Cellular senescence in ionizing radiation (Review)

ZHENGTING CHEN[1,2*], KE CAO[1,2*], YAOXIONG XIA[1*], YUNFEN LI[1*], YU HOU[1], LI WANG[1], LAN LI[1], LI CHANG[1] and WENHUI LI[1]

[1]Department of Radiation Oncology, The Third Affiliated Hospital of Kunming Medical University/Yunnan Cancer Hospital; [2]Key Laboratory of Lung Cancer Research of Yunnan Province, The Third Affiliated Hospital of Kunming Medical University/Yunnan Cancer Center, Kunming, Yunnan 650118, P.R. China

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Abstract. Radiotherapy (RT) is one of most common treatments for cancer. However, overcoming the failure and side effects of RT as well as radioresistance, recurrence and metastasis remains challenging in cancer treatment. Cellular senescence (CS) is permanent arrested state of cell division induced by various factors, including exposure to ionizing radiation (IR). CS induced by IR contributes to tumour cell control and often even causes side effects in normal cells. Improvement of the therapeutic RT ratio is dependent on more cancer cell death and less normal cell damage. In addition, the biological behaviour of tumour cells after IR has also been linked to CS. This review summarizes our understanding of CS in IR, which may be beneficial for providing new insight for improving the therapeutic outcomes of RT.

Correspondence to: Professor Wenhui Li or Professor Li Chang, Department of Radiation Oncology, The Third Affiliated Hospital of Kunming Medical University/Yunnan Cancer Hospital, Yunnan Cancer Hospital, 519 Kunzhou Road, Kunming, Yunnan 650118, P.R. China
E-mail: wenhuili64@yeah.net
E-mail: changli1981@126.com

*Contributed equally

Abbreviations: BM, bone marrow; BSE, bystander effect; CICs, cancer initiating cells; CS, cellular senescence; CSCs, cancer stem cells; DDR, DNA damage response; DSBs, DNA double-strand breaks; ECs, endothelial cells; EMT, epithelial-mesenchymal transition; HUVECs, human umbilical vein endothelial cells; IR, ionizing radiation; IRIS, IR-induced cellular senescence; MMPs, matrix metalloproteinases; Rb, retinoblastoma; MAPK, mitogen-activated protein kinase; MSCs, mesenchymal stem cells; PARP(i), poly(ADP-ribose) polymerase (inhibitor); SA-β-Gal, senescence-associated β-galactosidase; SASP, senescence-associated secretory phenotype; SIPS, stress-induced premature senescence; SLGA, senescence-like growth arrest; SNCs, senescent cells; TBI, total body irradiation; PF, pulmonary fibrosis

Key words: ionizing radiation, senescence-associated secretory phenotype, radiosensitivity, epithelial-mesenchymal transition, cellular senescence

Contents

1. Introduction

Senescence has been identified for decades (1), and cellular senescence (CS) represents a relatively stable state of proliferative arrest accompanied by failure to re-enter the cell division cycle. Generally, CS is divided into replicative CS resulting from telomere shortening and premature CS that is induced by various types of stress. Due to the increasing evidence of linkages between senescent cells (SNCs) and many age-related diseases, including cancer, atherosclerosis, osteoarthritis, and neurodegenerative diseases (2-5), methods for eradicating SNCs are a hot topic in research on these diseases (6). However, CS appears to play dual roles in cancer radiotherapy (RT). On the one hand, CS induced by ionizing radiation (IR), a major type of RT, can inhibit the proliferation of tumour cells and activate cancer immune surveillance. Many radiosensitizers are aimed at increasing CS when combined with IR (7,8). On the other hand, IR can induce senescence in surrounding and normal cells as well as in cancer cells, which leads to normal tissue fibrosis and organ dysfunction (9). Moreover, IR-induced CS (IRIS) may emerge as a method for helping cancer cells overcome RT and worsen the biological behaviour of tumour cells following IR treatment (10,11).

The aim of this review was to focus on CS in IR by evaluating radiosensitivity, IR-induced side effects, tumour cell biological behavioural changes after IRIS and underlying mechanisms. It was hypothesized that a comprehensive understanding may provide new insights into novel therapeutic modalities in RT to improve the outcomes of cancer patients.

2. IR-induced CS

IR kills tumour cells by causing lethal DNA damage, which can ignite the DNA damage response (DDR), and non-homologous

end joining (NHEJ) and homologous recombination (HR) are the two main pathways for repairing double-strand breaks (DSBs) induced by DNA damage. The accuracy of DNA damage repair by related downstream signalling pathways determines cell fate, including senescence and apoptosis (12). Generally, DNA DSBs are an especially potent stimulus for inducing CS (13). IRIS, is also a form of stress-induced premature senescence (SIPS) (14) and can occur in many types of cells, including cancer cells, fibroblasts, epithelial cells, endothelial cells (ECs), immune cells, and stem cells. Senescent cells (SCNs) always exhibit apoptosis resistance, metabolic activity, proinflammatory and profibrotic molecule secretion and neighbouring microenvironment alteration despite that they have no cell division capacity and permanently arrested proliferation (15) (Fig. 1).

