

## The Impact of Conventional and Carbon Ion Irradiation on Metastatic Potential of Tumor Cells

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### ABSTRACT

**AIM:** There are clinical and experimental studies indicating that ionizing radiation might promote metastatic processes of malignant tumors. The mechanism of radiation-induced metastatic potential of cancer remains substantially elusive. Carbon ion irradiation may suppress metastatic potential of cancer cells. A detailed understanding of the mechanisms and molecular pathways activated in metastatic progression is crucial in identifying new treatment options for anticancer therapy that target metastasis.

**MATERIALS AND METHODS:** The systematic review was

conducted by searching for keywords in Pubmed (January 2000 - May 2014) using the search terms: 'photon irradiation', 'particle irradiation', 'carbon ion irradiation', 'migration', 'invasion', 'angiogenesis' and 'metastasis'.

**RESULTS:** The following strategies to counteract the ionizing radiation-induced metastasis might improve the treatment result of photon irradiation. Combining PI3K/Akt inhibitors LY294002 with ionizing radiation might suppress radiation-induced MMP-9 mRNA expression. Inhibitors of integrin  $\alpha 2\beta 1$  and EGFR in combination with ionizing radiation might suppress invasiveness of lung cancer cells. The combination of inhibition of NF- $\kappa B/\beta 1$ -integrin signaling with radiotherapy might suppress the invasiveness induced by ionizing radiation. Radiation therapy combined with the tyrosine kinase inhibitors (TKI) vandetanib (anti-EGFR plus anti-VEGFR) to inhibit glioblastoma growth might improve the treatment results of photon irradiation. Combining of matrix metalloproteinase inhibitor Metastat or microtubule stabilizing agent Patupilone with ionizing radiation to target radiation-induced invasion and angiogenesis might improve the efficacy of radiotherapy. The combination of radiotherapy with vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) antiangiogenic agents resulted in reduced cell proliferation, migration and tube formation. Carbon ion irradiation provides both physical and biological advantages, such as the inverted dose-depth profile in tissue and an increased relative biological effectiveness (RBE) in the tumor region. In addition, carbon beam irradiation may have advantages such as inhibitory effects on metastatic potential of cancer cells, distinct from photon irradiation.

**CONCLUSIONS:** Many studies have demonstrated that ionizing radiation can promote invasion and/or metastasis. The strategies to counteract the ionizing radiation-induced metastasis might improve the treatment results of photon irradiation. Heavy-ion irradiation may be superior to conventional photon therapy in its possible effects for the prevention of metastasis of irradiated malignant tumor cells in addition to its physical dose distribution. Further investigation to elucidate the intricate molecular mechanisms involved in metastatic progression specifically associated with photon and carbon ion irradiation is warranted.

**Key words:** Photon irradiation; Particle irradiation; Carbon ion irradiation; Migration; Invasion; Angiogenesis; Metastasis

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## INTRODUCTION

The mechanisms of metastasis involve a selective and sequential series of steps, including separation from the primary tumor, invasion through surrounding tissues, entry into the circulatory system, and the establishment and proliferation in a distant location<sup>[1]</sup>. Metastasis poses great problem to cancer treatment and is the main cause of death of cancer patients<sup>[2]</sup>. Radiation therapy remains an important component of cancer treatment with approximately 50% of all cancer patients receiving radiation therapy during their course of illness. X-rays and gamma rays are routinely used photons in radiation therapy to treat various cancers. X-rays and gamma rays are sparsely ionizing radiations, considered low LET (linear energy transfer) electromagnetic rays<sup>[3]</sup>. Although radiation therapy is an essential modality of cancer treatment, its failure to cure is frequently associated with metastasis. The mechanism of this radiation-induced metastatic potential of cancer remains substantially elusive<sup>[4]</sup>. Radiation therapy is also carried out with heavier particles such as protons and heavy ions<sup>[5]</sup>. Particle irradiation exerts very distinct radiobiological effects that differ from photon irradiation<sup>[5]</sup>. High-LET carbon ions produce dense ionization along their trajectories, and cause complex and irreparable clustered DNA damage. Carbon ions are generally more genotoxic and cytotoxic to irradiated cells than low-LET photons<sup>[6]</sup>. Akino and colleagues reported that carbon ion irradiation suppressed metastatic potential of cancer cells<sup>[7]</sup>. A detailed understanding of the mechanisms and molecular pathways activated in metastatic progression is crucial in identifying new treatment options for anticancer therapy that target metastasis<sup>[8]</sup>.

