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−/CD44+/C211+ 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 (TD50) show significant discordance in median tumor control dose...
Intrinsic and extrinsic determinants of cancer stem cell 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 radiosensitivity, 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 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.

### Table 1
Cancer stem cell markers in common cancers.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CD133</th>
<th>CD44</th>
<th>ALDH1</th>
<th>α2β1 integrin</th>
<th>CD44/80</th>
<th>CD24</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gloma</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>[119–123]</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td>[6,124–126]</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td>[127–131]</td>
</tr>
<tr>
<td>HNSCC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[21,132]</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>[133–136]</td>
</tr>
</tbody>
</table>

(TCD<sub>50</sub>) 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 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.
Discovery of cancer stem cell radioresistance

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 analyze thousands of biological molecules simultaneously under standardized conditions and usually produce a list of candidate biomarkers, which need to be carefully validated using preclinical in vivo radiobiological experiments and large-scale analysis of clinical specimens. High density oligonucleotide arrays offer the opportunity to examine patterns of gene expression in CSC on a genome scale and compare it to the transcriptome of normal stem cell populations. For example, Liu et al. reported a 186-gene “invasiveness” gene signature (IGS) that discriminates between normal breast epithelium and breast CSCs characterized by CD44+/CD24− gene signature (IGS). This signature is associated with overall survival and metastasis-free survival in patients with breast cancer, and can be also applied to discriminate low- and high-risk patients with medulloblastoma, lung and prostate cancers indicating that a generalized list of “stemness” genes can serve as a useful prognostic tool for many types of tumors.

To perform CSC-based profiling in the context of radioresistance, Chen and colleagues have gauged the genomic traits of radioresistant cancer progenitors isolated from head and neck squamous cell carcinoma (HNSCC) tumors defined by high aldehyde dehydrogenase 1 (ALDH1) activity. Gene expression microarray analysis demonstrated that the epithelial-mesenchymal transition (EMT) pathway and EMT-related genes were significantly up-regulated in ALDH1+ HNSCC cells. Moreover, this study showed that the increased incidence of ALDH1 expression positively correlates with the clinical stage of HNSCC patients. These data demonstrated that metastatic potential can be partially due to a specific radioresistant subpopulation of CSCs. This finding was later supported by the work of Mihatsch et al., who demonstrated that ALDH1 activity is indicative of subpopulations of lung and breast cancer cells with increased radioresistance.

Our studies employing the comparative gene expression profiling revealed the transforming growth factor β (TGFβ) signaling as one of the few pathways overrepresented among the genes that are differentially regulated between the parental radiosensitive (RS) prostate cancer cells DU145 and its radioresistant (RR) derivative cell line DU145 as well as between ALDH1+ progenitor cells and ALDH− cell populations. This finding suggests that radioresistant and tumorigenic characteristics of cancer cells can be governed by the overlapping molecular mechanisms.

Although the level of gene expression underlies many alterations during signal transduction, its effects on CSC physiology might be more completely understood at the level of protein.

Fig. 1. TGFβ signaling is similarly overrepresented in radioresistant and tumor progenitor population. (A) TGFβ initiates its cellular response by binding and activating the cell surface receptors having intrinsic serine/threonine kinase activity. The activated TGFβ receptors stimulate the phosphorylation of Smad3 and Smad2 proteins, which form complexes with Smad4 and activate the transcriptional programs regulating multiple cellular functions, including self-renewal, tumorigenicity and radioresistance. (B) To better understand the biological pathways underlying the properties of radioresistant and tumorigenic cell populations, we performed comparative gene expression profiling of the parental and radioresistant prostate cancer DU145 cells, ALDH1+ and ALDH− cell populations isolated from the DU145 cancer cell line, and the cells grown under adherent condition and tumorsphere-forming conditions. TGFβ signaling was one of the few pathways overrepresented among the genes that are differentially regulated between DU145 and radiosensitive (RS) DU145 cells, between DU145 tumorspheres and DU145 monolayer cultures as well as between ALDH1+ and ALDH− cell populations. The overrepresentation analysis reveals statistically significant deviation of the observed number of genes associated with TGFβ signaling pathway (actual number) from the number of genes expected under random sampling conditions (expected number). This finding suggests that radioresistant and tumorigenic characteristics of cancer cells can be governed by the overlapping molecular mechanisms. To develop the radioresistant subline RR DU145, prostate cancer DU145 cells were treated with multiple X-ray doses of 4 Gy given once a week (40 Gy total dose). The surviving cells showed enhanced radioresistance in 2D clonogenic radiobiological assay; *p value <0.05.