Cell division cycle arrest. In the senescence process induced by IR, the cell cycle is interrupted by G2 arrest after inevitable DNA damage, accompanied by mitotic bypass into the G1 phase (16). Ataxia telangiectasia-mutated protein (ATM), p53, p21, p16-Rb, p38-mitogen-activated protein kinase (p38-MAPK), NF-κB signalling pathway factors, reactive oxygen species (ROS), senescence-associated secretory phenotype (SASP) factors and cyclin-CDK complexes are involved in this process (9,16,17). Different doses of IR and DNA damage can lead to various types of cells with mitotic cell cycle delays, including arrests in the G1, G2 or S phase. G2 arrest and G2 slippage has been linked to IRIS in most previous studies and reviews (18-21). SNCs can be identified by prominent β-galactosidase activity, increased p53, p21 and p16 expression, and decreased levels of Cdc2 and survivin. Notably, some features of IRIS in normal cells and cancer cells are summarized in Table I.

p53. The function of the tumour suppressor protein p53 is related to cell cycle control, DNA repair and apoptosis (40). p53 and phosphorylated retinoblastoma protein (pRB) are the main proteins involved in establishing and maintaining the state of irreversible growth arrest in replicative senescence in normal human cells, and p53 inactivation could reverse CS in BJ cells with a low level of p16 (41). Many studies (42-45) have been carried out to explore the influence of p53 on IR-induced effects. For example, HCT116 $p53^{+/+}$ cells were found to be much more susceptible to IRIS than $p53^{-/-}$ cells (43). IR-induced mitotic skipping during senescence-like growth arrest is associated with p53 function (24). Therefore, the mechanisms of p53, the guardian of the genome, and its related signalling pathways are well characterized in IRIS.

Other proteins/factors related to p53. Increasing evidence supports that insulin-like growth factor-binding protein 5 (IGFBP-5) plays a crucial role in CS via a p53-dependent pathway and especially functions in the coagulation factor Xa- or interleukin-6 (IL-6)-induced premature senescence of ECs, smooth muscle cells (SMCs), and fibroblasts (46-48). Exogenous IGFBP-5 or IGFBP-5 overexpression induces premature senescence in human umbilical vein endothelial cells (HUVECs) *in vitro*, and knocking down IGFBP-5 can partially alter the senescence process *in vitro* (48). Notably, IGFBP-5 is upregulated in the IRIS of HUVECs after chronic low-dose IR (49) and may therefore be a significant target to reduce IRIS in normal cells. In addition, the BRE gene (BRCC45) is also associated with the DNA damage-induced premature senescence of fibroblasts resulting from γ-IR (50). Downregulation of the lamin-B receptor (LBR) and LB1 is a primary response of cells to various stresses leading to senescence, and the loss of LB1 can even serve as a biomarker of senescence (51,52). Naturally, other factors involved in IRIS are independent of p53. For instance, oestrogen E2 suppressed IRIS by inhibiting the binding of cyclin E with p21 and the functional inactivation of p21, followed by permanent Rb hyperphosphorylation, but it did not affect p53 activation in MCF-7 breast cancer cells (53).

lncRNAs and miRNAs. Long non-coding RNAs and microRNAs also contribute to CS induced by IR (23,54). IRIS is modulated by miR-155 via the p53 and p38-MAPK pathways and partially regulates tumour protein 53-induced nuclear protein 1 (TP53INP1) expression in human WI-38 lung fibroblasts (23). The overexpression of miR-30e in HCT116 cells was revealed to markedly accelerate and augment the γ-IR-induced caspase-3-like DEVDase senescent phenotype because miR-30e upregulates p21 expression (55). However, miR-30e could not induce senescence in the poorly differentiated RKO colon carcinoma cells (55). This finding demonstrated that miR-30e controls IRIS and may be affected by the differentiation degree of the cell lines.

IR dose and fraction regimen. Other factors also affect the process of IRIS. For example, the IR dose plays a crucial role in inducing senescence or apoptosis upon cell exposure; a low dose (0.5-10 Gy) of IR induces senescence, while a very high dose (>10 Gy) induces apoptosis (30), and this phenomenon is related to the level of DNA damage and function of the DDR network. Recently, Velegzhaninov *et al* (56) reported that a single low dose (30-50 mGy) of gamma irradiation could suppress CS in normal human fibroblasts. Similarly, a single low-dose X-ray could promote the proliferation of normal cells but not of cancer cells (29). However, low-dose fractionated IR (5x1 Gy) induced temporal patterns of p53/p21 expression in MRC5 fibroblasts, resulting in more significant CS than that generated by a single 5 Gy pulse of IR, as indicated by an integrated stochastic model of DNA damage repair (57). Therefore, the fraction regimen also appears to affect IRIS and may respond differently in different cells. For example, lymphocytic leukaemia cells with exponential growth similar to that of rapidly proliferating tumour cells are not very sensitive to fraction size, while slow-growing fibroblasts and most late-responding cells show high sensitivity (31). Therefore, haematological toxicity occurs early during the RT process, and monitoring and preventing the development of leukopenia is of great importance. Other side effects of IRIS are discussed more specifically in section four.