## MATERIALS AND METHODS

### Literature Search strategy

We conducted a systematic review of the existing literature by searching for keywords in Pubmed (January 2000-May 2014) using the search terms: 'photon irradiation', 'particle irradiation', 'carbon ion irradiation', 'migration', 'invasion', 'angiogenesis' and 'metastasis'.

### Inclusion and exclusion criteria

We identified reports containing the impact of conventional and carbon ion irradiation on migration, invasion and metastasis of tumor cells for inclusion. Reports which were published in languages other than English, only published in abstract form, not related to metastasis/invasion of tumor cells, duplicate articles and containing insufficient detail were excluded. One reviewer (ML) screened all titles and abstracts to assess whether they were potentially eligible for inclusion and whether full text was required. Then abstracts and full texts for all potentially eligible studies were reviewed by two researchers (TC and MC), who independently evaluated these articles and extracted their data. Any disagreement during study selection and the data extraction process was resolved by discussion with a third author (SK).

## RESULTS

### Literature Search results

The search identified 551 potentially eligible articles. After application of the exclusion criteria, 50 reports were eligible. The collected data included the effects of photon and carbon irradiation on the processes of angiogenesis and metastasis, the influence of photon and carbon ion irradiation on migration and metastatic potential of tumor cells, the effect of photon and carbon ion irradiation on epidermal growth factor receptor signaling, the influence of photon and heavy ion irradiation on the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway, the effect of photon and carbon ion irradiation on the Rho signaling pathway, and the influence of photon and carbon ion irradiation on integrin family of adhesion molecule. Table 1 summarized the impact of photon and carbon ion irradiation of metastatic potential of tumor cells.

### The effects of photon and carbon ion irradiation on the processes of angiogenesis and metastasis

Many studies have demonstrated that ionizing radiation can promote invasion and/or metastasis<sup>[9-12]</sup>. The mechanism underlying the promotion of metastatic potential of cancer cells involved increased matrix metalloproteinase 2 (MMP-2) activity and upregulated expression of the cell-adhesion molecule integrin  $\alpha V\beta 3$ . MMPs constitute a family of Zn<sup>2+</sup>-dependent enzymes essential for extracellular matrix (ECM) turnover under normal and pathological conditions<sup>[10]</sup>. Matrix metalloproteinases (MMPs) play an important role in cell survival, apoptosis, angiogenesis, matrix remodelling and metastasis and are regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs)<sup>[13,14]</sup>. MMP-2 can degrade type IV collagen, one of the major components of the basement membrane, resulting in the promotion of tumor invasion and metastasis. There are many reports on the enhancement of MMP-2 activity by photon irradiation<sup>[6,15-17]</sup>.

Kaliski and colleagues demonstrated *in vivo* evidence of the antitumor efficacy of combining a MMP inhibitor Metastat with ionizing radiation to target radiation-induced invasion and angiogenesis<sup>[18]</sup>. Qian and colleagues reported that simultaneous administration of an MMP inhibitor, CGS27023A, suppressed the radiation-enhanced invasion through blockade of transition of MMP-2 from latent type to active type. Simultaneous administration of the MMP inhibitor during radiotherapy could be a potent adjuvant therapeutic approach to improve the efficacy of radiotherapy for pancreatic cancer<sup>[19]</sup>. Ionizing radiation (IR) in combination with microtubule stabilizing agent (MSA), patupilone, is a promising combined treatment modality of cancer therapy. In the study of Furmanova-Hollenstein and colleagues, enzymatic activity of secreted MMPs was determined after treatment with patupilone and irradiation in the human fibrosarcoma HT1080 and the human glioblastoma U251 tumor cell line. IR enhanced the activity of secreted MMPs up to 2-fold and cellular pretreatment with low dose patupilone counteracted specifically the IR-induced MMP activity. The cell invasive capacity of HT1080 and U251 cells was increased after irradiation with 2 Gy by 30% and 50%, respectively, and patupilone treatment completely abrogated IR-induced cell invasion. Patupilone counteracts an IR-induced MMP activation process by the reduction of secreted TIMP-1 (tissue inhibitor of metalloproteinase-1) and TIMP-2 (tissue inhibitor of metalloproteinase-2) proteins, which are required for activation of MMPs. Since IR-induced MMP activity could contribute to tumor progression and metastasis, treatment