(C) List of the TGFβ pathway genes according to KEGG (Kyoto Encyclopedia of Genes and Genomes) database, which are differentially regulated in the radioresistant and radiosensitive DU145 cells. (D) Smad3 protein is detected in the nuclear fraction and highly expressed in the radioresistant DU145 cells compared with more radiosensitive parental DU145 cells.
expression and protein–protein interaction. For example, recent interactome study suggested that protein interactome pattern regulates a balance between self-renewal and differentiation in glioblastoma. Facchinio et al. have shown that the polycistron group protein BM11 is enriched in CD133 positive glioblastoma stem cells and operates as a recruitment platform for the double strand break repair (DSB) response and non-homologous end joining (NHEJ) proteins resulting in increased cell radiosensitivity [23].

Moreover, the properties of CSCs can be regulated not only by gene expression or by the level of protein expression that does not necessarily reflect protein activity, but also via post-translational modifications regulating protein function, of which phosphorylation is one of the most prominent. A study of Nilsson et al. documented the changes occurring in phosphoproteome of glioblastoma progenitors upon treatment with a JAK/STAT inhibitor, stimulation with interleukin-6 (IL-6) and under hypoxia conditions [24]. This study demonstrates the power of phosphoproteomics for the analysis of treatment-induced changes of signaling pathways and interconnections between signaling pathways in a robust and comprehensive manner.

The recent discovery of microRNAs (miRNAs) has added an entirely new dimension to our knowledge about the regulation of gene expression and the control of various cell functions, such as apoptosis, proliferation, differentiation and therapy resistance. The miRNAs are regulatory, non-coding RNAs about 22 nucleotides in length which regulate the expression of a variety of genes by targeting mRNA transcripts. Over the past decade, it has become progressively clearer that these tiny genetic regulators are linked to the development of cancer. The miRNA profiles have been shown to be highly informative, reflecting the developmental history and differentiation state of the tumors, and providing molecular links between cancer and normal stem cells [25,26]. The fact that miRNAs expression may have adverse consequences for the functional properties of cancer cells has been recently highlighted for tumor radiosensitivity. Yan and coworkers for the first time demonstrated that miRNAs could be used to target the DNA repair machinery and thus sensitize tumor cells to radiation [27]. Since then, an accumulating body of research demonstrated that miRNAs can modulate tumor radiosensitivity [28–31]. In vivo experiments using xenograft models and clinical studies are needed to ascertain whether manipulation of miRNA expression can be a viable tool to augment current cancer therapies [32].

Emerging high throughput screening (HTS) technologies employing cell-based phenotypic assays and pathway-based readouts expand our knowledge regarding the role of genes and proteins in the regulation of CSC properties. To provide comprehensive large-scale genetic screens for specific changes of cell phenotypes, the RNA interference (RNAi) technique can be successfully employed. The development of RNAi techniques for gene silencing allows for systematic gene and pathway analysis in tumor cells to explore novel gene functions and signaling pathways that cannot always be identified by ectopic gene expression [33].

While such genetic screens provide important information about which genes are related to a given phenotype, molecular pharmacology can play an important role in the development of CSC-specific chemical therapy. The HTS of the small molecule libraries have yielded many useful “tool” small molecules regulating important features of cancer cells including stemness, invasiveness and resistance to therapy [34–37]. Moreover, biological molecules interacting with chemical compounds can potentially serve both as therapeutic targets and biomarkers. For example, a recent study of Sachlos et al. utilized a small molecule screen to identify compounds that impair leukemic stem cells while having no effect on normal blood stem cells. In this study a self-renewing state of human neoplastic and normal pluripotent stem cells (hPSC) was monitored by the expression of master pluripotency transcription factors Oct4 and Sox2. Using a library of 590 known compounds that induce differentiation, Sachlos et al. identified the small molecules thioridazine and mefloquine as the most effective regulators of pluripotency and validated these candidate compounds by using neoplastic hPSC and somatic leukemia stem cells from patients. The drug thioridazine antagonizes dopamine receptors that are expressed on leukemia stem cells as well as on breast cancer cells. These results can suggest that dopamine receptors may serve as a biomarker for diverse malignancies [38].

The HTS strategies are being widely introduced to experimental radiotherapy. The studies of chemical and biological radiosensitizers employed various approach to HTS, including analysis of γ-H2AX kinetics, cell proliferation and viability [35–37]. These studies revealed a number of promising candidates involved in the regulation of DNA repair, ROS production and apoptosis. However, the specificity, toxicity and efficacy of these radiosensitizers still need to be determined in future preclinical studies. It is important to identify molecular pathways that are distinctively different between CSCs and normal stem cells so that radiosensitizing agents can specifically target CSCs without causing a significant effect on normal stem cells. However, the HTS for the selective regulators of CSCs radiocurability has not been reported yet.