CSCs. Surviving non-tumourigenic cells were revealed to be more prone to CS, while breast cancer initiating cells (CICs) could be mobilized from the quiescent/G0 phase of the cell cycle to actively cycling cells after sublethal doses of radiation (33). CICs, also called cancer stem cells (CSCs), derived from many types of human cancers and cancer cell lines

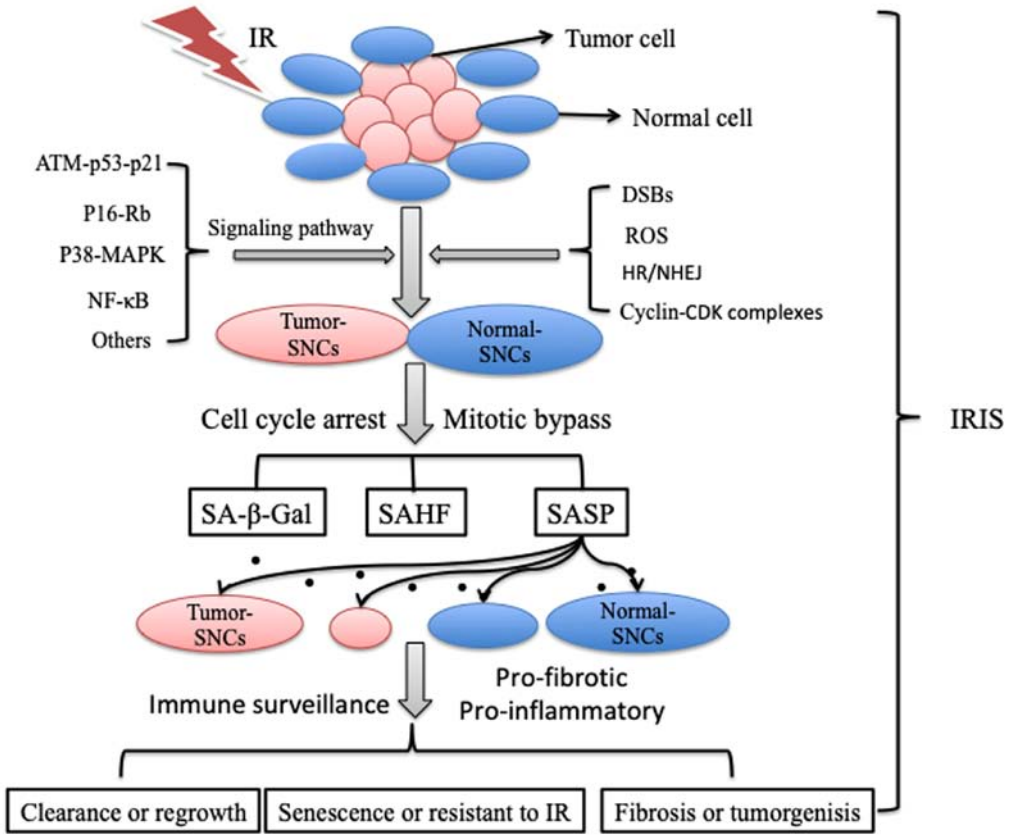

Figure 1. Cellular senescence is induced by IR. The exposure of both tumour cells and normal cells to IR can lead to DNA damage. NHEJ and HR are two main pathways for repairing DSBs, which are especially potent stimuli for inducing CS. Inevitable DNA damage triggers cell cycle arrest accompanied by mitotic bypass. ATM, p53, p21, p16-Rb, p38-MAPK, factors in the NF-κB signalling pathway, ROS and cyclin-CDK complexes are involved in this process. SNCs demonstrate senescence-associated heterochromatin foci, activated metabolism, the SASP and SA-β-Gal-positive staining. The SASP contributes to profibrotic and proinflammatory factors and plays a role in active immune surveillance. The SASP also alters tissues and the surrounding microenvironment through paracrine, autocrine, or endocrine methods. Finally, tumour SNCs may be cleared or regrown, and normal SNCs may be obliterated, induce fibrosis or promote tumourgenisis. Furthermore, non-senescent cells may become senescent or resistant to IR. ATM, ataxia telangiectasia mutated protein; CDK, cyclin-dependent kinase; DDR, DNA damage response; DSBs, DNA double-strand breaks; HR, homologous recombination; IR, ionizing radiation; IRIS, IR-induced cellular senescence; NF-κB, nuclear factor κ-B; NHEJ, non-homologous end joining; p38MAPK, p38 mitogen-activated protein kinase; pRb, retinoblastoma protein; ROS, reactive oxygen species; SA-β-Gal, senescence-associated β-galactosidase; SAHF, senescence-associated heterochromatin foci; SASP, senescence-associated secretory phenotype; SNCs, senescent cells; tumor-SNCs, tumour senescent cells; normal-SNCs, normal senescent cells.

demonstrate increased therapeutic resistance, partly because they can evade differentiation and senescence induced by the immune-suppression cytokine interferon (IFN) signalling pathway (58-60).

Studenckan *et al* (27) coined the term 'senoptosis', which refers to the phenomenon of γ-IR inducing deep senescence in human diploid fibroblasts (HDFs) with features of both senescence and apoptosis. Senescence-associated $CD4^+$ T (SA-T) cells, $PD\text{-}1^+$ and $CD153^+$ $CD44^{high}$ cells could serve as suitable biomarkers of immune ageing, as well as potential targets for controlling cancer (61). These observations could lead to new theories for predicting the prognosis of patients after treatment with a combination of immune therapy and RT.

The mechanisms underlying IRIS are becoming increasingly abundant and clear, ranging from the classical cell cycle regulation, DDR and DNA damage repair processes to related miRNAs, lncRNAs, IR factors and cell heterogeneity. Moreover, numerous unelucidated and unsolved problems related to the induction of CS by IR remain, and IRIS appear to be more complex in cancer cells than in normal cells partly because of the intricate biological features of tumour cells.