**Table 1** The impact of photon and carbon ion irradiation on metastatic potential of tumor cells.

The impact of irradiation on metastatic potential of tumor cells	Biological mechanisms	Reference
The effects of irradiation on the processes of angiogenesis and metastasis	(1) Photon irradiation enhanced MMP-2 activity resulting in the promotion of tumor invasion and metastasis.	[6,15,16,17]
	Strategy to counteract metastasis: Combination MMP inhibitor with ionizing radiation to target invasion and metastasis might improve the efficacy of radiotherapy.	[17,18]
The influence of irradiation on migration and metastatic potential of tumor cells	(2) Carbon ion irradiation decreased angiogenesis by affecting both tumor cells and endothelial cells.	[23]
	(1) Photon irradiation promoted cell migration and invasion concomitant with up-regulation of $\alpha v\beta 3$ integrin.	[26]
The effect of irradiation on epidermal growth factor receptor signaling	(2) Carbon ion irradiation decreased cell migration and invasion, inhibited MMP-2 activity, and decreased number of metastasis in vivo.	
	Carbon ion radiotherapy may be superior to conventional photon beam therapy in possible preventive effect on metastases of irradiated tumor cells.	
	(1) Migration of glioma U87 EGFR++ cells and LN229 EGFR++ cells: Photon irradiation increased migration of U87 cells and decreased LN229 cells.	[29]
The effect of irradiation on PI3K/Akt pathway	Heavy ion irradiation decreased migration of both cell lines.	
	(2) Phosphorylation of EGFR, Akt, ERK1/2: Photon increased phosphorylation.	[29]
	Heavy ion: phosphorylation remained unchanged.	
The effect of irradiation on Rho signaling pathway	(3) Treatment strategy: Photon radiotherapy with tyrosine kinase inhibitor vandetanib to inhibit GBM growth might improve the treatment result.	[35]
	(1) Photon irradiation increased invasiveness of tumor cells: mediated by PI3K/Akt/ NF- $\kappa$ B signal transduction pathway.	[39]
The effect of irradiation on integrin family of adhesion molecule	Strategy to counteract invasion: treatment with PI3K inhibitor LY294002 decreased tumor cell invasion.	[9]
	(2) Carbon ion irradiation suppressed the metastatic potential of tumor cells by suppressing the PI3K/Akt signaling pathway.	[40]
The influence of irradiation on integrin family of adhesion molecule	(1) Carbon ion irradiation suppressed invasiveness and motility of tumor cells via inactivation of EGFR/PI3K/Rho signaling pathway.	[7]
	Carbon beam irradiation suppressed the migration and invasion of non-small lung cancer cells more effectively than did photon irradiation.	
The influence of irradiation on integrin family of adhesion molecule	(1) Integrin $\alpha 2\beta 1$ and EGFR cooperatively promote invasiveness of ionizing radiation (IR)-survived lung cancer cells.	[9]
	(2) Photon irradiation induced up-regulation of $\beta 1$ -integrin in invasive breast cancer.	[44,45]
	(3) Carbon ion irradiation inhibited integrin expression and suppressed glioma cell migration.	[5]

combination of IR with patupilone might be of important clinical benefit for tumor therapy<sup>[17]</sup>.

Photons are reported to promote angiogenesis and increase metastasis by causing an increase in the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), hypoxia inducible factor 1 alpha (HIF-1 $\alpha$ ), basic fibroblast growth factor (bFGF), placenta-like growth factor (PLGF), and stem cell factor (SCF) derived from cancer cells<sup>[20-23]</sup>. In addition, overexpression of vascular endothelial growth factor (VEGF), an important growth factor in controlling angiogenesis, has been associated with tumor progression and metastasis. Photon irradiation enhanced the release and production of VEGF in human neuroblastoma cells, and these alterations have been associated with their increased metastatic potential<sup>[24]</sup>.