Nevertheless, a large number of studies were performed in the last few years to systematically identify genes or small molecules regulating CSC death and differentiation. However, their value for the enhancement of tumor radiocurability warrants further investigation. These studies employed screening of RNAi or chemical libraries with various readouts such as cell viability, reporter assays utilizing luminescence of fluorescence-based analysis of gene or pathway activation, and image-based analysis allowing the capture of multiple parameters at the single cell level.

In contrast to the radiobiological studies employing the clonogenic survival assays, cell viability assays have proven to be a fast and reliable readout in cell-based chemical screenings. Gupta and coworkers enriched CD44+/CD24−/low tumor progenitor population in a breast cancer cell line by inducing EMT upon the inhibition of the human E-cadherin gene CDH1. This study employed cell viability assay to screen a compound library in the modified and parental cell lines, and identified the monocarboxylic polyether antibiotic salinomycin as a selective inhibitor of the breast cancer progenitor population [34].

The reporter construct which transcription is specific for CSCs is an important tool to identify and monitor the CSC population. This reporter contains luciferase or green fluorescent protein (GFP) gene under control of a certain promoter that is activated in CSCs. After integration into the genome of the host cells upon transduction or stable transfection, this reporter system allows to analyze the expression from CSC-specific promoters. Thus, individual CSCs can be monitored based on their selective expression of the label such as luciferase or GFP [39]. A recent study employed high content screening to identify chemical modulators of the progenitor population in luminal breast cancer cells using a CK5 promoter-driven GFP reporter [40]. In another study mentioned above, pluripotent state of hPSC was monitored by the expression of the GFP gene driven by Oct4 and Sox2 promoters [38].

An alternative method for the identification of radiosensitive cell population with increased self-renewal capacity was proposed by Pajonk et al. [41,42]. They identified and characterized a small population of glioblastoma, breast and prostate cancer cells with intrinsically low 26S proteasome activity and stem cell properties, including self-renewal and tumorigenic capacity. This tumor-initiating population is resistant to proteasome inhibition and might explain the failure of the clinical trial the proteasome inhibitor bortezomib. The cell population with low 26S proteasome activity was tracked in vitro and in animal models using the proteasome function reporter system where the C-terminal degron of the mur-
ine ornithine decarboxylase (cODC) was fused to ZsGreen green fluorescent protein. The daily fractionated irradiation (5 × 3 Gy of Cobalt-60 γ-ray) resulted in the increase in ZsGreen-positive cell population in vitro and in vivo suggesting that low 26S proteasome activity is indicative of radopresistant tumorigenic cell fraction. If future studies will confirm an existence of this population in clinical samples, ZsGreen-cODC reporter construct can be a useful tool for the screening of molecular regulators affecting tumorigenic and radioresistant cell population.

Recent advances in high-content imaging technologies supported by high-performance computing have enabled rapid advances in the development of high-throughput image-based assays. Direct visualization of the cellular phenotype permits more comprehensive measurements of the responses to perturbations. The measurements can be performed specifically on single cells of interest. Xia and coauthors developed a new assay to identify and analyze high drug efflux cells possessing CSC properties based on fluorescence images [43]. Using this system, Xia and coauthors screened a library of pharmacologically active compounds for their effect on the high drug efflux cells in lung cancer. The screening successfully identified compounds, which reduce the drug efflux capability of lung cancer cells. Wurdak et al. described an interesting approach to monitor the differentiation of brain tumor-initiating cells by automated image analysis, which they called “cell dispersion” phenotype that was used as a readout in a kinome-wide RNA interference screen to identify genes that control self-renewal and tumorigenicity of brain tumor-initiating cells [44].

It can be hypothesized that quiescence or slow cycling state of CSC is associated with relative radioresistance. As it was demonstrated by Pece and coworkers, breast cancer stem cells retain the lipophilic fluorescent dye PKH26 as a consequence of their quiescent nature [45]. A study of Wang et al. demonstrated that PKH26+ nasopharyngeal cancer cells are highly clonogenic, spherogenic and radioresistant [46]. These findings suggest that PKH26 dye can be a useful tool for the discovery of new regulators of stemness and radioresistance.