3. CS and radiosensitivity

IRIS is the result of the inaccurate repair of damaged DNA after IR. Targeting accelerated and increased IRIS has been an important method for increasing the effectiveness of RT.

Poly (ADP-ribose) polymerase (PARP) is known to function in various DNA repair mechanisms, such as base excision repair, HR and NHEJ. PARP inhibitor (PARPi) has been used to treat tumours with BRCA1 or BRCA2 mutations (62) and can be used in combination with other treatment measures. Many studies have indicated that PARPis can sensitize most cancer cells to IR by prolonging growth arrest and CS (63-65). Concurrent therapy with blockade of DNA-dependent protein kinase (DNA-PK) and PARP-1 can accelerate the senescence of irradiated non-small cell lung cancer (NSCLC) cells and irradiated H460 xenografts further than that achieved with IR alone (66) (Table II).

Other evidence has also demonstrated that CS and irradiation have a synergistic effect when applied in combination with irradiation. Phosphorothioate-modified antisense oligonucleotide (PS62ASODN), which inhibits human telomerase reverse transcriptase (hTERT) to stimulate senescence,

Table I. Features of IRIS in normal cells and cancer cells.

Features	Normal senescent cells	Neoplastic senescent cells	(Refs.)
Morphological transformation	Larger, flattened, increased granularity, and increased cytoplasmic vacuolar content		(22,23)
Cell cycle arrest	a. S and G2/M-phase arrest, mitotic skipping, overexpression of cyclin D1, tetraploid cells b. Related to the DDR-network		(24-26)
Special description	Senoptosis[a]; Senescence-like growth arrest (SLGA)		(27,28)
IR dose dependent	Low dose resistant to CS, even promote proliferation	From low to high dose can lead to CS	(29,30)
Fraction size	Slow-growing fibroblasts and most late-responding cells exhibit high sensitivity	a. Rapidly proliferating tumour cells are not very sensitive b. Similar: single dose or fractioned irradiation	(31,32)
Cell type	Almost all normal cells can develop IRIS	a. non-tumorigenic cells more prone than CSCs b. degree of differentiation	(33-35)
SASP	A range of pro- inflammatory and pro-fibrotic chemokines, cytokines, growth factors and proteases, such as IL-1, IL-6/8, CXCL1, CCL2, MMPs, TGF-β, HGF, GM-CSF	Differ among different cells, high heterogeneity	(34,36)
Bystander effect	Especially in senescent fibroblasts and senescent ECs	Breast cancer cells, CRC cells, NSCLC cells	(37-39)

[a]Phenomenon of γ-IR-induced deep senescence in HDFs with features of both senescence and apoptosis. CCL2, CC chemokine monocyte chemoattractant protein (MCP)-1; CRC, colorectal cancer; CS, cellular senescence; CSCs, cancer stem cells; CXCL1, chemokine (C-X-C motif) ligand 1; DDR, DNA damage response; SLGA, senescence-like growth arrest; ECs, endothelial cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGF, hepatocyte growth factor; MMPs, matrix metalloproteinase; NSCLC, non-small cell lung cancer; IL-1α, interleukin 1α; IL-6/8, interleukin-6/8; IR, ionizing radiation; IRIS, IR-induced cellular senescence; Ref., reference; SASP, senescence-associated secretory phenotype; TGF-β, transforming growth factor-β; HDFs, human diploid fibroblasts.

enhanced the inhibition of tumour characteristics in liver cancer cells (67). Telomeric repeat-binding factor 2 (TRF2), a member of the shelterin complex that plays a key role in protecting and stabilizing chromosomal ends, markedly increased the radiosensitivity of human mesenchymal stem cells (hMSCs) compared to that of controls in both proliferation and senescence assays (68). Similarly, inhibition of the mammalian target of rapamycin (MTOR) pathway can augment the radiosensitivity of cancer cells by promoting CS (69). In glioblastoma (GBM) cells, silencing both histone deacetylase 4 (HDAC4) and erythropoietin receptor (EPOR) promoted IR-induced senescence and reversed radioresistance (70-71). Moreover, GBM cells treated with verapamil in combination with carmustine and irradiation were more vulnerable to IRIS than those subjected to individual or dual-combination treatment (72).

Irradiated non-small cell lung cancer (NSCLC) cells can be rendered more radiosensitive by inhibiting epidermal growth factor receptor (EGFR) in a p53-dependent senescence pathway (73). However, other evidence has revealed that senescence is a prominent mechanism of radiosensitization in 45% of NSCLC cell lines and occurs independent of the p53 status but is linked to p16 induction. Senescence and radiosensitization have also been linked to an increase in residual radiation-induced DNA damage, especially DSBs, regardless of the p53/p16 status (73). Notably, irrespective of the cell-based assay employed, caution should be paid to avoid misinterpreting radiosensitivity data in terms of reduced viability (74). Furthermore, similar to receptor tyrosine kinase (RTK) targeting strategies in cancer, IRIS could represent a potential alternative treatment outcome, both allowing tumour growth control and enabling patients to have a better quality of life (75). However, as the SASP incidence increases, IRIS appears to be a candidate mechanism contributing to Fanconi anaemia complementation group A (FancA)-mediated radioresistance in head and neck squamous cell carcinoma (11).

