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Intraoperative radiotherapy in breast cancer: Alterations to the tumor microenvironment and subsequent biological outcomes (Review)

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Abstract. Intraoperative radiotherapy (IORT) is a precise, single high-dose irradiation directly targeting the tumor bed during surgery. In comparison with traditional external beam RT, it minimizes damage to other normal tissues, ensures an adequate dose to the tumor bed and results in improved cosmetic outcomes and quality of life. Furthermore, IORT offers a shorter treatment duration, lower economic costs and therapeutic efficacy comparable with traditional RT. However, its relatively higher local recurrence rate limits its further clinical applications. Identifying effective radiosensitizing drugs and rational RT protocols will improve its advantages. Furthermore, IORT may not only damage DNA to directly kill breast tumor cells but also alter the tumor microenvironment (TME) to exert a sustained antitumor effect. Specific doses of IORT may exert anti-angiogenic effects, and consequently antitumor effects, by impacting post-radiation peripheral blood levels of vascular endothelial growth factor and delta-like 4. IORT may also modify the postoperative wound fluid composition to continuously inhibit tumor growth, e.g. by reducing components such as microRNA (miR)-21, miR-221, miR-115, oncostatin M, TNF-β, IL-6 and IL-8, and by elevating levels of components such as miR-223, to inhibit the ability of postoperative wound fluid to induce proliferation, invasion and migration of residual cancer cells. IORT can also modify cancer cell glucose metabolism to inhibit the proliferation of residual tumor cells. In addition, IORT can induce a bystander effect, eliminating the postoperative wound fluid-induced epithelial-mesenchymal transition and tumor stem cell phenotype. Insights gained at the molecular level may provide new directions for identifying novel therapeutic targets and approaches. A more comprehensive understanding of the effects of IORT on the breast cancer (BC) TME may further its clinical application. Hence, the present article reviews the primary effects of IORT on BC and its impact on the TME, aiming to offer fresh research perspectives for relevant professionals.

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Key words: breast cancer, intraoperative radiotherapy, intraoperative electron radiotherapy, bystander effect, metastasis, tumor microenvironment, anti-angiogenesis effect, microRNA

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1. Introduction

Breast cancer (BC) currently ranks as the most common malignancy among women worldwide, posing a significant threat to their health, and in 2020, it accounted for 11.7% of all new cancer cases (1). Previous research has reported that

BC progression is determined by tumor cells and influenced by the tumor microenvironment (TME) (2). Furthermore, the TME significantly influences the formation of the metabolic and chemical environment, consequently impacting tumor development and progression (3). The complex and multi-level interactions between BC cells and the stromal cells and immune cells in the TME, through direct contact or secretion of factors, can regulate tumor behavior, promoting metastasis and drug resistance (4). For instance, neutrophils have been shown to promote cancer cell EMT and drug resistance, thereby encouraging metastasis (5).

Breast-conserving surgery (BCS) is a prevalent method utilized for the treatment of early-stage BC (6). In 2018, over 266,000 women were diagnosed with early-stage breast cancer in the US, ~60% of whom chose BCS (7). However, surgery can trigger an inflammatory response, modifying the local microenvironment and increasing the invasiveness of residual cancer cells post-surgery (8). In addition, invasive surgical procedures and the related stress responses may facilitate tumor metastasis through the stimulation of angiogenic factor release and concurrent suppression of cellular immunity (9). Thus, the combination of surgery with chemotherapy, hormone therapy and radiotherapy (RT) has become a common treatment approach for BC (7). In 2018, 63% of stage I or II BC patients underwent BCS and radiation therapy (RT) (7).

Currently, BCS combined with postoperative external beam RT (EBRT) is the standard treatment modality and is typically administered using conventional fractionation, with a total dose of 45-50 Gy. Most patients require a tumor bed boost of 10-16 Gy, with the total treatment duration spanning 3-7 weeks (10). However, in comparison with traditional whole-breast irradiation, intraoperative RT (IORT) involves the delivery of high-dose internal brachytherapy radiation therapy to the postoperative region, including the tumor bed and any remaining lesions (11). Compared to EBRT, IORT only requires a few minutes to deliver the necessary radiation dose during surgery, with lower radioactive side effects, reducing the risks of complications such as infection, breast fibrosis, and dermatitis (12). Additionally, IORT can save about $15,000 in total costs compared to EBRT, alleviating patients' psychological stress and improving their psychological and quality of life (12,13). Therefore, IORT is considered to be an effective method for treating BC in clinical practice (12).

A study has reported that IORT not only eliminates neoplastic cells using radiation but also remodels the TME (14). This remodeling occurs via changes in the constituents and biological roles of the exudate at the surgical location, hindering the proliferation and invasion of tumor cells, thereby diminishing local recurrence (14). The efficacy of IORT was reported to be significantly lower in patients who received IORT after a delayed secondary incision than in those who received IORT during their primary surgery (12). This may be due to factors such as the postoperative shift of the tumor bed and the already established TME, highlighting the result that IORT can influence the reshaping process of the postoperative TME (12). Therefore, gaining a deeper and more comprehensive understanding of the impact of IORT on the TME, and using this knowledge as a foundation to seek more personalized treatment plans or radiosensitizing drugs, may be key to further leveraging the advantages of IORT. The present review aims to summarize a range of biological effects and alterations within the TME induced by IORT for BC. These aspects include direct IORT effects, bystander effects, impacts on tumor angiogenesis, miRNA expression and associated cytokines. The potential directions for future research concerning IORT were also proposed, with the goal of integrating these findings into relevant clinical investigations for more precise personalized treatments. The main content of this article is shown in Fig. 1.

