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REVIEW ARTICLE

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Role of p53, Cancer Stem Cells, and Cellular Senescence in Radiation Response

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Abstract

Currently, radiotherapy has been identified as the most common cancer treatment. However, the efficacy of this treatment modality is low in several malignancies due to the resistance of cancer to radiation. Multiple mechanisms, including cell-cycle checkpoint function, DNA repair, and cell death pathways, modulate the radio-responsiveness of cancer cells. This review considered the role of p53, cancer stem cells (CSCs), and cellular senescence in radiation response.(**International Journal of Biomedicine. 2023;13(3):31-45.**)

Keywords: p53 • cancer stem cells • cellular senescence • radioresistance

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Abbreviations

CSCs, cancer stem cells; **DSB**, double-strand break; **EMT**, epithelial-mesenchymal transition; **HCC**, hepatocellular carcinoma; **IR** ionizing radiation; **ROS**, reactive oxygen species; **SASP**, senescent-associated secretory phenotype.

Introduction

Currently, radiotherapy has been identified as the most common cancer treatment. However, the efficacy of this treatment modality is low in several malignancies due to the resistance of cancer to radiation. Multiple mechanisms, including cell-cycle checkpoint function, DNA repair, and cell death pathways, modulate the radio-responsiveness of cancer cells. This review considered the role of p53, cancer stem cells (CSCs), and cellular senescence in radiation response.

We reviewed published data on the role of p53, cancer stem cells, and cellular senescence in radiation response up to 2022, searching through PubMed, and references from relevant articles, using search terms with suitable keywords. The search terms were "cancer," "ionizing radiation," "cancer stem cells," "tumor protein p53," "DNA damage," "cellular senescence," and "radioresistance."

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Role of TP53 mutations in human cancer and resistance to radiotherapy

In cancer, the *TP53* (tumor protein p53) gene is one of the most mutated genes, and it is essential to find out the role that this gene plays in radioresistance. The *TP53* gene encodes a tumor suppressor protein, p53, a transcription factor, regulating downstream genes involved in cell-cycle arrest, DNA repair, and programmed cell death (apoptosis) (Image 1). Regulation of the apoptotic function of p53 is associated with selective activation of apoptotic target genes. p53 is considered the "guardian of the genome" to prevent the accumulation of oncogenic mutations that lead to malignant tumors.[2] *TP53* mutations occur in about half of all human cancers, almost always resulting in the expression of a mutant p53 (mutp53) protein.[3,4] Mutational inactivation is considered one of the most common molecular mechanisms behind the dysfunction of p53. *TP53* mutations are distributed in all coding exons of the *TP53* gene; 95% of mutations have been detectable within the genomic region (exons 5–8) encoding the DNA-binding domain of p53.[5] Of the mutations in this domain, the six amino acid residues are most

frequently mutated in human cancers, including Arg-175, Gly-245, Arg-273, and Arg-282.[6] These mutations found within the DNA-binding domain of p53 disrupt its proper conformation, and thus the mutp53 is defective in the sequence-specific transcriptional activation and has oncogenic potential.[7,8] While wild-type p53 is a very short-lived protein in the absence of stress, these missense mutations result in the production of a full-length, altered p53 protein with a long half-life.[9] Mutp53 acts as a dominant-negative inhibitor toward wild-type p53.[8]