Collectively, these findings indicate that many radiosensitizers function based on CS. Limited benefits suggest that more complicated mechanisms should be considered and explored because CS may facilitate radioresistance in tumour cells and increase the radiosensitivity of surrounding normal cells.

4. CS and IR side effects

CS induced by IR in normal cells leads to tissue fibrosis and organ dysfunction and increases the risk of secondary neoplasms in almost all bodily systems (42,64). As a result, decreasing these side effects induced by IRIS has been a

Table II. CS and radiosensitivity in typical types of cancer cells.

Type of cancer (cells)	Gene/medicine	Mechanisms/Targets	Role of CS (Se- or Re-)	(Refs.)
HNSCC	FancA	SASP	Re-	(11)
NSCLC (H460 and A549)	PAPRi+ inhibitors of DNA-PK	Promoting G2-M cell cycle arrest	Se	(66)
Liver cancer (Walker 256)	PS62ASODN	Against hTERT	Se-	(67)
hMSC	TRF2	Protecting and stabilizing chromosomal ends	Se-	(68)
GBM (U251MG, U87MG)	HDAC4 silencing	Sustain Double strand break repair	Se-	(70)
GBM (U87MG)	Verapamil+carmustine	Reducing intra-cellular ROS and calcium ion levels	Se-	(72)
GBM cells	EPOR silencing	Inducing G2/M cell cycle arrest	Se-	(71)
Breast cancer cells (MCF-7 cells)	Telomere-mitochondrion link	Telomere dysfunction hTERT suppression	Se-	(76)
Sarcoma cells	HSP90	Inducing CS	Se-	(77)

CS, cellular senescence; DNA-PK, DNA-dependent protein kinase; EPOR, erythropoietin receptor; GBM, glioblastoma; HDAC4, histone deacetylase 4; hMSC, human mesenchymal stem cells; HNSCC, human neck squamous cell carcinoma; HSP90, heat shock protein 90; FancA, Fanconi anaemia complementation group A, hTERT, human telomerase reverse transcriptase; TRF2, telomeric repeat-binding factor 2; NSCLC, non-small cell lung cancer; PAPRi, poly(ADP-ribose) polymerase inhibitor; PS62ASODN, phosphorothioate-modified antisense oligonucleotide; SASP, senescence-associated secretory phenotype; Se, radiosensitive; Re, radioresistant; Ref., reference.

direction for improving the therapeutic radiation ratio with the exception of radiosensitizers. An increasing number of researchers are exploring the deeper mechanisms underlying this process, and some interference targets have exhibited potential to suppress CS in normal cells (Table III).

Telomeres. The length of telomeres in somatic cells shortens over time due to increasing age or pathogenic factors, resulting in CS. Both chemotherapy and RT significantly impair telomere maintenance and function in normal human cells, which may lead to CS and ultimately result in tissue/organ damage and secondary malignancies in long-term survivors of cancer (78). However, the telomere length and the telomere length distribution in peripheral leukocytes was revealed to remain unchanged after RT (79). Residual NP-2 cells (human glioma-derived cells) exhibited CS without changes in telomere length after 6 Gy of C-ion irradiation (80).

Pulmonary fibrosis. IR-induced pulmonary fibrosis (PF) is a severe late side effect of thoracic RT. Irradiated mice administered with an inhibitor of B-cell lymphoma-2 (Bcl-2)/B-cell lymphoma-extra large (BCL-xL) via gavage after persistent PF developed reduced type II pneumocyte senescence, and PF was reversed (81). Both recombinant truncated plasminogen activator inhibitor-1 (PAI-1) protein (rPAI-1) and rapamycin, were revealed to prevent radiation-induced fibrosis in the lungs of mice (82,83). In terms of CS, these data indicate that PF is less challenging to treat and more preventable than ever.

Myelosuppression. Total body irradiation (TBI) induces long-term bone marrow (BM) suppression via the induction of premature senescence in haematopoietic stem cells (HSCs) in a p16-independent manner (84). The selective clearance of SCNs, including senescent BM-derived HSCs and senescent muscle stem cells, by a pharmacological agent or small-molecule inhibitor of p38 MAPK was beneficial in part through its rejuvenation of aged tissue stem cells and rescue of long-term myelosuppression (85,86).

Childhood cancer survivors. Childhood cancer survivors are at an increased risk of frailty, which is partly a result of RT (87); however, IR-reduced CS in children has more profound influences. The leukocyte telomere length (LTL) was shorter in childhood acute lymphocytic leukaemia (ALL) survivors who underwent treatment with cranial IR than in survivors in the control group, which may lead to the premature development of age-related chronic conditions in survivors (88). Notably, a regeneration defect in ageing germline stem cells after IR could be treated by the loss of FOXO in an adult model of stem cell injury induced by low-dose IR (89).

RB gene and other key genes. These researchers also justified that MSCs in which members of the RB gene family were silenced did not exhibit increased apoptosis, necrosis or senescence compared with untreated cells after exposure to X-rays at 40 and 2,000 mGy. These surviving MSCs exhibited accumulated DNA damage and may have undergone neoplastic transformation (90). Therefore, attention should be paid to cancer patients with RB gene mutations in terms of evaluating the onset of secondary neoplasms following RT. Another research group used weighted gene co-expression network analysis (WGCNA) to screen for differentially expressed genes between the senescence and non-senescence groups following RT and identified six hub genes: BANK1, Tomm70a, AFAP1, Cd84, Nuf2 and NFE2 (91). The authors provided an alternate method to search for key genes linked to IRIS and built a foundation for exploring these genes (91).

