glioma)	Eckol	↓ / (↑)	43
U87 (glioma)	Nordy	↓ / (N.A.)	44
HCT116 (colorectal)	Lovastatin	↓ / (↑)	45
SW1990 (gemcitabine-resistant pancreatic cell line)	Cyclopamine	↓ (↑)	46
Breast	SANT-1	↓ (↑)	38
Pancreas	SANT-1	↓ (↑)	37
Patient-derived (glioma)	Resveratrol	↓ / (↑)	47
Patient-derived (glioma)	AG490	↓ / (↑)	47
Patient NSCLC-derived cells (lung)	Cucurbitacin	↓ / (↑)	48
Patient HNSCC-derived cells (Head and neck squamous cell carcinoma)	Cucurbitacin	↓ / (↑)	49
MCF-7 (breast)	Metformin	↑/ (N.A.)	50
MCF-7 (breast)	Berberine	↓ / (N.A.)	51
FOLFOX-resistant cells (colon cancer)	Curcumin	↓/ (↑)	52
Glioma	ER-400583-00	↓(↑)	53
Glioma	WP1193	↓(↑)	54
Glioma	Angiogenesis inhibitors	↓(↑)	55
Glioma	All-*trans* retinoic acid	↓(↑)	56
Non-cancer			
3T3-L1 preadipocytes	Curcumin	↑/ (N.A.)	57

Combination therapy using stemness modulator drugs

Combination therapy using monoclonal antibodies against stem cell markers and standard anticancer drugs did not show any great benefit. Recent Phase II studies of combination therapy with gemtuzumab ozogamicin (GO), *e.g.* GO + vorinostat,[58] GO + ara-C,[59] GO + arsenic trioxide[60] and GO + fludarabine, cytarabine, idarubicin (FLAI-GO),[61] showed limited efficacy or, in the best cases, encouraging results that need further evaluation in multicenter trials.

Several combinations of an SMD + standard anticancer drug (SAD) have reported (Table 3)[62-72] potential advantages including a more potent anticancer effect with lower toxicity to normal cells. However, most of those studies did not evaluate the ability of the combination agents to actually deplete all cancer cells when that should be the ultimate goal. This is in part because most of the in vitro assays to test these drug combinations use short term assays (24-72 h) that are not adequate to evaluate the fate of surviving cells.[73] It is possible that the encouraging effect of these combinations (SMD+SAD) will be no better than any other standard combination (SAD1+SAD2) and that after treatment they will leave a fraction of resistant surviving cells that will lead to a tumor relapse. For a successful treatment (*i.e.* a cure) these cells need to be eliminated.

Targeting the microenvironment and lessons from the anti-angiogenesis hypothesis

The microenvironment plays an important role in determining the stemness properties of cancer cells and may constitute an important target.[74-76] Microenvironment targeting drugs (MMDs) are also being developed (Table 4)[77-85] but one can anticipate the same type of problems and ask the same questions as before for targeting CSCs/CS-LCs: how many microenvironments actually exist in a given tumor and how different are they from each other? How many drugs do we need to target all of them? In fact, cancer researchers have been targeting the microenvironment without success for almost 40 years with angiogenesis inhibitors. Angiogenesis is the formation of new blood vessels and since tumors need blood vessels to grow and spread it was postulated that blocking this process would stop cancer growth. The idea was received with great enthusiasm. Up till now, hundreds of angiogenesis inhibitors have been developed and tested but only a few have shown any benefit in clinical trials and none of them succeed in *curing* cancer. It seems that *angiogenesis inhibition is not enough*[86] either alone or in combination with other anticancer modalities. For advanced melanomas, around 70 angiogenesis inhibitors with different mechanisms of actions are in clinical experimental use but this disease still has a poor prognosis with an average survival of less than one year.[84] To date, angiogenesis inhibition has only improved progres-

Table 3. Examples of combination therapy with stemness modulatordrug and standard anticancer drugs.

Combination		Cancer type	Refs.
Stemness modulatordrug (SMD)	Standard anticancer drug (SAD)		
Salinomycin	Gemcitabine	Pancreatic	62
Salinomycin	Octreotide modified Paclitaxel	Breast	63
Salinomycin	Etoposide Doxorubicin	Hepatic Uterine sarcoma Breast	64
SANT-1	SAHA	Pancreatic	65
GSI-XII	Bortezomib	Multiple Myeloma	66
GSI-XII	ABT-737	Multiple myeloma	67
Curcumin	Dasatinib	Colon	52,68,69
Curcumin	Gemcitabine	Pancreatic	70
Curcumin	Gemcitabine Placlitaxel Tumor necrosis factor (TNF) TNF-related apoptosis inducing ligand	Bladder	71
Curcumin	5-FU plus oxaliplatin (FOLFOX)	Colon	72
ER-400583-00	Radiation	Gliomas	53
VEGFR2 targeting antibody	Cyclophosphamide	Gliomas	55

Table 4. Examples of microenvironment modulator drugs (MMDs).