2. Impact of IORT on angiogenesis

Angiogenesis is a vital process for cancer cell proliferation and dissemination. Certain concentrations of angiogenic factors within tumor tissue have been associated with tumor aggressiveness and patient prognosis (15). Vascular endothelial growth factor (VEGF) is a stimulator of vascular endothelial cell proliferation and angiogenesis, and delta-like 4 (DLL4) serves as a key angiogenesis inhibitor involved in the regulation of vascular maturation and tumor angiogenesis (16). IORT can impact angiogenesis; however, the effect of radiation on angiogenesis is complex, as it can have both anti-angiogenic and pro-angiogenic effects (17-20). A study by Nafissi *et al* (17) assessed the impact of IORT on angiogenesis and reported that patients with BC who underwent BCS and received 21 Gy of intraoperative electron RT (IOERT) experienced a significant decrease in the blood levels of DLL4 and an significant increase in VEGF levels compared with pre-surgery levels. These findings suggest that IOERT may lead to an increase in the formation of new blood vessels following BCS. However, certain studies have reported contradictory results. For instance, Belletti *et al* (18) reported a decrease in the levels of angiopoietin and VEGF receptor-3 in the wound fluid (WF) of patients following targeted intraoperative RT (TARGIT) treatment, indicating a potential decrease in neovascularization. Kulcenty *et al* (21) conducted a study on patients who underwent IORT and reported a significant reduction in the expression of the protein IL-8, which promotes the formation of endothelial cells (20), in the postoperative RT-WF of these patients. This finding suggests that IORT exhibits anti-angiogenic effects. In a mouse model assessing BC recurrence, a single high dose of radiation (20 Gy) was reported to decrease the local vascular density within the breast compared to normal breast tissue (22). Furthermore, higher doses of radiation within the range of 2-15 Gy have been reported to exhibit anti-angiogenic effects (23). However, tumors may also respond to radiation by protecting their blood vessels from radiation-induced damage, leading to a paradoxical pro-angiogenic effect (24).

The aforementioned differing results reported may be a consequence of variations in the RT apparatus and the dosages of RT. Thus, the relationship between IORT and angiogenesis in cancer treatment remains to be fully elucidated. However, the research indicates that IORT can potentially induce an anti-angiogenic effect, which may lead to a decrease in proliferation and metastasis of residual cancer cells. These findings provide new directions for future research on IORT treatment for BC, such as the determination of the impact of different radiation doses on treatment efficacy. Currently, in clinical practice, the mainstream radiation standards are based on TARGIT-A (20 Gy low-energy X-ray) and intraoperative