The traditional screening platforms are typically limited to two-dimensional (2D) cultures, which very roughly resemble the environment that tumor cells experience in living tissues and exclude the influence of cell–cell interaction, extracellular matrix (ECM) and stromal components. In contrast, three dimensional (3D) culture models providing direct interactions between cells, as well as between cells and the ECM proteins, can lead to an increase in CSC properties of the cultured cells, including cell self-renewal, migration, release of endocrine and paracrine factors regulating tumor development and angiogenesis, formation of hypoxic niches and therapy resistance [47–52]. 3D sphere forming conditions are particularly useful to enrich the CSC and radioresistant cell subsets (Fig. 2), especially when specific markers for these populations are not well understood. Sphere culture conditions are providing the foundation to develop marker-independent HTS assay with patient derived CSCs which may have high phenotypical heterogeneity. Moreover, sphere forming conditions might more accurately reflect in vivo mechanisms of action of the treatment modalities as it was demonstrated in the recent chemical screening of primary glioblastoma cell cultures [53,54].

In summary, genomics, proteomics and screening technologies are expected to provide us with information about new CSC-associated biomarkers, therapeutic targets and potential drugs that could specifically target CSCs or induce their differentiation. Further validation studies applying radiobiological assays combined with functional stem cell analysis will assess the role of the identified biological molecules and chemical drugs in the regulation of tumor radiosensitivity. To fill the gap between the radiobiological experiments and clinical practice, it is necessary to perform a large-scale analysis of the clinical specimens for the association of CSC biomarkers with the tumor grade, stage, and prognosis. A better understanding of the molecular mechanisms underlying CSC pathology as well as thorough clinical evaluation of the drug and marker candidates will be essential in the development of CSC-based anti-cancer therapies.

**CSC: a flexible target?**

There is emerging evidence that adult and embryonic stem cells are heterogeneous and consist of distinct subpopulations that exhibit distinct phenotype, self-renewal and differentiation biases [55]. Similar functional and phenotypical heterogeneity was also observed in CSCs. It was hypothesized that many types of tumors contain multiple cell lineages and each of these lineages can arise both from neoplastic transformation of the normal stem cell as well as from more differentiated progenitors by a process of multi-step clonal evolution [56–58]. In addition to the intratumor heterogeneity, it is becoming increasingly clear that CSC subsets can vary from patient to patient due to differences in individual genetic and epigenetic makeups [59].

These recent findings may explain why the experimental data depending on selected CSC marker may lead to contradictory results. For example, Bao et al. demonstrated more efficient DNA repair in CD133+ progenitor glioma cells. However, this observation was challenged by Ropolo et al. who showed that neither DNA base excision or single-strand break repair nor resolving of γ-H2AX nuclear foci were changed in CD133+ compared to CD133− cells [60,61]. In another study of tumor-initiating cells in ovarian cancer, Stewart et al. demonstrated that CD133 marked all cancer progenitor cells in several cancer specimens. However, in other cases, tumor initiating population was found in both the CD133+ and CD133− cell fractions [62].

Deep-sequencing technologies have enabled to reveal the phylogenetic history of the cells within an individual tumor and demonstrated that almost all tumors contain multiple clones which correspond to the distinct CSC lineages that co-exist within the tumor and contribute to intra-tumor heterogeneity [63–66]. The tumor cells within single genetic clone display inherent functional diversity in their repopulation potential and therapy resistance [67]. The possible relationship between distinct clones within an individual tumor warrant further investigation.

Recent studies in breast tumors and gliomas demonstrated that various extrinsic stressors, including hypoxia, inflammation and anti-cancer therapy, or (and) intrinsic mechanisms, such as activation of developmental pathways, can trigger cancer cell reprogramming and change their self-renewal and differentiation potency. For instance, hypoxia which may keep CSCs in a quiescent state could play an important role at the initial stage of radiotherapy followed by re-oxygenation during tumor eradication and recruitment of CSCs into the proliferation pool. An accelerated repopulation of tumor cells is one of the major reasons for the failure of conventional radiotherapy as discussed in [68] and in the review of Baumann et al. in this issue of Radiotherapy and Oncology journal.

In addition to oxygen tension, other microenvironment influences, such as cytokines, interleukins and growth factors might regulate the proliferative status of CSC. Similar to the normal stem cells, CSCs may undergo a switch from asymmetric to symmetric cell division becoming a mitotically active cell population after ionizing radiation, employing the activation of the developmental signaling pathways, like the Notch, WNT and Sonic hedgehog pathways [3,69]. In addition, irradiated tumor fibroblasts and macrophages start to produce growth factors and cytokines, such as PDGF, IL1β, TGFβ, CXCL12 and matrix metalloproteases (MMPs) which may lead to a highly proliferative and invasive behavior of CSCs [70–72]. These data may suggest that tumors containing a
large compartment of CSCs might benefit from the intensified radiotherapy schedules. Indeed, in a subset of tumors, hyperfractionated accelerated radiotherapy results in improved treatment efficacy, as shown in randomized trials in HNSCC and non-small cell lung cancer (NSCLC) patients [73,74].