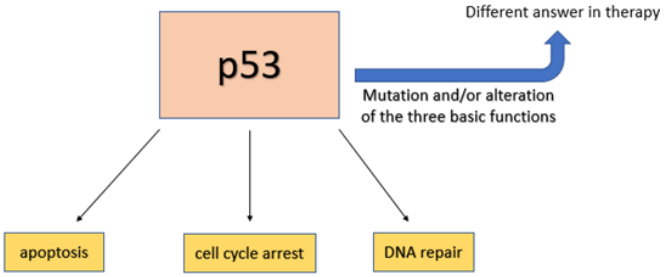

Image 1. *Three essential functions of a tumor suppressor protein p53.*

Mutp53 exhibits a radioresistant phenotype. Mutp53 proteins regulate the expression of several radioresistant genes.[10] Mutp53 activates the expression of NRF2, which is known to confer both chemo- and radioresistance.[11,12] Furthermore, in cells with wild-type p53, DNA damage caused by radiation therapy and most chemotherapeutic agents can lead to p53 accumulation and apoptosis. Loss of p53 function confers impaired apoptosis. Some Mutp53s have been reported to inhibit caspase-9 and p63/73-dependent induction of Bax and Noxa, contributing to the anti-apoptotic effects of mutp53 and the insensitivity of mutp53-containing cells to radiotherapy and chemotherapy.[13-15] Mutp53 proteins play a vital role in the formation and maintenance of CSCs,[16] which are known to play an important role in the development of radioresistance.[17,18] Wild-type p53 has been reported to repress the expression of several CSC markers, including CD44, c-KIT, NANOG, and OCT4. In contrast, mutp53 is associated with loss of repression of these CSC markers, subsequent CSC transformation, and increased radio- and chemoresistance.[19] Thus, the *TP53* gene is an important marker in determining the fate of cells before applying radiotherapy or some other treatment.

p53 molecular interactions

Melanoma is a type of cancer considered radioresistant. However, radiotherapy can be used as a complementary treatment for patients with advanced nodal disease, and that could reduce the risk of tumor prolapse. Melanoma resistance to radiotherapy can occur due to activation of the constitutive MAPK signaling pathway. Another cause of cancer radioresistance is p53 inactivation since wild-type p53 plays an important role in radiosensitization. In melanoma, p53 is rarely mutated, but the WT p53 is inactivated frequently. The melanoma cells' resistance to radiotherapy may very well be related to the MAPK pathway constitutive activation and/or to the p53 inactivation in approximately 90% of melanomas. Krayem et al.[20] carried out a study to evaluate in vitro and in vivo the effect of combining reactivation of p53 with MAPK inhibition on the efficacy of RT in BRAF-mutated melanoma with intrinsic and acquired resistance to BRAF inhibitors. To evaluate the benefit of combining RT with p53 activation and MAPK inhibition, a workflow was designed where melanoma cells were irradiated one day after drug exposure with one single dose of 2 Gy, 5 Gy, or 10 Gy. Protein analysis was done one day after irradiation to assess the direct effect of irradiation and effectors (vemurafenib and PRIMA-1Met). The combination of BRAF inhibition (vemurafenib, which completely shuts down the MAPK pathway) with p53 reactivation (PRIMA-1Met) significantly enhanced the radiosensitivity of BRAF-mutant melanoma cells. In contrast, radiation alone markedly promoted ERK and Akt phosphorylation, thus contributing to radioresistance. The combination of vemurafenib and PRIMA-1Met caused the inactivation of both MAPK kinase and PI3K/Akt pathways, and in combination with radiotherapy, it was able to significantly enhance melanoma cell radiosensitivity. The authors concluded that combining MAPK inhibition with p53 reactivation significantly enhances the radiosensitivity of melanoma both in vitro and in vivo.

In radiotherapy, using radioprotectors and radiosensitizers is an interesting strategy for alleviating adverse effects on normal tissues and reducing tumor resistance. Melatonin is a natural human hormone that shows protective properties against the toxic effects of chemotherapy and radiotherapy. Farhood et al.[21] conducted a review to clarify the mechanisms by which melatonin acts as a radioprotectant and radiosensitizer. Some in vitro studies have shown that melatonin has potent antitumor activity when used in conjunction with radiation. The mechanisms of its radiosensitive effect obviously involve activation of p53 by inhibition of MDM2, changes in the metabolism of tumor cells, suppression of DNA repair responses, and a number of other mechanisms. Additionally, SIRT1 suppression by melatonin is another pathway for p53-mediated apoptosis. The inhibition of COX-2 by melatonin plays an important role in p53-mediated apoptosis. During inflammation due to exposure to radiation, the expression of COX-2 and apoptotic genes such as iNOS and NF-kappaB is increased. The inhibition of these genes can sensitize tumor cells to radiotherapy. Melatonin can help to heal acute reactions during radiotherapy in organs like bone marrow, skin, and the gastrointestinal tract.

Human mitochondrial transcription factor A (TFAM) is needed for mitochondrial DNA replication and transcription, which are essential for mitochondrial biogenesis.[22,23] Inhibition of TFAM in OSC-2 cells leads to a decrease in cell viability and a pronounced induction of apoptosis after gamma irradiation.[24] P53 interacts with the TFAM promoter to activate TFAM transcription and binds to TFAM, thereby regulating cell death.[25-27] TFAM may influence ROS production and further influence cell proliferation and death by directly regulating mitochondrial electron transport chain (ETC) proteins.[28] TIGAR (TP53-induced glycolysis and apoptosis regulator)

promotes the pentose phosphate pathway and helps reduce intracellular ROS.[29,30] Jiang et al.[31] investigated how TFAM affects the sensitivity of tumor cells to IR and found that attenuated expression of TFAM slows tumor cell proliferation by causing G1/S phase arrest. A decrease in TFAM expression led to the inhibition of p53/TIGAR signaling, which further led to an increase in mitochondrial superoxide production and DSB DNA levels in irradiated tumor cells, regulating the sensitivity of tumor cells to radiation.