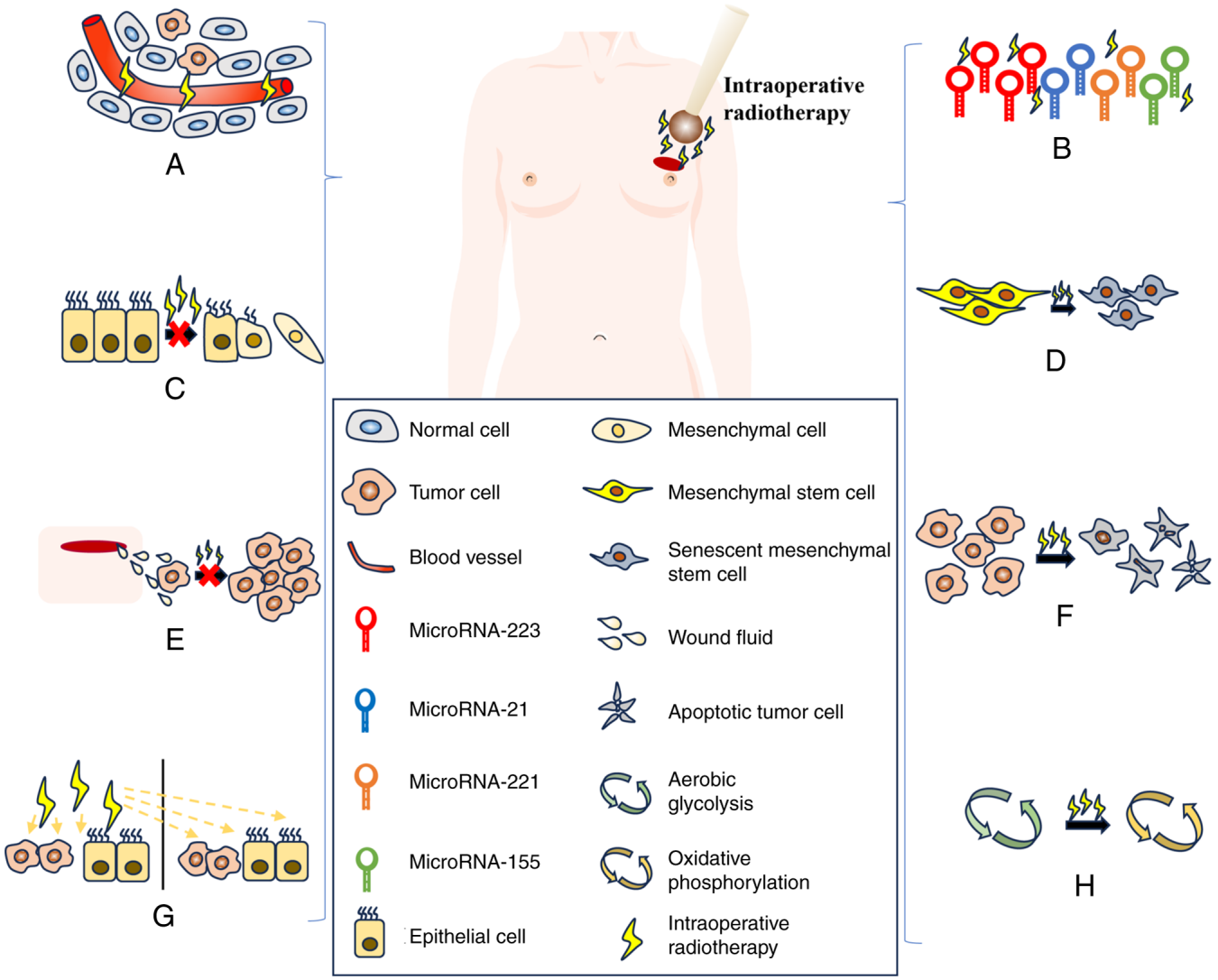

Figure 1. Biological effects of intraoperative radiotherapy on the tumor microenvironment of breast cancer. (A) Anti-angiogenic effects. (B) Regulation of microRNA expression. (C) Elimination of epithelial-mesenchymal transition in tumor cells. (D) Promotion of aging in adipose-derived stromal cells. (E) Elimination of the stimulating proliferative effect of wound fluid on tumor cells after surgery. (F) Promotion of apoptosis in tumor cells. (G) Bystander effect on unirradiated residual tumor cells. (H) Promotion of metabolic transition from aerobic glycolysis to oxidative phosphorylation in cancer cells.

irradiation for early BC (ELIOT; 21 Gy electron beam) (12,25). There are no consensus or guidelines for adjusting the dose according to the specific circumstances of the patient (26). In clinical settings, the intricate relationship between IORT and angiogenesis should be considered when deciding whether to use anti-angiogenic drugs postoperatively, as it is crucial to clarify the potential synergistic or attenuating effects it may have with anti-angiogenic medications.

3. Effect of IORT on micro (mi)RNA expression

miRNAs are a class of non-coding, single-stranded RNA molecules. Certain miRNAs have been identified as oncogenes or proto-oncogenes, making them important therapeutic cancer targets (27). Research has reported that ionizing radiation may lead to an increase or decrease in the miRNA expression profiles in certain cells such as human lung cancer cell lines, and pre-clinical models like mouse models, therefore making miRNA potential therapeutic targets or biomarkers for the radiation response of cancer (28,29).

In a study by Zaleska *et al* (30), postoperative WF obtained from BCS alone was compared to the RT-WF from patients who received IORT, and a significant downregulation in the expression of miR-21, miR-221 and miR-155 in BC cells treated with RT-WF was reported. Another study indicated that miR-223 was involved in mediating inflammatory responses and also functioned as an oncogene in tumor cells (31). In addition, Fabris *et al* (32) reported that 41 miRNAs exhibited differential expression in peritumor tissues between patients with BC who only underwent surgery and those who received IORT. Furthermore, the study reported that miR-223 targeted EGF, and overexpression of miR-223 inhibited residual tumor cell proliferation by reducing post-surgical EGF levels and subsequently suppressing the EGFR signal transduction activation, as demonstrated in Fig. 2. Overexpression of miR-21, an miRNA commonly associated with inflammation, has been reported to promote BC cell proliferation and metastasis

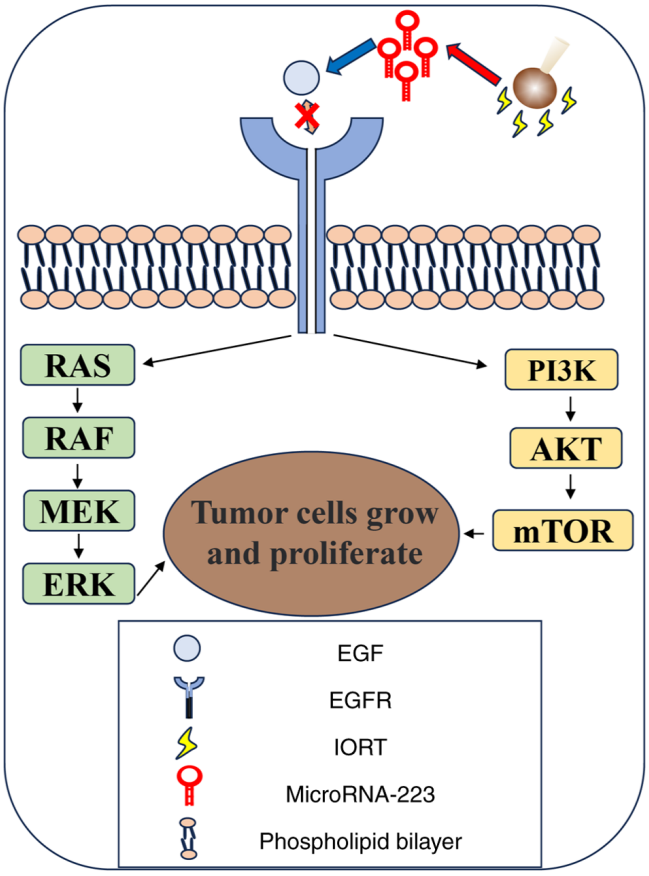

Figure 2. IORT regulates the proliferation of microRNA-223, thereby reducing EGF levels and ultimately attenuating the EGFR signaling pathway. IORT, intraoperative radiotherapy; EGFR, endothelial growth factor receptor.

in vivo (33). Furthermore, low levels of miR-21 are associated with a more favorable prognosis in patients who are HER-2 positive (34). In addition to miR-21, overexpression of miR-221 and miR-155 has also been associated with tumor growth, invasion and metastasis (35-37).