In contrast to photon irradiation, carbon ion irradiation decreases angiogenesis<sup>[23]</sup>. Takahashi and colleagues demonstrated a decrease in cell migration, adhesiveness, and the ability to form capillary-like tube structures was observed after 0.1 Gy carbon ion irradiation<sup>[25]</sup>. In the study of Kamlah and colleagues, an increase in the migration activity of endothelial cells was observed after photon radiation doses of up to 8 Gy, whereas carbon ion radiation compromised angiogenesis by affecting both tumor cells and endothelial cells even at a sublethal dose<sup>[23]</sup>. In their experimental setup, photon irradiation of human lung adenocarcinoma A549 cells, in contrast to carbon ion irradiation, resulted in enhanced tumor angiogenesis. This effect was likely mediated by photon-dependent induction and secretion of stem cell factor (SCF) in A549 cells, leading to c-Kit phosphorylation in endothelial cells. SCF signaling may represent a therapeutic target for the inhibition of tumor angiogenesis caused by photon irradiation<sup>[23]</sup>. Carbon ion irradiation may represent a therapeutic advantage over photon irradiation for the treatment of lung adenocarcinoma<sup>[23,25, 26]</sup>.

### The influence of photon and carbon ion irradiation on migration and metastatic potential of tumor cells

Cell migration is a fundamental process in early morphogenesis and cancer metastasis<sup>[27]</sup>.

Ogata and colleagues examined the biological properties of highly aggressive HT1080 human fibrosarcoma cells to assess their metastatic processes in terms of cell adhesion capability to extracellular matrix, expression of integrins, cell migration, cell invasive capability, and matrix metalloproteinase-2 activity in vitro. They also assessed the metastatic capabilities of LM8 mouse osteosarcoma irradiated with carbon ion or photon beam in the syngeneic mice. Low dose (0.5 Gy) photon irradiation promoted cell migration and invasion concomitant with up-regulation of  $\alpha v\beta 3$  integrin. Carbon ion irradiation decreased cell migration and invasion in a dose-dependent manner and strongly inhibited matrix metalloproteinase-2 activity. For cancer cells irradiated with carbon ion irradiation, the number of pulmonary metastasis was decreased significantly in vivo. These findings suggest that particle irradiation suppresses metastatic potential even at lower dose, whereas photon irradiation promotes cell migration and invasive capabilities at lower dose, and provide evidence that ion beam radiotherapy may be superior to conventional photon beam therapy in possible preventive effects on metastases of irradiated malignant tumor cells<sup>[26]</sup>.

In the study of Goetze and colleagues, two different tumor cell lines (U87 glioma and HCT116 colon carcinoma cells) were irradiated with 1, 3, or 10 Gy photons or 12C heavy ions. Twenty-four hours after irradiation a standardized Boyden Chamber assay for migration analysis was performed. Photons decreased HCT116 migration at higher doses and appear to increase U87 migration after 3 Gy. Heavy ions decreased migration of both cell lines dose-

dependently. The results suggested that irradiation of tumor cells can modulate their migratory behavior. Radiation-induced influences were cell line- and radiation type-dependent. An increased migration of U87 cells after photon irradiation gave rise to a higher probability of metastatic induction after irradiation of tumors. A reduced tumor cell migration of U87 and HCT116 cells after heavy ion irradiation could diminish the risk of radiation-induced metastasis<sup>[28]</sup>.

In the study of Akino and colleagues, A549 (lung adenocarcinoma) and EBC-1 (lung squamous cell carcinoma) cells were treated with 290 MeV/nucleon carbon ion beam or with 4-MV photon beam. Proliferative, migratory, and invasive activities were tested by cell proliferation assay, Boyden chamber assay, and Matrigel chemoinvasion assay, respectively. Photon irradiation increased cell proliferation of A549 cells at 0.5 Gy. On the contrary, carbon beam irradiation did not enhance proliferation. It reduced the migration and invasion capabilities of both A549 and EBC-1 cells more effectively than did photon irradiation. The results demonstrated that low dose photon irradiation increased cell proliferation of non-small-cell lung cancer (NSCLC) cells. Carbon beam irradiation effectively suppresses the metastatic potential of NSCLC cells<sup>[7]</sup>.