The aberrant gain of function identified in mtp53 has been shown to promote tumorigenesis; however, many downstream effects of mtp53 are still unknown.[32] A study by Gomes et al.[33] showed that the insulator protein CTCF (CCCTC-binding factor) plays a key role in suppressing the apoptotic p53 response by acting as a gene-specific repressor. Qu et al.[34] demonstrated that otopetrin 2 (*otop2*) plays an important role in the development of colorectal cancer (CRC). The authors determined that *otop2* is an important functional candidate in CRC oncogenesis and demonstrated that p53 plays an important role in governing the *otop2* transcription process by reprogramming the CTCF binding status and altering chromatin architecture.

Wu et al.[35] found an interaction between p53 and RAD18, a central regulator of translesion DNA synthesis, which is highly expressed in glioma cells and reduced cell radiosensitivity to ionizing.[36] Investigating the effects and mechanism of RAD18 in the radiation resistance of glioma and studying the role of p53 in this process, researchers showed that RAD18 functions as a promoter in glioma progression and reduces glioma cells' sensitivity to radiation through down-regulating P53. At the same time, cell growth promotion and cell apoptosis inhibition induced by RAD18 up-regulation were impaired when P53 expression was upregulated under radiation conditions.

Succinate dehydrogenase 5 (SDH5) has been reported to contribute to the development of several types of cancer.[37,38] Zong et al.[39] conducted a study showing that SDH5 can be detected not only in tumors but also in plasma by qRT-PCR, indicating its predictive effect in radiotherapy. The researchers showed that SDH5 modulates radiosensitivity by directly binding p53 and promoting phosphorylation of cytoplasmic p53 at Ser315, which ultimately accelerates the degradation of p53 via the ubiquitin/proteasome pathway and affects radiosensitivity. In SDH5 knockout mice, lung epithelial cells showed increased DNA damage after irradiation. Apoptosis and cell-cycle detection showed that decreased expression of SDH5 resulted in an apparent increase in apoptosis and a cell-cycle arrest in G2/M. SDH5 knockdown decreased p53 phosphorylation predominantly in the cytoplasm and increased its accumulation in the nucleus. SDH5 depletion inhibited p53 degradation via the ubiquitin/proteasome pathway, which promoted apoptosis and increased radiosensitivity in non-small cell lung cancer. Thus, the authors concluded that SDH5 is a novel regulator of p53 and that the loss of SDH5 enhances radiosensitivity by reducing p53 phosphorylation and delaying p53 degradation in lung cancer.

The significance of reducing p53 phosphorylation and degradation to increase radiosensitivity was also analyzed in a study by Xie et al.[40] who investigated the role of CDK16 in the radioresistance of human lung cancer cells. CDK16 negatively modulates p53 signaling pathway to promote radioresistance. CDK16, a member of the cyclin-dependent kinases family (CDK),[41] has demonstrated an essential role in tumorigenesis. A number of studies suggest that CDK16 may act as an oncoprotein in some types of cancer. In particular, the downregulation of CDK16 suppressed cell growth and proliferation in prostate, breast, and CRCs.[42-44] Xie et al.[40] found that CDK16, which is overexpressed in lung cancer and predicts poor prognosis in patients, binds to and phosphorylates p53 at the Ser315 site to trigger p53 degradation via the ubiquitin/proteasome pathway, and CDK16 depletion enhances radiosensitivity in a p53-dependent manner in lung cancer cells. In this regard, CDK16 is a possible target for cancer radiotherapy.

