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Cancer stem cells: Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments☆

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ABSTRACT

Inactivation of cancer stem cells (CSCs) is of utmost importance for tumor cure after radiotherapy. An increasing body of evidence complies with a higher radioresistance of CSCs compared to the mass of tumor cells, supporting the use of CSC related biomarkers for prediction of radiotherapy outcome. Treatment individualization strategies for patient groups with vastly different risk of recurrence will most likely require application of more than one biomarker. Specifically, inclusion of established biomarkers like tumor size for primary radio(chemo)therapy or human papilloma virus (HPV) infection status in head and neck squamous cell carcinoma seems to be of very high relevance. The high heterogeneity of CSC subclones along with changes of the functional behavior of individual tumors under treatment underlines the importance of the selection of the optimal timepoint(s) of biomarker evaluation, but also provides a potential therapeutic target for combined treatment approaches with irradiation.

Contents

Abbreviations: ABCG2, cATP-binding cassette sub-family G member 2; ALDH1A1, aldehyde dehydrogenase 1A1; ATM, ataxia telangiectasia mutated protein kinase; ATR, ATM-Rad-3-related protein kinase; CD, cluster of differentiation; CSC(s), cancer stem cell(s); Chk, checkpoint kinase; CXCR4, chemokine (C–X–C motif) receptor 4; DNA, deoxyribonucleic acid; DDR, DNA damage response; DSB, double strand breaks; DZNep, 3-Deazaneplanocin A; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; FAK, focal adhesion kinase; GBM, glioblastoma multiforme; HA, hyaluronan; HGFR, hepatocyte growth factor receptor; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme-A; ECM, extracellular matrix; HIF, hypoxia-inducible factor; HNSCC, head and neck squamous cell carcinoma; HPV, human papilloma virus; HR, homologous recombination; HRE, hypoxia response element; MGMT, O-6-methylguanine-DNA methyltransferase; NHEJ, non-homologous end joining; PET, positron emission tomography; PI3K, phosphatidylinositide 3-kinases; ROS, reactive oxygen species; SCL3A2, solute carrier family 3A2; SSB, single strand breaks; TCD50, tumor control dose 50%; TD50, transplantation dose 50%; WNT, wingless and INT-1.

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1. Introduction

Cancer stem cells (CSCs) play a pivotal role in tumor development, progression and recurrence after treatment. CSCs are defined as tumor cells that have an unlimited potential of cell division and an ability to repopulate the whole tumor [1]. For radiotherapy as well as for other potentially curative anti-cancer treatments, this definition implies that all CSCs need to be inactivated in order to permanently eradicate a tumor. The long established gold standard assay to determine CSCs is a functional assay, i.e. the tumor cell transplantation assay measuring the cell number needed to be injected to cause tumor takes in 50% (TD50) of the experimental animals. Technological advances allow us to identify and sort tumor cells for CSC-rich and CSC-low subpopulations, e.g. by cell surface marker expression. Such markers can in many cases also be identified by using staining techniques in tumor sections or by gene expression arrays, ensuring clinical applicability of such parameters for biomarker assays. However, limitations occur from the fact that threshold values for staining intensity cannot be determined by direct validation of the stemness of the stained tumors cells and from the general fact applying to all CSC marker assays, that the marker expression is not specific to CSCs but just determines cells that have a higher likelihood to express CSC features. Thus, stemness of cells has still to be validated by the functional assays mentioned above [2,3].

Because of their importance for tumor curability, CSCs can be utilized for strategies to optimize anti-cancer treatments by their evaluation as markers for patient stratification to specific intensified or de-escalated treatment strategies and/or by developing treatment approaches that specifically target tumor cells expressing putative CSC markers.

2. Radioresistance determined by cancer stem cells

If other confounding factors are constant, the radiation dose to eradicate a tumor inversely correlates with the logarithm of the number of CSCs, i.e. at the same radiotherapy dose tumors with a lower number of CSCs show higher local control rates compared with tumors with higher numbers of CSCs. In experimental and clinical data, this leads to a tumor volume dependence of the curability of tumors, because the absolute number of CSCs is expected to increase with tumor volume [2–6]. However, also densities of CSCs can differ between tumors, again impacting local tumor control after radiotherapy [6,7]. The dependence of local tumor control from the number of CSCs contained in a tumor is underlined by the log-linear correlation between the TD50 and the radiation dose necessary to permanently cure 50% of the tumors (tumor control dose 50%, TCD50) [3,8]. This correlation indicates that, even though CSCs seem to express a constant plasticity, TCD50 is a valuable correlate of the number of CSCs in a given tumor. Nevertheless, some of the described tumor models with equal TD50 show significant discordance in TCD50 values, suggesting that in addition to the total number of CSCs in a given tumor, the inherent radiosensitivity of CSCs as well as other radiobiological parameters may impact local tumor control after irradiation [2,8]. Such differences of intrinsic radiosensitivity between colony-forming cells in vitro (clonogenic cells) of tumors with the same histology have been shown by the variation of the surviving fraction at 2 Gy (SF2) in colony-forming in vitro assays, which in some studies correlate with clinically observed differences in radiosensitivity between tumor entities or within the same histology [3,9,10]. The same correlation between radiosensitivity of clonogenic cells in vitro and tumor radiocurability in vivo exists for tumors of different histologies [11–14], supporting a correlation between clonogenicity in vitro and stemness in vivo and the importance of intrinsic radiosensitivity of CSCs for radiocurability of tumors. Of note, such preclinical data on established tumor models are of high importance for our today's understanding of the biology of CSC, however, specific questions especially concerning detailed gene expressions or tumor to normal tissue interactions may in many cases require the use of primary xenografts. It is not the scope of this review to discuss the use of different experimental models in detail.