#### **The effect of photon and heavy ion irradiation on epidermal growth factor receptor signaling**

Epidermal growth factor receptor (EGFR) is a member of the cell-surface receptor family ErbB. Activation of EGFR by binding of its specific ligands, including epidermal growth factor (EGF), leads to dimerization of the receptor and subsequently to autophosphorylation of its tyrosine kinase domain. Following activation, the EGFR kinase stimulates a number of cellular signaling cascades. Thereby numerous cellular responses are precisely regulated, such as proliferation, cell survival and cell migration<sup>[29-31]</sup>. EGF-induced EGFR activation has been shown to promote tumor cell migration<sup>[32-34]</sup>. Stahler and colleagues demonstrated that 2 Gy photon irradiation increased migration of U87 EGFR ++ cells and decreased motility of LN229 EGFR ++ cells. Heavy ions decreased migration of both cell lines as a function of dose. There was a time-dependent increase of phosphorylation of EGFR, AKT and ERK1/2 in U87 EGFR ++ cells after 2 Gy photon irradiation. EGFR, AKT and ERK1/2 remained unchanged after heavy ion irradiation. The impact of irradiation on tumor cell migration depends on radiation type and cell line. Photon irradiation potentially contributes to treatment failure by increasing EGFR-related tumor cell migration<sup>[29]</sup>. Wachsberger and colleagues suggested that radiation therapy combined with the tyrosine kinase inhibitor (TKI) vandetanib (anti-EGFR plus anti-VEGFR) to inhibit glioblastoma multiforme (GBM) growth might improve the treatment result of photon irradiation<sup>[35]</sup>. Under comparable conditions, heavy ion irradiation reduced the mobility of GBM cells lines. The data implicate the beneficial role of heavy ion radiation therapy over photon irradiation<sup>[29]</sup>.

#### **The influence of photon and carbon ion irradiation on the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway**

The deregulation of PI3K/Akt pathway is associated with enhanced cell migration, invasion, and metastasis of cancer cells<sup>[36,37]</sup>. The activation of PI3K/Akt signaling regulates the nuclear localization of ANLN. The inhibition of PI3K/Akt signaling resulted in the dephosphorylation of ANLN and a decrease in its nuclear localization<sup>[38]</sup>. Cheng and colleagues investigated the molecular mechanism of radiation-enhanced cell invasiveness of hepatocellular carcinoma (HCC) correlating with clinical patients undergoing radiotherapy and subsequently developing metastasis. Radiation-

enhanced invasion capability was evident in HCC cells but not in normal hepatocytes. Radiation upregulated matrixmetalloproteinase-9 (MMP-9) mRNA level, MMP-9 protein level and MMP-9 activity. MMP-9 antisense oligonucleotides inhibited radiation-induced MMP-9 expression and thereby significantly inhibited radiation induced HCC invasion. Phosphatidylinositol 3-kinase (PI3K)/Akt inhibitors LY294002 and wortmannin suppressed radiation-induced MMP-9 mRNA expression. Nuclear factor- $\kappa$ B (NF- $\kappa$ B) decoy oligodeoxynucleotide suppressed radiation enhanced MMP-9 promoter activity completely. PI3K/Akt inhibitors inhibited radiation-induced NF- $\kappa$ B-driven luciferase promoter activity. A sublethal dose of radiation enhanced invasiveness of HCC cells, mainly through MMP-9 expression mediated by PI3K/Akt/NF- $\kappa$ B signal transduction pathway. It is possible to develop specific inhibitors to modulate unwanted metastatic signaling while retaining the therapeutic benefit of radiation for treating localized disease<sup>[39]</sup>. Signaling molecules, including extracellular signal-regulated kinase-1/2 (ERK1/2) and Akt, exhibited higher activation in lung adenocarcinoma cells that survived ionizing radiation (IR cells). Inhibition of Akt activation by treating with phosphoinositide 3-kinase (PI3K) inhibitor LY294002 decreased IR cell invasion<sup>[9]</sup>.