Table III. Cellular senescence and IR side effects.

Side effect	Irradiation	Experimental cell/animal	Targets	Method	(Refs.)
Premature neurodegenerative diseases	Single 20 Gy	Brain microvascular endothelial cells (bEnd.3 cells)	A Disintegrin And Metalloprotease 10 (ADAM10)	Downregulation of ADAM 10	(4)
Hippocampus damage	5 Gy of cranial IR	Whole-brain irradiation mouse	p53, p21, and IL-6 were increased	Knockout of the TRP53 or p21 gene	(42)
Neuropsychological deficits	X-rays	Neural stem and progenitor cells (NSPCs)	Caspase-1 activation	-	(65)
PF	17 Gy X-ray	C57BL/6J mice, type2 pneumocytes	MMP-3, IL-1, TGF-β	Inhibitor of Bcl-2/ BCL-xL; rPAI-1; Rapamycin	(81-83)
Adult stem cell injury	50 Gy	*Drosophila melanogaster* (GSCs)	FOXO and mTOR homologue	FOXO RNAi	(89)
IR-induced DNA damage	2.5 Gy and 10 Gy γ-IR	Human breast cancer and fibroblasts	Different responses to LLLT following exposure to IR	LLLT	(93)
Radiation-induced collagen contraction	2 Gy	fibroblasts, CRC cells	Fibroblasts and CRC cells present different responses to medicine	Manganese porphyrins -MnTnBuOE-2-PyP	(96)
PF	12.5 Gy thorax irradiation	C57BL/6J mice	Pathogenesis (model)	RNA sequencing of lung tissue	(97)
IR-induced cardiovascular disease	10 Gy X-rays	Human coronary artery endothelial cells	SASP, STAT3, BSE	Proteomics analysis	(98)
IR-Induced damage in the prostate	2 Gy X-rays	Mouse prostate fibroblast cells	TGF-β1signaling pathway	ROS scavenger (MnTE-2-PyP)	(99)
BM suppression, HSC	6.5 Gy of-IR	Mouse model	ROS-p16 pathway	MnTE	(100)
BM injury	4 Gy γ-TBI	Ly5.2 mice	Inhibit HSC senescence	Metformin	(101)

Bcl-2/BCL-xL, B-cell lymphoma 2/B-cell lymphoma-extra large; BM, bow marrow; BSE, bystander effect; FOXO, Forkhead box O; CRC, colorectal cancer; GSCs, germline stem cells; mTOR, mammalian target of rapamycin; HSC, hematopoietic stem cell; MMP-3, matrix metalloproteinase-3; MnTE, Mn(III) meso-tetrakis-(N-ethylpyridinium-2-yl) porphyrin; IR, ionizing radiation; LLLT, low-level laser therapy; PAI-1, plasminogen activator inhibitor-1; PF, pulmonary fibrosis; Ref. reference; RNAi, RNA interference; ROS, reactive oxygen species; TBI, total body irradiation; TGF-β, transforming growth factor-β; SASP, senescence-associated secretory phenotype; STAT3, signal transducer and activator of transcription 3.

Radiation sources. Different radiation sources used in IR have different effects on normal cells. Alessio *et al* (92) revealed that IR with α particles created less apoptosis and senescence in BM-MSCs; that is, α particles may spare healthy stem cells more efficaciously than X-rays. Low-level laser therapy (LLLT) enhanced viability and proliferation and reduced senescence of fibroblasts following γ-IR exposure, while LLLT resulted in decreased proliferation and increased senescence in breast cancer cells (MDA-MB-231 cells) (93). It is worth mentioning that the greater biological efficacy of C ions compared to that of low linear energy transfer (LET) radiation (X-rays) may be misevaluated in 2D culture experiments (94). Relevant models and beams are necessary to promote the use of charged particles with increased patient safety.

The application of senolytic agents that selectively kill senescent cells may improve organ function, including SNCs induced by IR (81,95). Other therapeutic methods, including antioxidants, free radical scavengers, mTOR inhibitors, anti-inflammatory agents, stem cell therapy and senomorphics, also have the potential to reduce side effects induced by IRIS (9). MnTnBuOE-2-PyP could inhibit radiation-induced collagen contraction and CS in fibroblasts but could not protect colorectal cancer cells from IR damage (93,96), potentially providing new options for reducing IR-induced damage. However, further investigations need to be performed in humans to evaluate their safety and efficacy.

