Cancer stem cells: The potential of carbon ion beam radiation and new radiosensitizers (Review)

SUNG-JAE BAEK[1,2], HIDESHI ISHII[2,3], KEISUKE TAMARI[1,2], KAZUHIKO HAYASHI[1,2], NAOHIRO NISHIDA[2], MASAMITSU KONNO[2], KOICHI KAWAMOTO[2,4], JUN KOSEKI[3], TAKAHITO FUKUSUMI[2,5], SHINICHIRO HASEGAWA[2,4], HISATAKA OGAWA[2,4], ATSUSHI HAMABE[2,4], MASAAKI MIYO[2,4], KOZO NOGUCHI[2,4], YUJI SEO[1], YUICHIRO DOKI[4], MASAKI MORI[4] and KAZUHIKO OGAWA[1]

Departments of [1]Radiation Oncology, [2]Frontier Science for Cancer and Chemotherapy, [3]Cancer Profiling Discovery, [4]Gastroenterological Surgery and [5]Otorhinolaryngology and Head and Neck Surgery, Osaka University, Graduate School of Medicine, Suita, Osaka 565-0871, Japan

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Abstract. Cancer stem cells (CSCs) are a small population of cells in cancer with stem-like properties such as cell proliferation, multiple differentiation and tumor initiation capacities. CSCs are therapy-resistant and cause cancer metastasis and recurrence. One key issue in cancer therapy is how to target and eliminate CSCs, in order to cure cancer completely without relapse and metastasis. To target CSCs, many cell surface markers, DNAs and microRNAs are considered as CSC markers. To date, the majority of the reported markers are not very specific to CSCs and are also present in non-CSCs. However, the combination of several markers is quite valuable for identifying and targeting CSCs, although more specific identification methods are needed. While CSCs are considered as critical therapeutic targets, useful treatment methods remain to be established. Epigenetic gene regulators, microRNAs, are associated with tumor initiation and progression. MicroRNAs have been recently considered as promising therapeutic targets, which can alter the therapeutic resistance of CSCs through epigenetic modification. Moreover, carbon ion beam radiotherapy is a promising treatment for CSCs. Evidence indicates that the carbon ion beam is more effective against CSCs than the conventional X-ray beam. Combination therapies of radiosensitizing microRNAs and carbon ion beam radiotherapy may be a promising cancer strategy. This review focuses on the identification and treatment resistance of CSCs and the potential of microRNAs as new radiosensitizers and carbon ion beam radiotherapy as a promising therapeutic strategy against CSCs.

Correspondence to: Professor Kazuhiko Ogawa, Department of Radiation Oncology, Osaka University, Graduate School of Medicine, 2-2 (D10) Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: kogawa@radonc.med.osaka-u.ac.jp

Professor Hideshi Ishii, Department of Cancer Profiling Discovery, Osaka University, Graduate School of Medicine, Center of Medical Innovation and Translational Research (0814B), 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: hishii@gesurg.med.osaka-u.ac.jp

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1. Introduction: Cancer stem cells (CSCs)

Cancer treatments have markedly improved in recent decades. Surgery is considered the main treatment for primary tumors. Yet, surgery has limitations since it can be highly invasive, sometimes causing the loss of function of organs. Improvements in chemoradiotherapy are promising as this modality is less invasive and may preserve organ function. Utilizing a combination of various cancer therapies, such as surgery, radiotherapy, systemic chemotherapy and molecular targeted therapy, locoregional cancer control rates have greatly improved (1). However, relapse and metastasis, which worsen patient outcomes, are of concern. It is important to prevent cancer recurrence and metastasis as the resultant tumors are often more viable and resistant to chemoradiotherapy. The reasons for therapeutic resistant of cancer cells are controversial. However, the cancer stem cell (CSC) theory may provide an accurate explanation.