Ogata and colleagues demonstrated that carbon ion irradiation of human lung adenocarcinoma cells A549 decreased their invasion more effectively than photon irradiation did. Carbon ion irradiation reduced the nuclear localization of ANLN at lower dose. Low-dose carbon ion irradiation also reduced the level of phosphorylated Akt compared to untreated controls, whereas photon irradiation did not. These results implied that carbon ion irradiation effectively suppressed the metastatic potential of A549 cells by suppressing the PI3K/Akt signaling pathway<sup>[40]</sup>.

#### **The effect of photon and carbon ion irradiation on the Rho signaling pathway**

Migratory and invasive activities are important for metastasis. Carbon beam irradiation suppressed the migration and invasion capabilities of both A549 (lung adenocarcinoma) and EBC-1 (lung squamous cell carcinoma) cells more effectively than did photon irradiation. Downregulation of ANLN was induced by carbon beam irradiation<sup>[7]</sup>. ANLN, a gene encoding the human homologue of anillin, an actin-binding protein in *Drosophila*, is overexpressed in the majority of the primary non-small cell lung cancers (NSCLCs)<sup>[41]</sup>. ANLN is involved in the activation of Rho and the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway<sup>[38,42]</sup>. Rho signaling plays a vital role in cell migration and invasion, which are essential features to the development of metastasis<sup>[7]</sup>. Induction of exogenous expression of ANLN enhanced the migrating ability of mammalian cells by interacting with RhoA, a small guanosine triphosphatase<sup>[38]</sup>. Selective suppression of ANLN could be a promising approach for the development of a therapeutic agent as a useful adjunct to conventional therapies<sup>[38]</sup>. Carbon beam irradiation may suppress invasiveness and motility via inactivation of EGFR/PI3K/Rho signaling pathway<sup>[7]</sup>. Carbon beam irradiation may have biologic advantages such as inhibitory effects on metastatic potential of cancer cells, distinct from photon irradiation. Identification and enhancement of these unique capabilities of particle radiation may contribute to improved prognosis because of the suppression of cancer cell metastasis<sup>[7]</sup>.

#### **The influence of photon and carbon ion irradiation on integrin family of adhesion molecule**

Rieken and colleagues reported that photon irradiation enhanced the

risk of tumor cell migration and subsequently promoted locoregional spread via enhanced  $\alpha$ V $\beta$ 3 and  $\alpha$ V $\beta$ 5 integrin expression<sup>[5]</sup>.

**Cooperation of integrin  $\alpha$ 2 $\beta$ 1 and EGFR:** Expression levels of the integrin  $\alpha$ 2 and  $\beta$ 1 subunits were significantly elevated in lung adenocarcinoma A549 cells which survived 10 Gy ionizing radiation (IR cells). Knockdown of  $\alpha$ 2 expression or functional blockade of integrin  $\alpha$ 2 $\beta$ 1 resulted in a round morphology of IR cells, and abrogated their invasion in the collagen matrix, suggesting the molecule's essential role in cell spread and invasion in 3D collagen. Epidermal growth factor receptor (EGFR) also presented enhanced expression and activation in IR cells. Treatment with EGFR tyrosine kinase inhibitor, PD168393, decreased the ratio of elongated cells and cell invasiveness. Signaling molecules, including extracellular signal-regulated kinase-1/2 (ERK1/2) and Akt, exhibited higher activation in IR cells. Inhibition of Akt activation by treating with phosphoinositide 3-kinase (PI3K) inhibitor LY294002 decreased IR cell invasion. Li and colleagues demonstrated that integrin  $\alpha$ 2 $\beta$ 1 and EGFR cooperatively promote invasiveness of IR-survived lung cancer cells, mediated in part by the PI3K/Akt signaling pathway, and might serve as alternative targets in combination with radiotherapy<sup>[9]</sup>.