5. IRIS and tumour cell biological behaviour

In fact, the SLGA response to IR may reflect a key mechanism of residual-cell survival, ultimately resulting in radioresistance, tumour regrowth and dormant tumour recurrence (102). Recently, the phenomenon that SCNs can regrow after exposure to IR has attracted increasing attention, which reflects that CS plays 'opposing roles' in RT and other genotoxic therapies (23,103,104). SNCs appearing in the context of

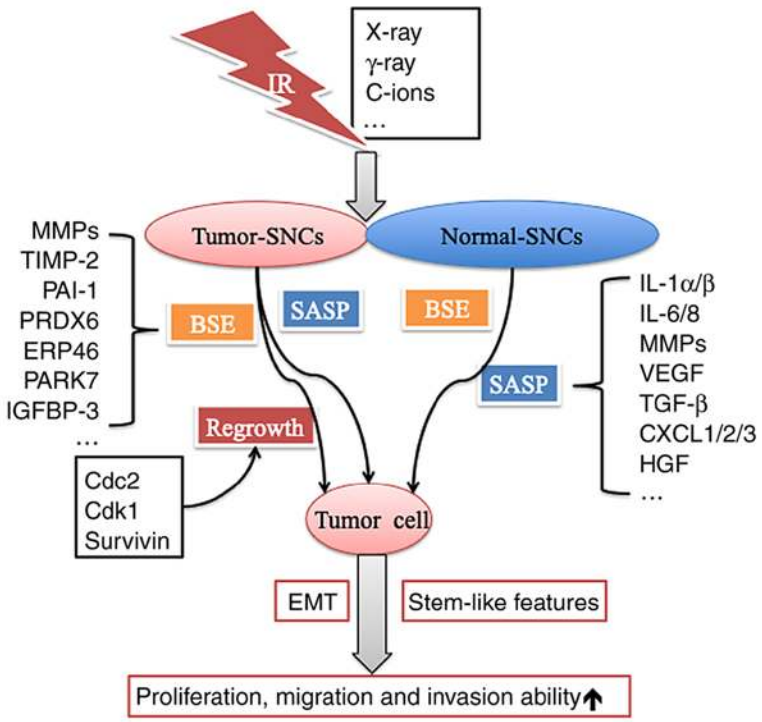

Figure 2. IRIS and biological characteristics of tumour cells. On the one hand, SCNs, including tumor-SNCs and normal-SNCs acquire the ability to secrete many types of factors (e.g., SASP factors: IL-1α/β, IL-6/8, MMPs, VEGF, TGF-β, CXCL1/2/3 and HGF) and facilitate tumour cell growth and invasion *in vitro* and *in vivo*. On the other hand, the cells regrown after IRIS may also develop EMT and stem-like features with enhanced proliferation, invasion and migration capacities, than those existing before IR. Upregulation of survivin, Cdc2, and Cdk1 may help senescent tumour cell regrowth. In addition, the IR-induced BSE may have important implications in this progression, and MMPs, TIMP-2, PAI-1, ERP46, PARK7, may participate in this process. BSE, bystander effect; Cdc2, cell division cycle 2; Cdk1, cyclin-dependent kinases; CXCL1/2/3, the chemokine (C-X-C motif) ligand 1/2/3; EMT, epithelial-mesenchymal transition; ERP46, endoplasmic reticulum protein 46; HGF, hepatocyte growth factor; IL-1α/β/6/8, interleukin-1α/β/6/8; IR, ionizing radiation; IGFBP-3/5, insulin-like growth factor-binding protein-3/5; IRIS, IR-induced cellular senescence; MMPs, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor 1; PARK7, Parkinsonism-associated deglycase; SASP, senescence-associated secretory phenotype; SNCs, senescent cells; TIMP-2, tissue inhibitor of metalloproteinase 2; VEGF, vascular endothelial growth factor; tumor-SNCs, tumour senescent cells; normal-SNCs, normal senescent cells.

neoadjuvant chemoradiotherapy for rectal cancer can promote epithelial-mesenchymal transition (EMT) and further affect the residual tumour microenvironment (105).

Some DNA damage foci induced by IR may persist for a long time. However, the repair of DSBs in SCNs may ultimately result in recovery and regrowth after combination IR/PARPi treatment (106,107). Furthermore, the cells regrown after IRIS may exhibit more aggressive biological behaviours, such as enhanced proliferative ability and increased invasion and migration capacities, than those existing before IR. SCNs also acquired the ability to secrete many types of factors to facilitate growth and invasion *in vitro* and *in vivo* (5) (Fig. 2).

Normal cells are more sensitive to the IR dose regarding the changes in proliferative ability induced by IRIS, while tumour cells seem to dull to the IR dose and segmentation mode. Fractionated radiation and single IR (e.g., 6 or 3x2, 12 or 6x2 Gy) exposures have equivalent abilities to inhibit tumour growth via IRIS *in vitro* and *in vivo* (32). Ablative doses (18 Gy) of radiation exhibit more inhibitory effects on the proliferative, migratory and invasive capacities of lung cancer-associated fibroblasts (CAFs) because CAFs play significant roles in cancer cell invasion and metastasis (108). A low dose of 30 mGy γ-IR was revealed to increase the overall proliferative potential of normal human fibroblasts (HELF-104) (56), while γ-IR could inhibit the growth of primary prostate epithelial cells by inducing senescence, not apoptosis (109).

Apart from proliferative arrest, the SASP is another prominent feature of senescent cells (110). The SASP includes cytokines, chemokines, growth factors and proteases and can trigger the activation of a complex signalling network (111). Irradiated ECs may adversely affect non-irradiated surrounding cells via the SASP, which has been linked to radiation-induced cardiovascular disease (98). The cytokine IL-6, an SASP component, is highly upregulated in many cancers and is considered one of the most important cytokines involved in pro- and anti-tumourigenic effects (112). Senescence-associated IL-6 and IL-8 cytokines can be triggered by paracrines, autocrines, and endocrines, which reinforce the senescent milieu and inflammatory microenvironment in breast cancer cells (36).