In a previous review on miRNA and radiation (28), it was reported that miR-21 expression was upregulated in BC, esophageal cancer and lung cancer tissue samples after RT. While changes in the expression of miR-221 reported were inconsistent among studies, it tended to decrease according to most studies. Qu *et al* (38) reported that after undergoing surgery, chemotherapy and RT, the plasma levels of miR-155 significantly decreased in patients with BC compared to before treatment. However, the specific effects of RT could not be determined. Based on these data, it may be inferred that IORT serves a pivotal role in regulating miRNA to exert its anti-tumor effects. In particular, miR-223 is a noteworthy miRNA. It has been reported to serve a key role in BC proliferation, drug resistance and metastasis (38). Therefore, miR-223 is a potential target in BC therapy. However, current research on the relationship between miR-223 and radiation is limited. In summary, the study by Zaleska *et al* (30) offers a new clinical therapeutic perspective, suggesting that IORT may have synergistic or antagonistic effects when combined with certain miRNA-targeted treatments. Furthermore, the combined use of IORT with drugs targeting miR-223 may enhance the therapeutic effect of IORT, thereby advancing its application.

4. Epithelial-mesenchymal transition (EMT) of BC cells using IORT elimination of WF

EMT is a biological process where epithelial or endothelial cells transform into mesenchymal cells. During this transition, cells lose their polarity and exhibit downregulation of epithelial markers, including cadherin (CDH)1, epithelial cell adhesion molecule and keratin, whilst upregulating mesenchymal markers such as CDH2, snail family transcriptional repressor 1 (SNAI1) and vimentin (VIM). EMT confers stem cell-like characteristics to mesenchymal cells, enhances tumor cell aggressiveness and promotes tumor dissemination and metastasis (39). Furthermore, EMT is closely associated with the aggressive phenotype of cancer stem cells (CSCs). Kulcenty *et al* (40) reported that treatment of BC cell lines with WF resulted in an upregulation of mesenchymal markers (CDH2, SNAI1 and VIM) and downregulation of the epithelial marker CDH1 *in vitro*. Hence, it can be inferred that WF may have the potential to induce EMT in BC cells. However, in the IORT-treated group, both the BC cells and the induced WF group demonstrated higher levels of epithelial markers and lower levels of mesenchymal markers compared to the RT-WF group. These results indicate that IORT attenuated the EMT process induced by postoperative WF in BC cells. In addition, the study reported that radiation-induced bystander effects counteracted the stimulatory influence of WF on the CSC phenotype and EMT in BC cells.

5. Impact of IORT on adipose stromal cells

Adipocytes are an essential component of the TME both before and after BCS (41). A study reported that adipocytes can interact with tumor cells and contribute to the development, progression and metastasis of BC (42). The mesenchymal stem cells (MSCs) derived from adipose tissue in the breast are referred to as breast adipose stromal cells (bASC) (43). bASCs have the ability to differentiate into cancer-associated fibroblasts and actively participate in the regulation of the TME for tumor cells (44).

In a study by Uhlig *et al* (45), bASCs exposed to IORT demonstrated a senescent-like morphology, indicating a loss of their ability to proliferate when cultured *in vitro*. This suggests that IORT may have eliminated the capacity of bASCs to adhere and proliferate, demonstrating the radiosensitivity of bASCs. These findings indicate that IORT may influence the active components of stem cells in the tumor bed, thereby reducing tumor recurrence. A further study by the same group reported that IORT-stimulated mammary bASCs demonstrated significantly reduced proliferation, migration and wound-healing compared with that of the group treated with WF alone (46). In addition, the stimulated MSCs demonstrated significantly lower levels of secretory 'regulated on activation, normal T cell expressed and secreted' (RANTES), growth-regulated oncogene α (GROα) and VEGF, suggesting a potential mechanism by which IORT influenced the TME. GROα, one of the secretory factors assessed, has been reported to increase the aggressiveness of triple-negative BC when overexpressed, whilst knockdown of GROα attenuated these effects (47). RANTES, also known as chemokine ligand (CCL)5, serves a significant role in the interaction between MSCs and the tumor

stroma, thereby facilitating the metastasis of BC cells (41). The mechanism by which IORT affects MSC function may be associated with the bystander effect it produces.

6. IORT alters WF composition, expression of associated factors and inflammation

Several studies have demonstrated that surgical removal of the primary tumor from the breast induced a wound-healing response and inflammatory process (8,9). This, in turn, altered the local TME and stimulated the proliferation of remaining cancer cells, promoting tumor recurrence and metastasis. Conversely, IORT mitigated these effects.

IORT reduces the stimulatory effect of WF on tumor cell proliferation. Agresti *et al* (8) reported that the composition of WF varied based on the pathological type of tumors. The study analyzed 34 cytokines, growth factors and chemokines in the WF of 27 patients undergoing BCS. The increased expression of macrophage inflammatory protein (MIP)-1α, MIP-1β, interferon (IFN)γ-induced protein 10 (IP-10), IL-6, granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1/monocyte chemotactic and activating factor and bone bridge proteins were reported in more aggressive tumors, specifically those with HER-2 overexpression. In addition, patients who underwent mastectomy had significantly higher levels of IL-1 receptor antagonist, IL-1β, IFN-γ, IL-6, G-CSF, bone bridging protein, IP-10 and MIP-1β in their WF compared with that of those who underwent partial mastectomy. These factors may promote cancer development and progression, and a study by Belletti *et al* (18) on the effects of postoperative WF on BC cells reported that the fluid promoted the proliferation, movement and invasiveness of the cells. However, when the patients underwent IORT, the fluid collected from their wounds did not demonstrate these effects. It was reported that the WF obtained from patients treated with IORT had diminished levels of numerous proteins linked to tumor development and movement, such as hepatocyte growth factor, leptin and RANTES. This reduction would have resulted in impaired activation of the signaling pathways leading to STAT3 and p70S6 kinases, ultimately inhibiting tumor growth and metastasis. These results demonstrate that IORT may modulate the abundance of growth factors and cytokines within WF, resulting in antitumor effects. Therefore, IORT may be recommended for highly invasive subtypes of BC.