Radiation therapy always causes DNA damage, and cells repair the damaged DNA by activating the cell-cycle checkpoint signaling pathway and stopping the cell cycle to maintain the stability of the genome and the accuracy of chromosome inheritance.[45] Cell-cycle arrest is a common and direct response of most tumor cells affected by radiation.[46] Among the molecules involved in processes of DNA damage repair, the p53-binding protein 1 (53BP1) and mediator of DNA damage checkpoint 1 (MDC1) are distinguished. Following radiation-induced DNA damage, both MDC1 and 53BP1 could transmit the DNA damage signals to the downstream molecules such as cell-cycle checkpoint kinases CHK1 and CHK2. p53 is also involved in the modulation of the cell cycle and the regulation of the cell-cycle checkpoints. A study by Yang et al.[47] aimed to investigate the effects of MDC1 and p53 binding protein 1 (53BP1) silencing on p53, cell-cycle checkpoint kinases (CHK1 and CHK2), and CHK2-T68 expression in the epithelial cell line of human esophageal carcinoma (Eca-109). 53BP1 downregulation significantly reduced p53 and enhanced CHK1 and CHK2 expression in Eca-109 cells. 53BP1 downregulation also significantly regulated CHK1, CHK2, and p53 in xenograft nude mice models exposed to γ-ray irradiation, compared to the untreated group, with p53 negatively correlated with CHK1 and CHK2. The data obtained showed that 53BP1 regulates the cell-cycle arrest by modulating the expression of p53, CHK1 and CHK2 in both Eca-109 cells and xenograft nude mouse models.

The gene associated with retinoid-interferon-induced mortality-19 (GRIM-19) is a tumor suppressor that mediates cell apoptosis in multiple cancer types. A study by Chen et al.[48] investigated the role and underlying mechanism of GRIM-19 in the progression of osteosarcoma, one of the most aggressive types of primary bone cancer that frequently responds poorly to radiotherapy. Overexpression of GRIM-19 accelerated radiation-induced osteosarcoma cell apoptosis by p53 stabilization ex vivo and in vivo.The forced expression of GRIM-19 diminishes the activity of MDM2, a specific p53 protease, resulting in the accumulation of p53 and activation of p53-mediated apoptosis. So, restoring p53 function by inhibiting its interaction with MDM2 is a promising therapeutic strategy for cancer. Yi et al.[49] analyzed the capability of APG-115 to enhance radiation response in gastric cancer in vitro

and in vivo. The authors found that APG-115 radiosensitized p53 wild-type gastric cancer cells. Increasing apoptosis and cell-cycle arrest were observed after the administration of APG-115 and radiation.

In a clinical study performed by Wang et al.,[50] the p53 protein expression in cervical cancer after RT was significantly correlated with cervical space-occupying lesions and tumor size shown in transvaginal color Doppler ultrasound, providing helpful clinical data for monitoring cervical cancer.

p53 molecular interactions are summarized in Table 1.

Table 1.

p53 molecular interactions

Gene/ Protein	Action	Source
MAPK	Inactivation of both MAPK kinase and PI3K/Akt pathways with p53 reactivation significantly enhances the radiosensitivity of melanoma	Krayem et al. (2019) [20]
SIRT1	SIRT1 suppression by melatonin induces p53-mediated apoptosis	Farhood et al. (2019) [21]
TFAM	A decreased TFAM expression led to the inhibition of p53/TIGAR signaling, elevated mitochondrial superoxide and DSB DNA production, and arrest in the G1/S phase.	Jiang et al. (2019) [31]
otop2	p53 governs the transcription process of otop2, a functional candidate in colorectal cancer oncogenesis, by reprogramming the CTCF binding status and altering chromatin architecture.	Qu et al. (2019) [34]
RAD18	p53 upregulation weakens the role of RAD18 in inhibiting apoptosis	Wu et al. (2019) [35]
SDH5	The loss of SDH5 enhances radiosensitivity by reducing p53 phosphorylation and delaying p53 degradation in lung cancer.	Zong et al. (2019) [39]
53BP1	53BP1 downregulation significantly reduced p53 and enhanced CHK1 and CHK2 expression in Eca-109 cells.	Yang et al. (2019) [47]
GRIM19	Overexpression of GRIM-19 diminishes the activity of MDM2, a specific p53 protease, resulting in the accumulation of p53 and activation of p53-mediated apoptosis.	Chen et al. (2018) [48]
APG-115	APG-115 radiosensitizes p53 wild-type gastric cancer cells, increasing apoptosis.	Yi et al. (2018) [49]
CDK16	CDK16 binds to and phosphorylates p53 at Ser315 site to inhibit the transcriptional activity of p53.	Xie et al. (2018) [40]

Sensitizing drugs and modulation of adverse effects of radiotherapy

Cell-cycle checkpoints play a critical role in cell survival after exposure to radiation.[51,52] Most types of cancer have defects in cell-cycle checkpoints, which contribute to the development of radioresistance.[53] Targeting cell-cycle checkpoint defects is the anticancer therapy of the future.[54-57] For example, it was reported that three human cholangiocarcinoma (CCA) cell lines with various cell-cycle defects differ markedly in their sensitivity to radiation. The different radiation sensitivities were associated with existing G1 or G2 checkpoint defects in analyzed cells.[55] CCA cells with a defective G1 checkpoint but an intact G2 checkpoint were the most radioresistant cells. In addition, inhibition of checkpoint kinase 1/2 (Chk1/2) selectively increased the radiation sensitivity of CCA cells with G1 checkpoint defect.

