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Review

Discovery of the cancer stem cell related determinants of radioresistance

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ABSTRACT

Tumors are known to be heterogeneous containing a dynamic mixture of phenotypically and functionally different tumor cells. The two concepts attempting to explain the origin of intratumor heterogeneity are the cancer stem cell hypothesis and the clonal evolution model. The stochastic model argues that tumors are biologically homogenous and all cancer cells within the tumor have equal ability to propagate the tumor growth depending on continuing mutations and selective pressure. By contrast, the stem cells model suggests that cancer heterogeneity is due to the hierarchy that originates from a small population of cancer stem cells (CSCs) which are biologically distinct from the bulk tumor and possesses self-renewal, tumorigenic and multilineage potential. Although these two hypotheses have been discussed for a long time as mutually exclusive explanations of tumor heterogeneity, they are easily reconciled serving as a driving force of cancer evolution and diversity. Recent discovery of the cancer cell plasticity and heterogeneity makes the CSC population a moving target that could be hard to track and eradicate. Understanding the signaling mechanisms regulating CSCs during the course of cancer treatment can be indispensable for the optimization of current treatment strategies.

Cancer stem cells (CSCs) have been identified as the major players for tumorigenesis, therapy resistance and metastasis [1–4]. Comparable to their normal counterparts, they possess the ability to self-renew and to differentiate into all tumor cell subpopulations to maintain the bulk tumor mass. This stem cell concept of tumorigenesis was proven the first time in 1994 by Dick and colleagues who demonstrated that only $CD34^{+}CD38^{-}$ acute myeloid leukemia (AML) cells are able to engraft immunodeficient mice and initiate leukemia. Furthermore, using an *in vivo* limiting dilution assay they found that only one out of one million leukemia cells is a tumor-initiating cell, which they called AML CSC [5]. The CSC concept was applied the first time to solid tumors in 2003 by Clarke and his colleagues. In this study they identified $CD44^{+}CD24^{-/low}$ breast cancer CSCs as the only tumor-initiating population that was able to generate new tumors by serial passaging in immunodeficient mice, while most of the other tumor cells were unable to initiate tumor growth on their own [6]. During the last years similar discoveries were made in other tumor types including brain, colon and prostate as well as other types of cancer as summarized in Table 1. Functional association of the CSC markers with signaling mechanisms governing CSC properties is important for the development of novel targets for therapeutic intervention, and many studies have been set up to fill this gap in our knowledge. The most investigated and functionally characterized surface markers of human CSCs are listed in Table 1.

In many cases radiotherapy can completely destroy the tumor, i.e. obtain local control. However, if the tumors are large or located close to the critical normal tissue, local tumor control is often impeded, resulting in tumor recurrence. The potential impact of the number of CSCs within a given tumor on local tumor control after radiotherapy was first demonstrated by radiobiological studies more than 20 years ago, as described in the review of Baumann et al. in this issue of Radiotherapy and Oncology journal. As suggested by preclinical and clinical observations, the dose necessary to completely destroy irradiated tumors increases for the large tumors that can be explained by an increase in the absolute number of CSCs with increasing tumor size. The radiobiological studies demonstrated that radioresistance of experimental tumors correlates with their transplantability *in vivo*, which is defined by the tumor stem cell content. Moreover, based on the fact that some tumor models with equal median transplantation dose (TD_{50}) show significant discordance in median tumor control dose (TCD_{50}) values, not only the absolute number of CSCs, but also their intrinsic radiosensitivity might play a role in local tumor control after irradiation [7–10].

In the early 1990s, a few translational studies demonstrated that clonogenic *ex vivo* assays based on the pretreated tumor biopsies may predict clinical outcome for patients treated with radiotherapy [11–13]. However, these findings were challenged in a

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Table 1
Cancer stem cell markers in common cancers.

	CSC marker						
Tumor type	CD133	CD44	ALDH1	α2β1 integrin	α6 integrin	CD24	References
Glioma	+		+		+		[119–123]
Breast cancer	+	+	+		+	-	[6,124–126]
Colon cancer	+	+	+		+		[127–131]
HNSCC		+	+				[21,132]
Prostate cancer	+	+	+	+			[133–136]
Protein family	Transmembrane glycoproteins	Transmembrane glycoprotein, hyaluronic acid receptor	Aldehyde dehydrogenase	Heterodimeric transmembrane glycoprotein receptors	Heterodimeric transmembrane glycoprotein receptors	Transmembrane glycoprotein	
Function in CSC	Endocytosis, co-regulation of growth factors, PI3K pathway activation, self-renewal	Adhesion, co-regulation of growth factors EMT, STAT3 signaling activation, migration, drug resistance, self-renewal	Aldehyde detoxification, drug resistance, oxidative stress response	ECM interaction, cell–cell interaction, cytoskeletal rearrangement, co-regulation of growth factors, signal transduction	ECM interaction, cell–cell interaction, cytoskeletal rearrangement, co-regulation of growth factors, Signal transduction	Adhesion, migration, invasion, CXCR4 signaling, DNA damage-induced nuclear factor-kappaB (NF-kB) signaling	
References	[137–140]	[141–145]	[146]	[147]	[147]	[148–150]	