**$\beta$ 1-integrin via NF- $\kappa$ B signaling:**  $\beta$ 1-integrins are critical mediators of normal cell-extracellular matrix (ECM) interactions and have been shown to play a multifaceted role in malignant progression<sup>[43]</sup>. Ionizing radiation results in up-regulation of  $\beta$ 1-integrins in invasive breast cancer, leading to increased cancer cell survival<sup>[44,45]</sup>. Phosphorylated-Akt (p-Akt) was up-regulated in clinical ductal carcinoma in situ (DCIS) specimens and was associated with recurrent disease. When Akt overexpressing human mammary epithelial (MCF10A-Akt) cells that survived ionizing radiation were repropagated in three-dimensional laminin-rich extracellular matrix (IrECM), a malignant phenotype emerged, characterized by invasive activity, upregulation of fibronectin,  $\alpha$ V $\beta$ 1-integrin, matrix metalloproteinase-9 (MMP-9) and loss of E-cadherin. Ionizing radiation induced nuclear translocation and binding of nuclear factor-kappa B (NF- $\kappa$ B) to the  $\beta$ 1-integrin promoter region, associated with upregulation of  $\alpha$ V $\beta$ 1-integrin. Inhibition of NF- $\kappa$ B or  $\beta$ 1-integrin signaling abrogated emergence of the invasive activity. Nam and colleagues suggested that fibronectin- $\beta$ 1-integrin via NF- $\kappa$ B feedback signaling was critical mediator of invasive progression after ionizing radiation, and could be therapeutic target to suppress invasive recurrence after radiation therapy of DCIS<sup>[46]</sup>. Ahmed and colleagues demonstrated that NF- $\kappa$ B was required for  $\beta$ 1-integrin transactivation in T4-2 breast cancer cells after irradiation. Inhibition of NF- $\kappa$ B reduced clonogenic survival, and induced apoptosis and cytostasis in formed tumor colonies. T4-2 tumors with inhibition of NF- $\kappa$ B activity exhibited decreased growth in athymic mice, which was further reduced by ionizing radiation with downregulated  $\beta$ 1-integrin expression. Upon  $\beta$ 1-integrin inhibition, radiosensitization was regulated by NF- $\kappa$ B via increased transcriptional activity, and a loop-like  $\beta$ 1-integrin-NF- $\kappa$ B- $\beta$ 1-integrin pathway was activated after irradiation. Ahmed and colleagues suggested a promising approach to enhance the radiotherapeutic effect by targeting NF- $\kappa$ B/ $\beta$ 1-integrin pathways<sup>[47]</sup>.

**Carbon ion irradiation inhibits integrin expression:** High-LET carbon ions have been shown to be more effective for cell killing than photons. Goetze and colleagues demonstrated that carbon-ion irradiation inhibited integrin expression, thus leading to the inhibition of migration ability *in vitro*<sup>[28]</sup>. Rieken and colleagues reported that carbon ion irradiation caused decreased integrin expression and suppressed glioma cell migration<sup>[5]</sup>.

## DISCUSSION

Metastasis is a multi-step process that includes local invasion, intravasation, survival in circulation, extravasation, and ultimately proliferation of micrometastases<sup>[27]</sup>.

The mechanisms and molecular pathways of radiation induced metastatic progression involved the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway, integrin expression, epidermal growth factor receptor signaling, angiogenesis, etc. There are strategies to counteract the ionizing radiation-induced metastasis which might improve the treatment results of photon irradiation. Combining PI3K/Akt inhibitor LY294002 with ionizing radiation might suppresses radiation-induced MMP-9 mRNA expression<sup>[39]</sup>. Integrin  $\alpha$ 2 $\beta$ 1 and EGFR cooperatively enhanced invasiveness of ionizing radiation-survived lung cancer cells, and might serve as alternative targets in combination with radiotherapy<sup>[9]</sup>. The combination of inhibition of NF- $\kappa$ B/ $\beta$ 1-integrin signaling pathway with radiotherapy might suppress the invasiveness induced by ionizing radiation<sup>[46,47]</sup>. Radiation therapy combined with the tyrosine kinase inhibitors (TKI) vandetanib (anti-EGFR plus anti-VEGFR) to inhibit glioblastoma multiforme (GBM) growth might improve the treatment results of photon irradiation<sup>[35]</sup>. The combination of matrix metalloproteinase inhibitor Metastat or microtubule stabilizing agent Patupilone with ionizing radiation to target radiation-induced invasion and angiogenesis might improve the efficacy of radiotherapy<sup>[17,18]</sup>. Combining inhibition of VEGF receptor-2 (VEGFR-2) with ionizing radiation might improve the treatment results of radiotherapy<sup>[21]</sup>. Several studies demonstrated that the combination of radiotherapy with vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) antiangiogenic agents resulted in enhanced apoptosis, reduced cell proliferation, reduced endothelial cell migration and tube formation compared with single pathway inhibition<sup>[48-50]</sup>.