Cancer cells frequently contain G1 checkpoint defects due to loss of p53 function, resulting in radioresistance.[58] A study by Hematulin et al.[59] evaluated the radiosensitizing potential of etoposide, widely used as an antitumor chemotherapy drug,[60] in p53-defective CCA KKU-M055 and KKU-M214 cell lines with G1 checkpoint defects, which differ in G2 checkpoint status. KKU-M055 cells had an effective G2 checkpoint with marked accumulation of cells in the G2/M phase, together with induction of Chk2, Wee1 and Cdc2 phosphorylation after irradiation, and without the activation of the p53-p21 axis in response to radiation. In contrast, a defective G2 checkpoint was demonstrated in KKU-M214 cells, which failed to arrest the cell cycle in the G2/M phase after irradiation. The observed induction of p53 phosphorylation did not contribute to the induction of cell-cycle arrest in KKU-M214 cells in the G2/M phase. Treatment with etoposide increased the sensitivity of two p53-defective CCA cell lines to radiation, regardless of the function of the G2 checkpoint, and G2/M arrest was not the determining mechanism for the radiosensitization activity of etoposide. It was found that apoptosis was the dominant mode of death for KKU-M055 cells with an intact G2 checkpoint, while mitotic catastrophe was the dominant mode of death for KKU-M214 cells with a G2 checkpoint defect. Thus, etoposide can be used as a tumor radiosensitizer regardless of the functionality of the tumor's G2 checkpoints.

Histone deacetylase inhibitors (HDACi) are a group of agents that target histone deacetylase, which affects chromatin structure resulting in gene expression regulation. Radiosensitization by HDACi has been demonstrated in numerous preclinical and clinical studies.[61-64] Moreover, HDACi can also modulate cellular functions independent of gene expression by acting on non-histone protein deacetylation. Thus, HDACi are involved in regulating different altered pathways in cancer, such as apoptosis, cell cycle, and DNA repair.

Valproate (VPA) is an antiepileptic that, in addition to its anticonvulsant properties, is an effective HDACi. A study by Terranova-Barberio et al.[65] examined the combination of VPA with capecitabine metabolite 5′-deoxy-5-fluorouridine (5′-DFUR) in combination with radiotherapy on CRC cells: HCT-116 (p53-wild-type), HCT-116 p53−/−(p53-null), SW620 and HT29 (p53-mutant), which also made it possible to study the role of p53 in the combination setting. Combined treatment with equipotent doses of VPA and 5′-DFUR resulted in synergistic effects in CRC lines expressing p53 (wild-type or mutant). In HCT-116 p53-null−cells, antagonist effects were observed. Radiotherapy further potentiated the antiproliferative, pro-

apoptotic, and DNA damage effects induced by 5′-DFUR/VPA combination in p53 expressing cells.

As noted earlier, in human cancer, more than 50% of tumors contain a mutation or deletion of the *TP53* gene, which increases the likelihood of uncontrolled cell division,[4] since *TP53* plays a central role in mediating the response to DNA damage through the transactivation of numerous genes that inhibit growth or apoptosis, including *p21* gene.[66] Therefore, establishing the dependence of the activity of antitumor drugs on the cellular expression of p53 is of great importance. A study performed by Choo et al.[67] demonstrated the in vitro radiosensitizing effects of VPA on the human breast cancer MCF7 cell line and also revealed that VPA increased the level of DNA breakage, apoptosis, and senescence. VPA also induced tumor suppressor protein p53 and p21 expression and activated checkpoint kinase 2 (CHK2) in MCF7 cells. The treatment with VPA also increased p21 levels and CHK2 activity in p53-null colon cancer HCT116 cells, suggesting that VPA may be used to treat various types of cancer with altered p53 status. VPA-induced radiosensitization was largely dependent on the activity of CHK2. Thus, VPA may exhibit clinical utility concerning increasing the anticancer efficacy of radiotherapy by affecting the level of p53; in addition, the treatment with VPA and irradiation may enhance the radiosensitivity of p53-altered types of cancer.