IORT induces tumor cell apoptosis and inhibits their expansion and spread. Tumor necrosis factor (TNF) is a small protein molecule mainly produced by activated macrophages, NK cells, and T lymphocytes (48). This can lead to natural killer cell dysfunction in BC, resulting in the failure of immunotherapy. Death receptor 5 (DR5; also known as TRAIL receptor 2) is a receptor in TNF that induces cancer cell death via exogenous pathway activation by cystatin proteases (49). Kulcenty *et al* (19) reported that DR5 protein expression was significantly induced in BC cells treated with RT-WF compared to BC cells treated with WF. By contrast, a previous study demonstrated notably diminished levels of IL-6 in the WF of patients following IORT (18), and this is closely associated with tumor stem cell proliferation and drug resistance (50). In addition, IL-8 is an inflammatory chemokine, associated with the EMT and CSC phenotypes of BC cells (51,52). A notable decrease was observed in RT-WF in the protein expression of both IL-6 and IL-8 after treatment (19,21). This reduction in IL-6 expression led to the inhibition of STAT 3 activity, thereby affecting tumor growth, and it also resulted in decreased EMT and CSC phenotypes (21).

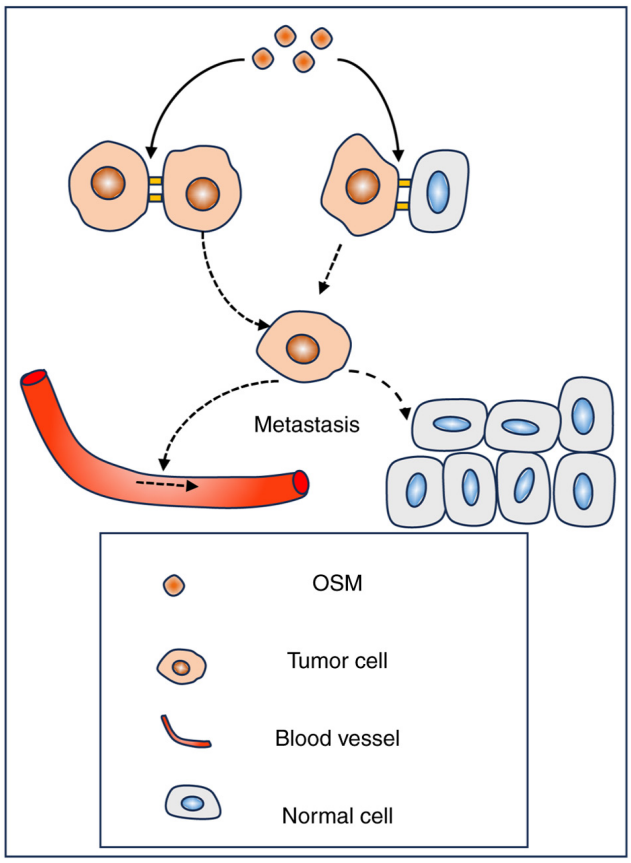

Figure 3. OSM loosens intercellular connections, promoting the metastasis of tumor cells. OSM, oncostatin M; IORT, intraoperative radiotherapy.

Oncostatin M (OSM) is a key factor in the reprogramming of the TME in BC, promoting tumor progression (53). A study reported that OSM can loosen cell-cell and cell-matrix junctions in BC cells, ultimately leading to increased aggressiveness of tumor cells (54), as demonstrated in Fig. 3. IL-1β exhibits a context-dependent function in cancer progression. A study reported that it promotes the development of bone metastases (55), whilst another has reported that it may hinder the colonization of tumor cells that induce metastasis (56). Therefore, the precise role of IL-1β may depend on its specific context. Wuhrer *et al* (46) reported a significant decrease in OSM levels and a significant increase in leptin and IL-1β levels in BC cells that underwent RT-WF, compared to BC cells treated with WF. The differences in leptin were hypothesized to be due to the adipose tissue being exposed to radiation (18). In addition, it was reported that RT-WF not only reduced the proliferation of MSCs, but also attenuated wound healing.

The aforementioned findings suggest that IORT has the potential to induce apoptosis in tumor cells and prevent their proliferation and metastasis by influencing the composition

of WF constituents, modulating the expression of cytokines and affecting signaling pathways. However, the possibility that these effects may coincide with a prolonged wound recovery process following the surgical procedure should be considered and the specific mechanisms underlying this remain to be elucidated through more in-depth research. Furthermore, more research should be conducted on the relationship between OSM and RT, as the OSM/OSM receptor signaling pathway has been reported to be a critical avenue for remodeling the TME of BC and therefore, inhibition of this pathway may offer a new strategy for treating BC (53). The suppressive effect of IORT on OSM could perhaps be an effective measure to realize this therapeutic strategy.

IORT reduces the incidence of radiation inflammation. In a study with a 4-year follow-up period, a single application of IORT did not have a significant impact on the leukocyte count in peripheral blood samples in comparison with conventional external irradiation (57). Meng *et al* (58) reported that a single high-dose irradiation was more effective in treating cancer compared with multiple irradiations. It was elucidated that a single high-dose radiation treatment diminished the activation cycle of the autotaxin-lysophosphatidic acid axis. Conversely, multiple irradiations facilitated inflammatory responses, which may have protected cancer cells from cell death induced by radiation. In an *in vivo* mouse model study, Krall *et al* (59) reported that the systemic inflammatory response initiated after BCS could augment tumor cell proliferation and this process could potentially lead to recurrent metastasis. However, a reduction in tumor resurgence and relapse among patients with BC who received anti-inflammatory treatment during the perioperative period was also reported.