The CSC theory explains that a small population of cells in a tumor has stem cell properties, such as self-renewal, multiple differentiation and tumor initiation capacities. The idea of CSCs has attacted interest recently, but it was conceptualized

in 1971 with the discovery of mouse myeloma tumor stem cells (2). When injected into mice, not all leukemia cells but only a small population of leukemia cells initiated tumors, and these tumor-initiating cells were termed CSCs. In humans, CSCs were first discovered in leukemia by Bonnet and Dick in 1997 (3). The recurrence rates of patients with residual tumors after surgery and the rates of distant metastases of patients with circulating cancer cells after successful treatment of the primary tumor were not 100%, although these patients were part of a high-risk group (4-9). These data suggested that not all cancer cells have the potential to cause recurrence and metastasis (10). Recent studies have revealed that CSCs play an important role in maintaining tumor growth (11). Growing evidence suggests that CSCs are resistant to chemoradiotherapy and cause tumor metastasis and recurrence (12). Overall, CSCs are a stem cell-like population in cancer with tumor initiation, self-renewal and multiple differentiation capacities which cause metastasis and recurrence, and it appears that the targeting of CSCs during cancer treatment is a promising strategy for a permanent cancer cure.

In this review, we discuss how the idea of CSCs can be used in future clinical practice. We focus on radiotherapy, particularly the potential of carbon ion beam therapy in relation to CSCs.

2. Methods for identifying CSCs: Biomarkers

To date, in order to target CSCs, markers of CSCs have been studied. CSCs have been identified in several types of tumors, and biomarkers have been established, such as those identified by SP assay (13-15): CD44 in breast CSCs (16); and CD133 in hematopoietic CSCs, (17) neural stem cells (18) and colon cancer cells (19,20). These biomarkers are sometimes related with the prognosis of tumors.

CD133 is a major marker of CSCs in various types of cancers, including glioblastoma, rectal cancer and lung cancer (21-24). CD133 is also expressed in differentiated epithelial cells, and some CD133-expressing cancer cells have CSC properties (16-25).

MicroRNA expression in CSCs can also be used for the identification of CSCs. MicroRNAs are endogenous RNAs, which contribute to oncogenic transformation, tumor suppression, and cell differentiation as well as pluripotency (26-28).

Several microRNAs are known as CSC regulators. miR-181 was found to be highly expressed in CSCs in HCC, in embryonic liver tissues, and pluripotent hepatic stem cells in the human liver (29). The expression of miR-130b in HCC positively regulates $CD133^+$ CSCs with respect to self-renewal, tumor initiation and chemoresistance properties (30). miR-34a was found to inhibit the growth of CSCs and metastasis in prostate cancer by directly repressing CD44 expression (31). miR-200b negatively regulates CSCs in breast cancer by reducing CSC formation (32). These microRNAs regulate CSCs positively or negatively, and identification of their mechanism of action may make it possible to target or eliminate CSCs specifically.

CSCs can be identified by measuring expression levels of those biomarkers, although these markers are not specific. A combination of these markers may be more useful for identification of CSCs.

3. The resistance mechanisms of CSCs

CSCs are a small population present in cancer cells with unique properties: i) self-renewal capability, ii) cell differentiation ability and iii) cancer initiation potential (33,34). These properties of CSCs contribute to chemoradiotherapy resistance and cause tumor recurrence. Enhanced DNA repair capacity and reduced reactive oxygen species (ROS) levels may be responsible for the radioresistance of CSCs (35-38).

Several pathways such as OCT4, WNT, NOTCH, Sonic Hedgehog (SHH), B lymphoma Mo-MLV insertion region 1 homolog (BMI1), and SNAIL1/SLUG are known to be linked to the radiation resistance of CSCs (39). Wang *et al* reported that inhibition of the NOTCH pathway with γ-secretase inhibitors sensitized glioma CSCs to radiation (40). Chen *et al* observed that the survival rate of $CD133^+$ cells was higher than that of $CD133^-$ cells after radiation in lung cancer. In the same study, OCT4 knockdown improved the treatment effects of chemoradiotherapy on $CD133^+$ cells (41). CD133 is expressed in radiation-resistant CSCs of glioma (36) and colon cancer (42). The DNA repair- and cell cycle-regulating proteins Chk1/Chk2 have been proven to be related to such resistance. It has also been demonstrated that the inhibition of these kinases improves treatment sensitivity (36). Zhang *et al* reported that the EMT-inducing transcription factor zinc finger E-box binding homeobox 1 (ZEB1) is a regulator of radiosensitivity and DNA damage response in breast cancer cells (43). In the same study, downregulation of ZEB1 was found to radiosensitize breast cancer cells, indicating that ZEB1-targeting agents could be used as tumor sensitizers.