In contrast to photon irradiation, carbon ion irradiation decreases angiogenesis<sup>[23]</sup>. Takahashi and colleagues demonstrated a decrease in cell migration, adhesiveness, and the ability to form capillary-like tube structures was observed after carbon ion irradiation<sup>[25]</sup>. Carbon ion irradiation decreased cell migration and invasion in a dose-dependent manner<sup>[26]</sup>. A reduced tumor cell migration of U87 and HCT116 cells after heavy ion irradiation could diminish the risk of radiation-induced metastasis<sup>[28]</sup>. In addition, carbon beam irradiation effectively suppresses the metastatic potential of non-small-cell lung cancer (NSCLC) cells<sup>[7]</sup>. There was a time-dependent increase of phosphorylation of EGFR, Akt and ERK1/2 in U87 EGFR ++ cells after 2 Gy photon irradiation. EGFR, Akt and ERK1/2 remained unchanged after heavy ion irradiation. Photon irradiation potentially contributes to treatment failure by increasing EGFR-related tumor cell migration. Under comparable conditions, heavy ion irradiation reduced the mobility of GBM cells lines<sup>[29]</sup>. Carbon ion irradiation effectively suppressed the metastatic potential of A549 cells by suppressing the PI3K/Akt signaling pathway<sup>[40]</sup>. Carbon beam irradiation may suppress invasiveness and motility via inactivation of EGFR/PI3K/Rho signaling pathway<sup>[7]</sup>. Carbon ion irradiation decreased integrin expression and suppressed glioma cell migration<sup>[5]</sup>.

Heavy ion radiation is characterized by a high linear energy transfer (LET) leading to a high RBE due to generation of clustered DNA damage that is difficult to repair by cellular enzymatic repair systems<sup>[23]</sup>. Carbon ion irradiation provides both physical and biological advantages, such as the inverted dose-depth profile in tissue and an increased relative biological effectiveness (RBE) in the tumor region. Therefore, carbon ion irradiation is promising

for the treatment of radioresistant cancers<sup>[23]</sup>. In addition, carbon ion irradiation may have advantages such as inhibitory effects on metastatic potential of cancer cells, distinct from photon irradiation<sup>[7]</sup>. Further intensification of these unique capabilities of heavy ion radiation may contribute to the improvement of outcome due to the suppression of cancer cell metastasis.

## CONCLUSIONS

There are clinical and experimental studies indicating that ionizing radiation might promote metastatic processes of malignant tumors. The following strategies to counteract the ionizing radiation-induced metastasis might improve the treatment results of photon irradiation. Combining PI3K/Akt inhibitors LY294002 with ionizing radiation might suppress radiation-induced MMP-9 mRNA expression. Inhibitors of integrin  $\alpha 2\beta 1$  and EGFR in combination with ionizing radiation might suppress invasiveness of lung cancer cells. The combination of inhibition of NF- $\kappa$ B/ $\beta 1$ -integrin signaling with radiotherapy might suppress the invasiveness induced by ionizing radiation. Radiation therapy combined with the tyrosine kinase inhibitor (TKI) vandetanib (anti-EGFR plus anti-VEGFR) to inhibit glioblastoma growth might improve the treatment results of photon irradiation. Combining matrix metalloproteinase inhibitor Metastat or microtubule stabilizing agent Patupilone with ionizing radiation to target radiation-induced invasion and angiogenesis might improve the efficacy of radiotherapy. The combination of radiotherapy with vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) antiangiogenic agents resulted in reduced cell proliferation, migration and tube formation. Heavy-ion irradiation may be superior to conventional photon therapy in its possible effects for the prevention of metastasis of irradiated malignant tumor cells in addition to its physical dose distribution. Further investigation to elucidate the intricate molecular mechanisms involved in metastatic progression specifically associated with photon and carbon ion irradiation is warranted.

## CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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