7. Direct, bystander and cancer cell metabolism effects of IORT

IORT inhibits the division of cancer cells. A study by Pan *et al* (60) reported that after *in vitro* irradiation of BC cells with a single dose of 2/4/6 Gy, the proportion of normal BC cells decreased and the proportion of cells undergoing apoptosis or necrosis increased with increasing doses of irradiation. Furthermore, among the examined cells, there was a rise in the proportion of cells arrested in the G1 phase and a decline in the proportion of cells in the S and G2 phases, indicating an inhibition of the mitotic process. In addition, the total number of newly dead cells gradually increased on days 2 and 3 after RT. Based on the observation of cancer cell survival after four weeks, it was hypothesized that a single dose of brachytherapy may have a long-term inhibitory effect on proliferation and invasion, and a pro-apoptotic effect. Additionally, it was reported that this effect increased with higher doses of brachytherapy.

IORT affects unirradiated cells through bystander effects. In addition to its direct effects, ionizing radiation has the capacity to influence non-irradiated cells situated adjacent to those exposed to radiation. This occurrence is recognized as the radiation-induced bystander effect (RIBE) and is facilitated by intercellular gap junctions, along with the secretion of cytokines and chemokines (61), as illustrated in Fig. 4.

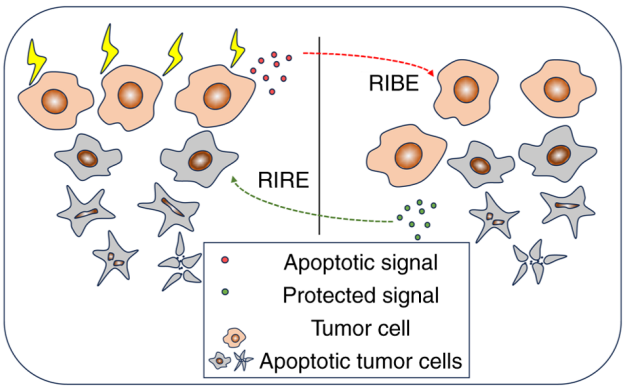

Figure 4. Intraoperative radiotherapy can act directly on tumor cells to induce apoptosis. The signals secreted by irradiated cancer cells induce apoptosis in non-irradiated cancer cells through RIBE. The signals released by non-irradiated cancer cells rescue irradiated cancer cells through RIRE. RIBE, radiation-induced bystander effect; RIRE, radiation-induced rescue effect.

Kulcenty *et al* (40) reported that co-culturing WF-treated BC cells with RT-WF-treated BC cells eliminated the original EMT-inducing effect. Moreover, *in vitro* scratch assays demonstrated a reduction in the migration of BC cells treated with WF after undergoing RT-WF.

During RT, bystander cells that are not directly exposed to radiation can display effects akin to those of directly irradiated cells, including heightened genomic damage, modified apoptosis rates, increased mutation frequency, DNA damage, diminished cloning efficiency and oncogenic transformation (61). However, there is a distinct difference between the radiation-induced bystander effect and the direct effect of radiation. Al-Abedi *et al* (62) reported that radiation-generated RIBE enhanced the EMT phenotype of bystander cells and increased the invasiveness of bystander MCF-7 cells. By contrast, Feghhi *et al* (63) reported that using culture media from electron beam-irradiated cells reduced the survival rate of non-irradiated MCF-7 cells but promoted their migration. It has been hypothesized that, to produce this bystander effect, the radiation dose needs to reach a certain threshold, resulting in an 'all-or-nothing' state (61).

The radiation-induced rescue effect (RIRE) is a phenomenon bearing a strong association with RIBE. The occurrence of RIRE permits cells exposed to radiation to derive advantages from signals emitted by nearby, non-irradiated cells. Chen *et al* (64) reported an improved survival rate for radiation-exposed fibroblasts when grown alongside non-irradiated cells in a shared environment. Similarly, when radiation-exposed HeLa cells were cultivated together with non-irradiated fibroblasts, a reduction in the formation of micronuclei within the HeLa cells was reported. This indicated that bystander cells may rescue irradiated tumor cells through the RIRE, as illustrated in Fig. 4. Consequently, this effect could potentially contribute to the recurrence of residual tumor cells.

In addition, when exposed to radiation stress conditions such as RT, cells can display autophagy-inducing behavior. The induction of autophagy may result in tumor cells transitioning into a reversible dormant condition, allowing them to survive instead of undergoing apoptosis (65,66).

However, this behavior may result in later tumor recurrence (66). A study reported that markers of autophagy are notably increased in bystander hepatocellular carcinoma cells exposed to 3 Gy of γ-rays (67). This demonstrates that bystander hepatocellular carcinoma cells can produce an autophagic response.

The mechanism of the RIBE produced during RT is complex. The aforementioned studies demonstrated that diverse cells could generate different biological effects when exposed to varying doses of radiation. However, despite the advantages of IORT, the specific RIBE produced has remained to be fully determined (14,40). Therefore, further research is required to provide an improved understanding of the clinical implications associated with IORT.

Effect of IORT on DNA damage and glucose metabolism in cancer cells. Ionizing radiation primarily damages DNA in cells. Studies conducted by Piotrowski *et al* (14) and Kulcenty *et al* (68) reported that both RT with and without RIBE stimulation in cancer cells induced DNA double-strand breaks and heightened the expression of genes responsible for DNA damage repair [such as the genes ERCC excision repair 2, TFIIH core complex helicase subunit (ERCC2), ERCC8 and RAD51 recombinase]. Upon exposure to RT-WF and WF + RIBE stimuli, two DNA repair mechanisms were activated, nucleotide excision repair and homologous recombination.