Recent studies have revealed that certain CSC-related mechanisms are epigenetic. Suvà *et al* observed that CSCs can be reprogrammed from normal cancer cells in glioblastoma. They also showed that inhibition of the core transcription factors can suppress cancer stem cell properties, and these proteins can be used as therapeutic targets (44). Seguin *et al* reported that integrin β3 expression and the related KRAS-RalB-NF-κB pathway are both necessary and sufficient for CSC formation and erlotinib resistance. Bortezomib reverses both tumor stemness and erlotinib resistance by inhibiting this pathway (45). These findings suggest that the therapeutic resistance of tumors is related to genetic alterations, and epigenetic removal of CSC properties would enhance the therapeutic sensitivity of tumors.

4. MicroRNAs as radiosensitizers of CSCs

MicroRNAs (miRNAs) are small non-coding RNAs that regulate epigenetic gene expression (26). A growing body of evidence suggests that miRNAs are associated with tumor initiation and progression (46-49). Cui *et al* reported that nanoparticle-delivered miR-200c serves as an effective radiosensitizer of gastric cancer cells and suppresses CSC-like properties (50). CSC-targeted therapies including the miR-200 family tend to damage normal stem cells. Damage to normal cells must be considered in clinical practice, and it is difficult to selectively damage cancer cells while sparing normal cells. By exploiting cellular uptake differences depending on gelatinase levels, Cui *et al* showed that it possible to spare normal cells via the gelatinase strategy. Gelatinases are overexpressed

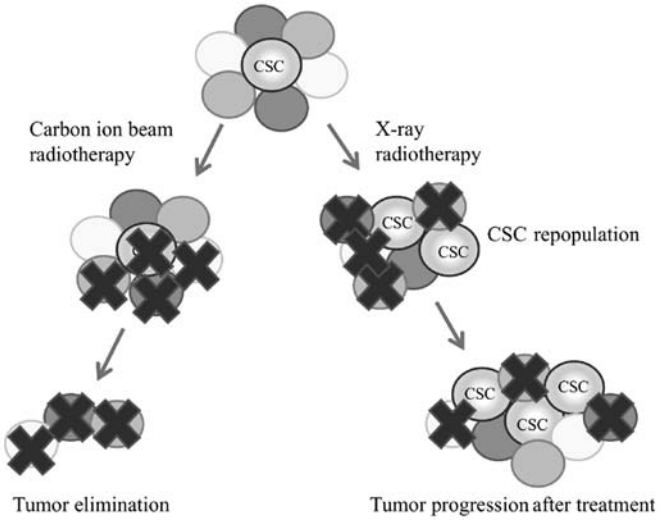

Figure 1. Difference in the biological effects of X-ray and carbon ion beam irradiation. OER of X-ray is between 2.5 and 3.5, which implies that approximately 2/3 of DNA damage is induced indirectly through ROS. Thus, hypoxic cells with low ROS levels are relatively X-ray resistant. OER decreases as LET increases. Carbon ion beam-induced damage is mostly through direct interaction as a high LET beam induces damage regardless of ROS levels. Moreover, a carbon ion beam has strong biological effects; the RBE of a carbon ion beam ranges between 3 and 5, whereas RBE of X-ray is 1.10. OER, oxygen enhanced ratio; ROS, reactive oxygen species; LET, linear energy transfer; RBE, relative biological effect.