Analogous to differentiated somatic cells, which can be reset to pluripotent cells by the process of induced dedifferentiation, non-CSC tumor cells can be reprogrammed under “induced conditions.” Different stimuli coming from the microenvironmental niche and anti-cancer therapies can trigger cancer cell reprogramming, which results in the generation of induced CSC (iCSC) cell population from non-CSC tumor cells [66,75]. There is growing evidence for the possibility of dedifferentiation of non-CSCs by experimental manipulations, such as overexpression of oncogenes (e.g. NANOG, hTERT, OCT4), exposure to inflammatory cytokines (e.g. IL-6, IL-1β, TNFα), and experimentally induced EMT by culturing the cells under hypoxic conditions [39,76–82]. Factors contributing to the phenotypical and functional diversity of CSCs are schematically summarized in Fig. 3.

Finally, the multiplicity of the microenvironmental conditions can affect CSC properties and contribute to CSC heterogeneity within a tumor that will be discussed in the next paragraph.

Microenvironment and cancer stem cell phenotype

The microenvironmental conditions can affect CSC properties and contribute to CSC heterogeneity within a tumor. Status and behavior of normal stem cells are determined by the local spatio-temporal environment. Unique but dynamic niches play an essential role in the survival and maintenance of stemness regulating the balance between homeostasis of the stem cell pool and the induction of proliferation and differentiation processes [83]. The dynamic behavior of the tumor microenvironment might determine phenotypic and functional properties of normal stem cells transforming them into the malignant cells and contributing to cancer development and metastatic spread of tumor cells from the site of their origin [84–86]. Modulators in this scenario are ECM compounds, stromal cell components including vascular elements as well as secreted para- and autocrine factors, such as bone morphogenetic proteins (BMPs) and Wnt proteins. Alterations in the micromilieu conditions, i.e. in extracellular pH, oxygen partial pressure and ion strength, which have been intensively studied in radiobiology and radiotherapy over the past decades, also contribute to stem cell regulation. The maintenance of CSCs may be governed by the distinct microenvironmental niches at different
In low oxygenated areas triggered by the local oxygen concentration, which considers the fact that adult normal stem cells are localized in low oxygenated areas, cancer cells in chronically hypoxic areas are often cell cycle arrested and more resistant to therapy. Hypoxia is a fundamental pathophysiological phenomenon strongly associated with the development and aggressiveness of various solid malignancies and also implicated in radio- and chemoresistance [3].

Hypoxia can evolve resistance against cytotoxic treatments due to various intrinsic mechanisms, extrinsic stimuli, epigenetic changes and mutational alterations. A highly dynamic nature of the intrinsic and extrinsic determinants of radioresistance along with CSC plasticity and heterogeneity in the pathophysiological heterologous environment make CSC population(s) a target that may be hard to treat. Controlling the phenotypical and functional changes and mutational alterations. HIFs (in particular HIF1α) are the master transcription factors whose dysregulation contributes to an enhanced glycolytic flux. The cancer cell’s capacity to switch between oxidative phosphorylation and aerobic glycolysis and use the alternative substrate as fuel for energy production may result in metabolic reprogramming of CSCs [110–113]. The phenotype switching driven by the complex microenvironmental stimuli makes it difficult to target CSCs on one hand but opens a therapeutic window on the other hand [112,114–118]. Tumor microenvironment and tumor cell metabolism thus remain one of the central challenges for curative treatment and to eradicate putative CSCs.

**Conclusion**

In conclusion, many tumors contain heterogeneous CSC populations which can evolve resistance against cytotoxic treatments due to various intrinsic mechanisms, extrinsic stimuli, epigenetic changes and mutational alterations. A highly dynamic nature of the intrinsic and extrinsic determinants of radioresistance along with CSC plasticity and heterogeneity in the pathophysiological heterologous environment make CSC population(s) a target that may be hard to treat. Controlling the phenotypical and functional properties of CSCs during radiation therapy is ultimate for optimization and individualization of treatment strategy. Genomics, proteomics and high-throughput studies validated by functional...
radiobiological assays and clinical observations are expected to provide more information about new CSC-associated biomarkers and CSC-targeted therapies in order to enhance the efficacy of radiation treatment and provide more tailored therapy for cancer patients.

References