Moreover, it was reported that BC cells exposed to RT-WF and WF + RIBE treatment exhibited increased oxidative phosphorylation levels in comparison with those treated solely with WF. It has been reported that cancer cells may be more adapted to aerobic glycolysis as their primary means of glucose metabolism instead of oxidative phosphorylation and this phenomenon is known as the Warburg effect (69). During this metabolic process in cancer cells, glucose molecules are converted into pyruvate via glycolysis and then reduced to lactate with the help of lactate dehydrogenase, ultimately leading to a decrease in the pH of the TME (69), as illustrated in Fig. 5. This reduction in pH can enhance the metastatic capacity of cancer cells (70,71). Kulcenty *et al* (68) hypothesized that it is therefore possible that IORT facilitates a metabolic shift in BC cells from glycolysis to the oxidative phosphorylation pathway, due to direct and bystander effects. The metabolic shift may contribute to a decrease in glucose consumption and lactate secretion in BC cells, which consequently modifies both the cellular metabolism and the pH of the TME. These findings suggest that the alteration of metabolism in cancer cells is another role served by IORT.

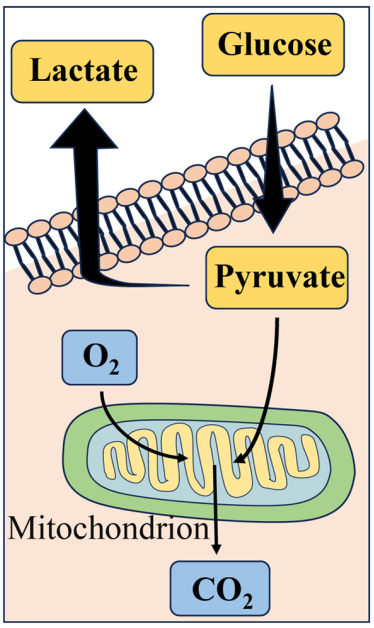

Figure 5. Schematic diagram of glycolysis and oxidative phosphorylation in tumor cells.

8. Discussion

Recent research from the TARGIT-A and ELIOT trials have reported that the effectiveness of IORT for patients with BC is not inferior to traditional EBRT (12,25). The data from the TARGIT-A trial demonstrated that over an average follow-up period of 8.6 years, the overall mortality rate in the IORT group was significantly lower than that in the EBRT group (3.9 vs. 5.3%; hazard ratio, 0.76), with no significant differences in BC mortality rate and distant metastasis (12). The local recurrence rate in the IORT group was markedly higher than that in the EBRT group (3.3 vs. 1.3%; hazard ratio, 2.55), but this was within an acceptable range. In the TARGIT-A trial, the non-inferiority margin for the difference in local recurrence rates between the two groups was set at 2.5%. In the ELIOT trial (25), after a median follow-up of 12.4 years, the ipsilateral breast tumor recurrence rate was significantly higher in patients receiving single-dose IORET compared to those receiving whole-breast external radiotherapy (12.6 vs. 2.4% at 15 years; hazard ratio, 4.62). However, there was no significant difference in overall survival between the two groups (83.4 vs. 82.4% at 15 years).

The application of IORT has yet to be standardized internationally, primarily due to insufficient large-scale clinical trials and foundational research on this technique. IORT is an RT technique that has become more commonly used over the last 30 years and has filled certain gaps between conventional surgical treatments and traditional EBRT (72). For instance, in the traditional surgical + EBRT treatment, patients are required to endure long periods of psychological distress and complications, whereas implementing IORT is able to markedly shorten patients' treatment cycles, enhance their quality of life and reduce economic losses (73).

However, for optimal therapeutic and economic benefits, whilst ensuring patient quality of life and mental health, integrating IORT with EBRT may be a novel paradigm worth exploring. The ongoing TARGIT-B trial is a large-scale clinical trial evaluating the combination of IORT with post-operative tumor bed boost (74). However, there is still insufficient research supporting the wider clinical application of IORT and therefore, understanding the potential mechanisms of IORT is pivotal for the advancement of this technology.

Furthermore, the present review demonstrated variations in the composition of RT-WF across different studies, possibly due to differences in IORT equipment or radiation doses.