Taking into account that tumors can potentially arise from a single CSC, a failure of radiation treatment might be attributed to the incomplete eradication of the entire CSC population [3,15]. The cure rate of irradiation results from its ability to induce DNA damage in tumor tissue through production of the water-derived reactive oxygen species or by direct ionization of the DNA molecules [16]. At the cellular level, DNA damage has long and short term consequences. An acute effect is related to the disturbance of DNA metabolism such as DNA replication and RNA transcription. Based on the status of DNA repair and the nature of the DNA lesions, the outcome of DNA injury can be temporary or permanent cell arrest, immediate or delayed cell death or mutagenesis of the surviving cells, which in turn might lead to genomic instability and subsequently results in tumor development in a long term perspective [17].

The biological consequences of irradiation leading to cell death are highly influenced by the activation of the DNA damage response (DDR) mechanisms. Among the various types of DNA damages produced by ionizing irradiation, i.e. single strand breaks (SSBs), double strand breaks (DSBs), damaged nucleotide bases or abasic sites, DSBs represent the principal lesions that might lead to cell death if not adequately repaired [16]. Both normal and tumor cells can repair DSBs by either error-free homology-directed recombination (HR) or error-prone non-homologous end joining (NHEJ) mechanisms which are acting in an overlapping and complementary manner [18]. In addition to the activation of DNA repair process, DNA damage induces checkpoint kinase signaling pathways such as ataxia telangiectasia mutated (ATM)-checkpoint kinase 2 (Chk2) and ATM-Rad3-related (ATR)-checkpoint kinase (Chk1) which delay cell cycle progression in order to allow DNA repair [18]. When DNA damage is beyond the cell repair capacity, proteins of DDR signaling mediate cellular death or loss of reproductive capacity through activation of different pathways such as apoptosis, senescence or mitotic catastrophe [19]. Therefore, the efficacy of the DNA repair machinery activated by the DNA damage signaling network is critical and determines cell death or repair. A high DNA repair capacity has been described for CSC populations in different tumor entities including glioblastoma, prostate, lung and breast cancers and mainly attributed to the activation of the ATR-Chk1 and ATM-Chk2 signaling pathways [20–30], as summarized in Table 1.

Radiation-induced cell death occurs not only by a direct energy transfer to the DNA but, in case of conventional photon radiotherapy, mainly as a result of generation of the free radicals, including the chemically reactive products of oxygen metabolism called reactive oxygen species (ROS) [31]. Under physiological conditions, these metabolites are involved in the signaling events regulating different cellular processes such as differentiation, proliferation, autophagy and survival [32]. However, if ROS production is beyond the capacity of the cellular antioxidant system, it may lead to the irreversible oxidative stress and cell death [33]. The physiological ROS level is maintained by the scavenging molecules such as glutathione, dismutase, peroxidase, thioredoxin, catalase, and superoxide [33]. In addition, adverse effect of ROS can be also minimized by an adequate DDR response [34]. A highly efficient ROS scavenging system or generally lower ROS levels in CSC may contribute to the high resistance of CSC populations to genotoxic stress described for different tumor entities [35–38] (Table 1).

Besides the above-described intrinsic mechanisms underlying CSC radioresistance, the CSC fate under physiological conditions and during treatment is tightly regulated by a broad range of extrinsic microenvironmental stimuli [39,40]. Within a tumor, CSCs can reside in specific niches occupying different locations including hypoxic, perivascular and invasive tumor areas that can dynamically change during tumor development and treatment [41,42]. A CSC niche is defined by various soluble factors, extracellular matrix (ECM) elements and direct cell–cell interactions via cell surface molecules. Depending on oxygen tension, a niche occupied by CSCs may define differentiation, self-renewal and treatment resistance of the CSCs. The CSCs residing in the hypoxic