Furthermore, the IR-induced bystander effect (BSE) may have important implications in RT (113). The IR-induced BSE describes how cells not exposed to IR show biological changes under the influence of molecular signals secreted by irradiated neighbouring cells (113,114). Several pathways are involved in the paracrine circuit that induces senescence in neighbouring cells, such as the matrix metalloproteinase-2 (MMP-2)/tissue inhibitor of metalloproteinase-2 (TIMP-2), IGFBP3/PAI-1, and peroxiredoxin 6/endoplasmic reticulum protein 46 (ERP46)/Parkinsonism-associateddeglycase(PARK7)/cathepsin

D/major vault protein pathways (115). Moreover, lung fibroblasts with premature senescence resulting from IR may strongly enhance the growth of malignant human lung cancer cells (A549 and H1299) *in vitro* and in immunocompromised mice through increasing the expression of matrix MMPs (38).

In other words, it is common that various tumour cells can undergo SLGA after different types of IR. However, these tumour SNCs may recover their proliferative ability and exhibit more aggressive biological behaviour when the environment is suitable. While the SASP exhibited by tumour SCNs and normal SCNs is mostly responsible for this process, the BSE induced by IR also plays a crucial role via various pathways. However, the complexity of the SASP and various mechanisms of action still restrict our understanding of IRIS (35). The mechanism underlying IR-induced BSE and tumour cell escape from IRIS remains unknown, and further research is urgently required to solve this problem.

6. Other related mechanisms

Although the p16-pRB and p53-p21 tumour suppressor pathways are widely recognized as the main mechanisms underlying SLGA, it is still unclear what makes this arrest stable and what makes CS act as a double-edged sword in cancer treatment (116), especially in terms of improving the efficacy of RT. There may be other related mechanisms contributing to IR-induced senescence.

Mitochondrial dysfunction. Mitochondria play an important role in radiation-induced cellular damage, and different qualities of radiation affect the changes in mitochondrial dynamics (117). Cells exposed to low-dose X-rays and replicative senescent cells exhibit a residual capacity to use fatty acids and glutamine as alternative fuels, respectively (118). Several mitochondrial signalling pathways have been revealed to induce CS (119). DNA cleavage occurring in senescent HDFs after γ-irradiation was triggered by a modest decrease in the mitochondrial membrane potential, which was strong enough to release mitochondrial endonuclease G (EndoG). Then, EndoG translocated into the nucleus to induce the nonlethal cleavage of damaged DNA (27).

IR-induced senescence in quiescent ECs is mediated by at least 2 different pathways dependent on the mitochondrial oxidative stress response and p53 activation (120). hTERT suppression caused by either C ion irradiation or MST-312 impairs mitochondrial function, and telomere-mitochondrion links play a role in the induction of senescence in MCF-7 cells after C ion irradiation (76).

Ferritinophagy. Ferroptosis is a form of regulated necrotic cell death controlled by glutathione peroxidase 4 (GPX4). Ferritinophagy is a lysosomal process that promotes ferritin degradation and ferroptosis. Iron accumulation in SNCs is driven by impaired ferritinophagy. The autophagy activator rapamycin could prevent both the iron accumulation phenotype of SNCs and the increase in TfR1, ferritin and intracellular iron, however, rapamycin failed to re-sensitize these cells to ferroptosis (121).

Acyl-CoA synthetase long-chain family member 4 (Acsl4) is preferentially expressed in a panel of basal-like breast cancer cell lines and predicts their sensitivity to ferroptosis. Acsl4 inhibition is a viable therapeutic approach for preventing ferroptosis-related diseases (122).

Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS). cGAS is a DNA sensor in the DDR process. Genomic DNA damage leads to cGAS activation, stimulation of inflammatory responses, CS and cancer via the cGAMP/stimulator of interferon genes (STING) pathway (123). cGAS deletion also abrogated SASPs induced by IR. cGAS mediated CS and inhibited immortalization, and cGAS activated antitumour immunity (124). cGAS recognized cytosolic chromatin fragments in SNCs. The activation of cGAS, in turn, triggered the production of SASP factors via STING, thereby promoting paracrine senescence (125).

7. Future perspectives

Although our understanding of CS in IR is still initial, similar to RT in the treatment of cancer, IRIS functions as a 'double-edged sword' and crucially influences the comprehensive results of RT. First, because the SASPs created by different types of SCNs are highly different, SCNs play a complicated role in the response of cancer to RT via SASPs. Developing effective pharmacological methods, such as senolytic agents, to remove accumulated SNCs or weaken SASP intensity may be a promising method (126). In addition, combining prosenescence therapy with checkpoint immunotherapy may contribute to eradicating cancer cells from the viewpoint of CS (127). Finally, more well-designed preclinical and clinical trials have the potential to facilitate the development of targeted SNC therapy, which will ultimately improve the clinical outcomes of cancer patients subjected to RT.

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Authors' contributions

ZC, LC, YX and WL conceived and designed the study. KC, YL, LW, LL and YH researched the literature. ZC, LC and WL wrote the manuscript. KC, YX and YL performed data analysis and designed the figures. ZC, KC, YX, YL, YH, LW, LL, LC and WL revised and edited the article. All authors read and approved

the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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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