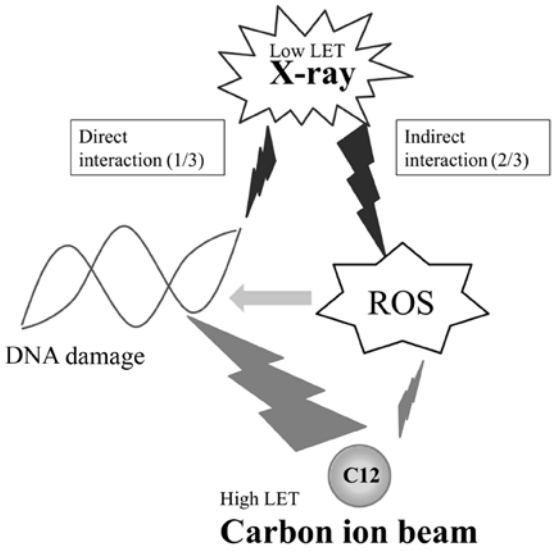

Figure 2. Repopulation of CSCs induced by X-ray radiotherapy and the ability of carbon ion beam radiotherapy in eliminating cancer cells. X-ray-resistant CSCs may survive X-ray radiotherapy while differentiated cancer cells are killed. Thus, the proportion of CSCs at the tumor site increases and causes cancer progression, metastasis and recurrence. Carbon ion beam radiotherapy is more effective against CSCs; therefore, CSC repopulation is less likely to occur. These observations explain the reason why carbon ion beam radiotherapy is a promising modality for cancer elimination. CSCs, cancer stem cells; ROS, reactive oxygen species; LET, linear energy transfer.

in numerous cancers but are undetectable in normal cells (50). Yao *et al* (52) demonstrated that miR-205 is related with radiosensitivity in breast cancer. Downregulation of miR-205 is observed in radio-resistant cancer cells, and it is highly associated with poor distant relapse-free survival in breast cancer patients. Such findings suggest that miRNAs could be utilized as promising radiosensitizers with minimal side effects, while further *in vivo* studies and clinical trials are required for clinical utilization.

5. Radiotherapy: The potential of carbon ion beam therapy in relation to CSC-targeted treatment

X-ray-induced DNA damage mostly occurs through ROS interaction. CSCs have lower ROS levels and enhanced protection from oxidative damage and therefore exhibit better radiation resistance compared with normal cancer cells (35,53,54). Nevertheless, fractionated radiotherapy may result in the repopulation of CSCs (55). Although normal cancer cells are killed by radiotherapy, radioresistant CSCs can survive and proliferate during such treatment. Thus, an increased proportion of CSCs makes a tumor more aggressive (Figs. 1 and 2).

High-LET heavy ions may be the key to treating the therapeutic resistance of CSCs. Heavy-ion radiotherapy such as carbon ion radiotherapy has higher relative biological effectiveness (RBE), ranging between 3 and 5 (56,57), and therefore is more effective against hypoxic radioresistant cells than conventional X-ray radiotherapy. Carbon ion radiotherapy for malignant tumors has yielded favorable results in several clinical trials (58-63). It was reported that Bcl-2-overexpressing HeLa cells are more resistant to γ-rays (0.2 keV/μm) and helium ions (16.2 keV/μm) than neomycin resistance gene-expressing HeLa cells, whereas heavy ions (76.3-1610 keV/μm) yield similar survival regardless of Bcl-2 overexpression. This implies that heavy-ion radiotherapy may be equally effective against CSCs (64) and that carbon ion beam radiotherapy may not induce CSC repopulation in contrast to X-ray radiotherapy.

Notably, when cancer cells are irradiated by X-rays and a carbon ion beam, genomic expression patterns are changed differentially. Carbon ion beam-specific gene expression patterns may be promising therapeutic targets, which have not been well studied to date.

6. Conclusions

CSCs are responsible for tumor recurrence, metastasis and treatment resistance; thus, they are key targets by which to ensure the permanent elimination of cancer cells. Several CSC markers have been recently discovered. Although these markers are not specific, CSCs can be reliably identified using combinations of these markers. Furthermore, specific epigenetic alterations, which are believed to be responsible for the therapeutic resistance of CSCs, are now better understood. These markers and alterations are promising therapeutic targets. By inhibiting the expression of the responsible genes, CSCs can be radiosensitized. Targeting CSCs while sparing non-CSCs is a challenging task as these cell populations exhibit similar expression patterns and biological properties. Recent studies and insights into epigenetic processes and cellular metabolism are providing clues regarding novel promising targeted therapeutic agents that specifically inhibit the growth of CSCs; nanoparticle-delivered miR-200c is a good example. Nevertheless, most studies are currently at the

in vitro level, and translational research is needed to use these ideas in clinical practice.

Carbon ion beam radiotherapy is a promising method for the elimination of CSCs, with strong effects on CSCs. The tumor control rates of carbon ion beam radiotherapy may be further improved by the use of CSC-targeting drugs such as microRNAs as radiosensitizers. Such combination therapies for carbon ion beam radiotherapy have not yet been adequately researched, and further studies are warranted to establish an optimal combination.

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