Cell line/cancer	Agent	Refs.
Pancreas	PF-562,271	77
Different models of cancer	Monoclonal antibody (AB0023)	78
Lung	Pazopanib	79
Human colon and gastric cancer xenograft	TSU68	80,81
Head and neck squamous cell carcinoma Non-small cell lung cancer	Erlotinib	82
Glioblastoma	QLT0254	83
Glioblastoma	QLT0267	83
Melanoma	Angiogenesis inhibitors	84
Gliomas	Angiogenesis inhibitors	85

sion-free survival in highly vascular tumors such as gliomas,[85,87,88] ovarian cancer[89] or hepatocellular carcinoma,[90] but no cure has been achieved. The response of the cancer community to the cancer stem cell hypothesis has been in some ways similar to the idea introduced around 40 years ago to treat cancer by inhibiting angiogenesis. The pharmacological search for the elimination or modulation of cancer stem cells seems to sum up the angiogenesis story: the pioneer identification of putative stem cells in human acute myeloid leukemia in 1997,[91] and later in 2003 in breast cancer[92] and brain tumors,[5] gave rise to extensive research on the biology of CSCs/CS-LCs as well as on the development of pharmacological agents targeting these cells. Equally disappointing, after almost 15 years of cancer stem cell research, anticancer drugs targeting CSCs/CS-LCs have shown only modest clinical benefit.

Substances that inhibit or indirectly modulate the expression of the hypoxia-inducible factor-1 (HIF-1), such a konokiol,[93] manassantin B and 4-O-demethylmanassantin B,[94] laurenditerpenol,[95] curcumin,[96] trans-3,4,5'-trihydroxystibene,[97] resveratrol,[98] SU5416,[99] are also modulators of the microenvironment. However, they showed little efficacy either alone or in combination with standard anticancer agents or radiation. Around 30 HIF inhibitors are currently being investigated in clinical trials[90] but it has already been shown that some of them fail to cure cancer.

The finding that a subset of glioblastoma stem-like cells show characteristics of endothelial progenitors and are capable of maturation into endothelial cells,[100] if confirmed for other types of tumors, further supports the idea that all cancer cells should be eliminated at once and that specific subpopulations of cancer cells or specific microenvironments per se are minor targets in the complex tumoral tissue.

Anticipated problems for targeting cancer stem cells/cancer stem-like cells or the tumour microenvironment

The heterogeneous nature of CSCs/CS-LCs may represent an important problem for the selective targeting of specific cancer cell subpopulations, stemness-related signaling pathways or specific tumor microenvironments. For instance, metastatic colon cancer HCT116 cells express the majority of known CSC markers but also show markedly phenotypic variation indicating that CSCs represent a heterogeneous population.[101] These signaling pathways per se are not valuable targets for anticancer therapy. It is likely that, due to the high intratumoral heterogeneity, the functional status of a particular signaling pathway can be highly active in one cell phenotype, relatively inactive in another and close to *normal* in a third phenotype within the same tumor. A small molecule targeting this particular pathway will only be effective on the cells that belong to the first group. As discussed above, a similar outcome can be expected with MMDs.

A modular tumor model

In gliomas, radioresistance seems to be the result of interaction between cancer cells and the microenvironment that creates a *microenvironment-stem cell unit* and there is extensive data to suggest that CSCs/CS-LCs reside in specific niches (microenvironment).[102] If this is true, it is obvious that there are other specific niches where non-CSCs and other cancer cell subpopulations reside. This suggests that complex, highly heterogeneous tumors may be organized in modules of specific cancer cell phenotype in their specific microenvironment (modular tumor model (MTM)). Each module is a microenvironment-cancer cell unit enriched for cells with a specific stemness phenotype. The interaction between the microenvironment and their associated cells determines the chemosensitivity of the entire module for a particular anticancer agent. Figure 1 shows the schematic represen-