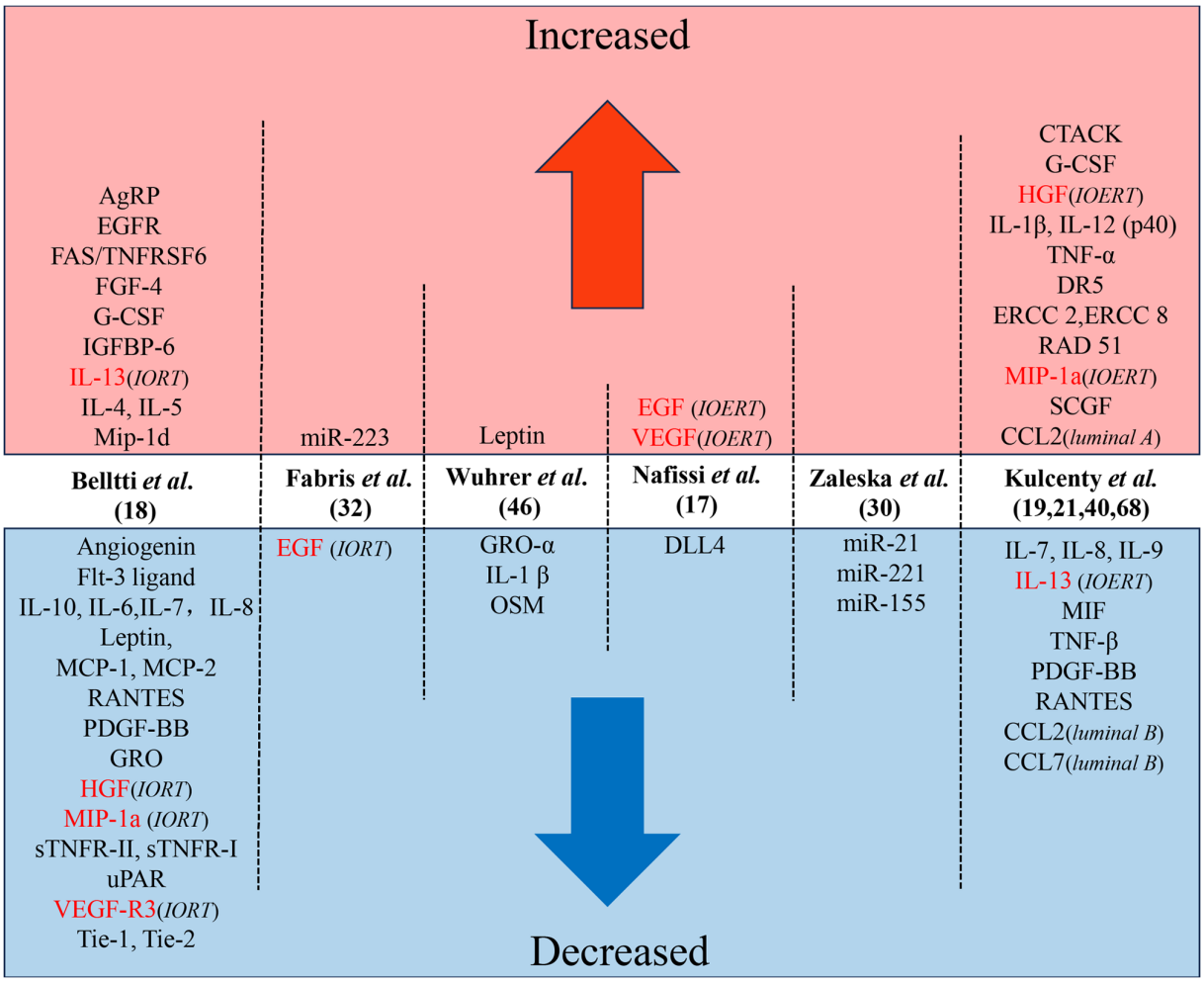

Figure 6. Changes in cytokines, genes and other compounds reported in numerous studies. The highlighted parts represent different components in different studies. IORT, intraoperative radiotherapy; IOERT, intraoperative electron radiotherapy; EGFR, epidermal growth factor receptor; FAS/TNFRSF6, FAS cell surface death receptor/tumor necrosis factor receptor superfamily member 6; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; IGFBP-6, insulin like growth factor binding protein 6; IL, interleukin; Mip, macrophage inflammatory protein; MCP, monocyte chemoattractant protein; PDGF-BB, platelet-derived growth factor B; GRO, growth regulated oncogene; HGF, hepatocyte growth factor; sTNFR. soluble tumor necrosis factor receptor; uPAR, urokinase-type plasminogen activator receptor; Tie, tyrosine kinase with immunoglobulin like and EGF like domains; OSM, oncostatin M; SCGF, stem cell growth factor; CCL, C-C motif chemokine ligand; CTACK, cutaneous T cell-attracting chemokine; MIF, macrophage migration inhibitory factor.

Belletti *et al* (18) reported a notable decrease in hepatocyte growth factor (HGF) and MIP-1α levels and an increase in IL-13 concentration in RT-WF, whereas Kulcenty *et al* (21) reported a significant increase in HGF and MIP-1α levels and a decrease in the IL-13 concentration in RT-WF, as illustrated in Fig. 6. Thus, it is hypothesized that these changes in the TME may explain the differences in the results of the TARGIT-A and ELIOT trials. Furthermore, WF had significantly higher concentrations of HGF and MIP-1α, and lower levels of IL-13 in both studies. Kulcenty *et al* (21) additionally noted a marked disparity in the levels of HGF in the RT-WF between patients with luminal A and B BC. The concentrations of small molecules, including IL-9, platelet-derived growth factor-BB, RANTES, TNF-β, CCL2 and CCL7, were reported to differ between the WFs of the two groups. This suggested that there may be differences in how different types of BC respond to IORT.

The aforementioned studies illustrate the intricate biological effects that result from direct irradiation of the tumor bed and surrounding tissues through IORT during surgery, which may be the primary mechanism underlying its antitumor effects. However, the entire biological foundation underlying this phenomenon has remained to be fully elucidated, necessitating additional studies. Detailed molecular-level information obtained from these studies would form a foundation for further investigations into tumor recurrence and metastasis, and for the identification of new therapeutic targets and treatment modalities. Through comprehending the interplay between BC cells and their TME following IORT, novel approaches to BC therapy may be identified.

Lastly, it is noteworthy to mention some limitations in this review. Firstly, due to time constraints, we may not have incorporated the most recent research findings. Secondly, the heterogeneous quality of reviews might lead to a lack of depth or comprehensiveness in the interpretation of certain literature. Additionally, given the constraints in length and focus, this review may not have encompassed all pertinent literature, thereby possibly omitting some crucial information or research.

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Availability of data and materials

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Authors' contributions

YY, XH, SK, ZZ, MH, CL and NL searched the literature. YY, XH and SK wrote the manuscript. ZZ, MH, CL and NL critically revised the manuscript. FG and WC conceived the idea for the review and provided the final approval. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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