number of other studies [14–19]. This controversy of the *ex vivo* data could be explained by the lack of the extrinsic stimuli coming from the CSC niche and regulating CSC properties *in vivo*.

Since then a growing body of *in vitro* experimental evidence demonstrated that CSCs isolated from the established cell lines and tumor specimens can be protected from the treatment modalities by multiple intrinsic and extrinsic mechanisms, such as resistance to the oxidative DNA damage, enhanced DNA repair, activation of the anti-apoptotic signaling pathways and by the tumor microenvironment, as summarized in Table 2.

Retrospective clinical studies for the different types of cancer have shown that analysis of CSC-specific markers in pre-therapeutic biopsies might be an important tool for the prediction of clinical outcome and appropriate treatment selection, as described in detail in the review of Baumann et al. in this issue of Radiotherapy and Oncology journal.

However, it has not yet been proven directly that clonogens, which determine tumor recurrence after radiotherapy are the same as the cells with the CSC phenotype. To fill this gap between the cell-based experimental data and clinical observations, *in vivo* radiobiological assays are needed to be established where tumor control probabilities after different radiation doses are analyzed in parallel with the frequency of tumorigenic CSCs defined by specific phenotypic markers and functional features, including tumorigenicity, self-renewal and differentiation potential. However, it may be complicated by the fact that the phenotypical and functional properties of CSCs may be dynamically regulated during the course of radiotherapy. Understanding the complex mechanisms regulating CSC population during the course of cancer treatment will turn CSCs into a powerful tool for therapeutic and diagnostics improvement.

Strategies to identify cancer stem cell regulators and markers of radioresistance

The CSC hypothesis provides a strong clinical rationale for the identification of CSC specific antigens to develop new predictive biomarkers and therapeutic strategies. However, despite that a large number of CSC markers have been characterized during the last decades, only a few CSC-related antigens were validated in a clinical setting. This could be due to the high heterogeneity of CSC populations and lack of CSC markers with a high level of specificity. Nevertheless, despite the high inter-tumor heterogeneity of CSC marker expression, the current findings suggest that analysis of the number of CSCs in pre-therapeutic biopsies might be important for the prediction of tumor radiocurability, estimation of the total radiation dose required, and the selection of the optimal therapeutic strategy [3,4].

In the search for novel CSC-specific predictive biomarkers, high throughput technologies, such as DNA microarray, mass-spectrometry based proteomics and high-throughput genetic screening have become an area of increasing interest in a radiation oncology setting. Such high throughput *in vitro* assays in conjunction with bioinformatics methods for data handling and analysis allow to

Table 2
Intrinsic and extrinsic determinants of cancer stem cell radioresistance.

Molecular determinants of radioresistance	Mechanism	CSC population	References
Intrinsic determinants	Enchased DNA repair capability through ATM and CHK1/CHK2 phosphorylation	■ $CD44^+/CD24^{-/low}$ human breast cancer ■ $CD133^+$ human glioma ■ CD29high/CD24high mouse mammary tumor	[60,61,151–153]
	Protection from oxidative DNA damage by enhanced ROS scavenging	■ $CD44^+/CD24^{-/low}$ human breast caner ■ $Thy1^+CD24^+$ mouse mammary tumor ■ $CD44^+$ human gastric cancer	[154,155]
	Activation of the cell survival pathways, including PI3K/Akt, WNT/β-catenin, notch signaling pathways	■ $CD133^+$ human glioma ■ $CD29^{high}/CD24^{high}$ mouse mammary tumor	[156,157]
Extrinsic determinant	Hypoxic environment	■ $CD133^+/nestin^+$ human medulloblastoma ■ $CD133^+$ human glioma ■ $Nestin^+$, $CD15^+$ human glioma	[158,159,105,160]
	Growth factors and cytokines	■ $CD133^+$ human glioma ■ Glioblastoma stem cells enriched in neurospheres	[161,162]

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