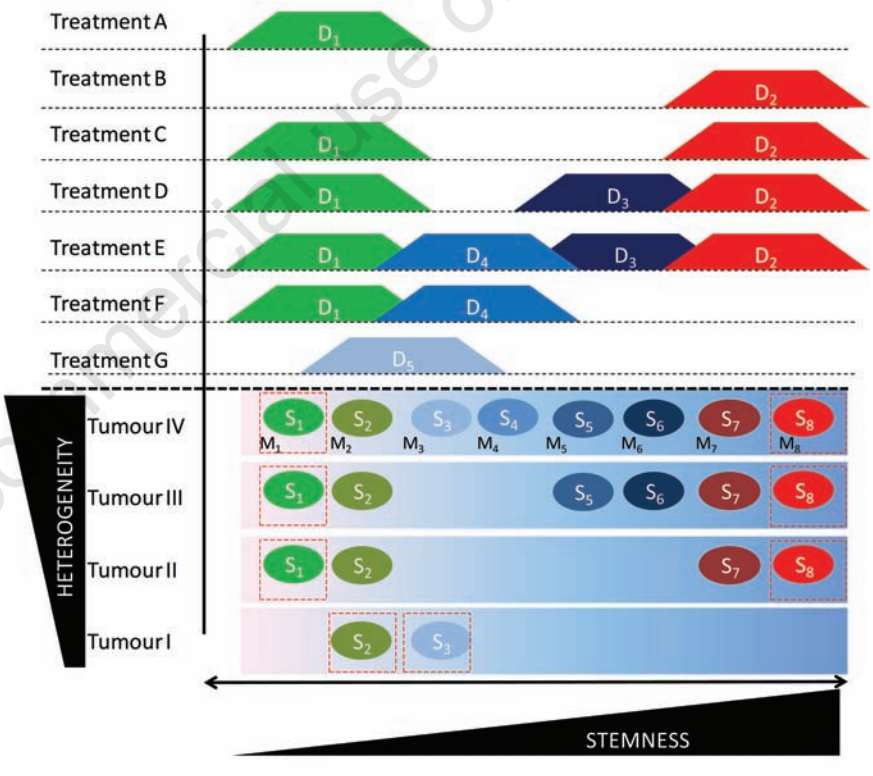

Figure 1. The modular tumor model (MTM). S1-S8 represent cancer cells with different stemness phenotypes (subpopulations) in their respective microenvironment (M1-M8) that creates specific microenvironment-cancer cell units (e.g. M1/S1or M8/S8). D1-D5 represents drugs or a combination of drugs that preferentially eliminate a specific cell phenotype or target a specific microenvironment (that in turn eliminates a subpopulation of cancer cells). Their position in the chart indicates the target (e.g. D1 targets S1-S2 and/or M1-M2). Treatments A-G represent the use of D1-D5 as single agents or in combination and the predicted outcome depending on the tumor heterogeneity. A highly homogeneous tumor (Tumor I) will be easily cured with one (D5, treatment G) or two drugs (D1 and/or D4, treatment F). As tumor heterogeneity increases (from Tumor I to Tumor IV), the number of drugs necessary to eradicate the tumor also increases. Treatments A and B (single agents) will be ineffective. Treatment D will be effective for a Tumor I, II and III but ineffective for Tumor IV since it will spare the M3/S3 and M4/S4 modules. For simplicity, only 8 different continuous modules (showed as a gradient from M1/S1 to M8/S8) have been presented in this model.

tation of tumors of increasing heterogeneity and an increasing number of microenvironment-cancer cell units. Due to the plastic nature of cancer cells, changes in the microenvironment (*e.g.* drugs) (Table 2) can eliminate or affect the stemness of the associated cells. Highly homogeneous tumors are organized in only a few modules and highly heterogeneous tumors in many. The increasing complexity dictates the number of drugs necessary to eliminate all modules. It is likely that the most curable cancers (*e.g.* testicular cancer[103] or acute promyelocytic leukemia (APL)[104] are the most simple and are organized in just a few microenvironment-cancer cell modules (similar to Tumor I in Figure 1). Indeed, APL, a rare but highly homogeneous cancer, can be cured in most cases with a combination of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO).[104,105] These drugs also target the leukemia-initiating cells.[106,107] For highly heterogeneous tumors, usually associated with a poor prognosis, it will be necessary to identify and characterize all relevant modules in order to develop effective cancer therapies.

Conclusions

The complex cellular heterogeneity of cancers and the growing evidence that tumors contain cells with different stem cell phenotypes, that in turn reside in specific microenvironment rather than a pure CSC and a non-CSC subpopulation, challenges and limits the clinical use of SMDs and/or MMDs. Despite the enormous interest and apparent strong rationale to develop SMDs and/or MMDs, the chances of obtaining a cure are slim since we do not know how many subpopulations (or phenotypes) of cancer cells and specific microenvironments need to be targeted. At present, there is still a translational gap between the basic research and pre-clinical anticancer drug screening. On a basic level, we are aware of the complexity of tumors and recognize the need for therapies aimed at eliminating all cancer cells. But most in vitro pre-clinical studies and tests in animal models are usually designed to target only a few cancer cell phenotypes. This translates into poor clinical outcome since most clinical trials are also designed for testing single agents or combinations of just a few (usually 2-3) drugs that will likely eliminate only a few cancer cell subpopulations. Unless we develop a more integrated approach to eliminate all cancer cells at once rather than target a specific pathway, cell subtype or microenvironment, the next 25 years will only serve to repeat the experience of 40 years of angiogenesis research. We will get a better understanding of cancer stem cell biology and tumor microenvironment, and produce and test hundreds or thousands of novel drugs, but very limited clinical success will be achieved. The real challenge is to find a way to integrate advances in cancer biology into successful therapies that save lives. The MTM may serve as a useful guide to develop cancer treatment regimes in a more rational way, based on our knowledge of the complexity of tumor biology in terms of cancer stem cells and their interaction with the tumor microenvironment.

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