Recent studies have shown that simvastatin, a 3-hydroxy-3-mehtylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor often used to treat lipid disorders, exhibits anticancer effects by regulating proliferation, apoptosis, and metastasis in various tumors,[68-71] and also enhances radiosensitization by suppressing BIRC5 (survivin) and CTGF (connective tissue growth factor) in gastric cancer and colorectal cancer.[72] Lee et al.[73] investigated whether the combination of simvastatin and IR would radiosensitize HCT116 p53+/+ and p53-/- colon cancer cells. Simvastatin potently stimulated radiosensitization of HCT116 p53-/- cells and xenograft tumors. The combination of simvastatin with IR decreased G2/M arrest and delayed the repair of IR-induced DNA damage, and no differences between the HCT116 p53+/+ and p53-/- cells were evident. Simvastatin also exhibited MDM2 suppression, regardless of p53 status, resulting in inducing radiosensitization. In addition, simvastatin caused accumulations of the FOXO3a, E-cadherin, and tumor suppressor protein p21, downstream factors of MDM2, in HCT116 p53-/- cells. Thus, these findings suggest the possibility of applying simvastatin as an MDM2 inhibitor and radiosensitizer for p53-deficient colorectal tumor treatments.

Nutlin-3, a small molecular weight cis-imidazoline analog, was designed to compete with Mdm2 for binding to p53.[74] Nutlin-3 induces the regulation and activation of the p53 pathway and is found to be effective and non-genotoxic in stabilizing p53 and enhancing apoptosis using experimental models in tumors expressing wild-type p53.[75]

Taste disturbance is one of the most common complications after radiation therapy, leading to decreased appetite and quality of life in patients with head and neck cancer. Faccion et al.[76] showed that checkpoint kinase 2 (Chk2) deficiency reduces p53 expression and inhibits cell apoptosis, partly contributing to the radioprotective effect on taste cells. In particular, Chk2 −/− mice showed less loss of type II and type III taste cells, lower expression of p53, caspase-3, and cleaved caspase-3, and lower levels of apoptosis. However, Chk2 deficiency did not alter oxidative stress levels, antioxidant capacity, and oxidative DNA damage in taste receptors. Chk2 appears to be a new target for correcting radiation-induced taste dysfunction.

Sensitizing drugs and modulation of adverse effects of radiotherapy are summarized in Table 2.

Table 2.

Sensitizing drugs and modulation of adverse effects of radiotherapy

Drug	Action	Source
Etoposide	Etoposide radiosensitizes p53-defective cholangiocarcinoma cell lines regardless of the functionality of the tumor's G2 checkpoint.	Hematulin et al. (2018) [59]
Valproate (VPA)	VPA is HDACi. Valproate up-regulates wild-type p53 and down-regulates mutp53 levels.	Terranova-Barberio et al. (2017) [65]
Simvastatin	Simvastatin exhibits MDM2 suppression, regardless of p53 status, inducing radio-sensitization	Lee et al. (2018) [73]
Nutlin-3	Nutlin-3 induces the regulation and activation of the p53 pathway and enhances apoptosis in tumors expressing wild-type p53.	Yee-Lin et al. (2018) [75]

Response predictive molecular markers

Various studies have shown that wild-type p53, but not mutant p53, can repress survivin expression at the transcriptional level[77] and that loss of survivin function partially mediates a p53-dependent apoptosis pathway.[78] Survivin is the smallest member of the apoptosis inhibitor protein family that plays a key role in regulating cell division and inhibiting apoptosis by blocking caspase activation. Overexpression of survivin in human lung cancer cells blocks p53-dependent apoptosis in a dose-dependent manner,[78] suggesting that survivin regulates (at least in part) the p53-dependent apoptosis pathway. Faccion et al.[76] found that high p53 expression levels and nuclear survivin localization correlated with the subtype of anaplastic astrocytoma, whereas cytoplasmic survivin localization correlated with the glioblastoma subtype. In addition, patients carrying tumors with a high cytoplasmic survivin expression, a high nuclear survivin expression, or a high p53 expression and who did not receive radiotherapy exhibited poorer short-term and long-term survival rates. Hence, patients whose tumors overexpress these proteins may benefit from radiotherapy, irrespective of age and/or histological classification.

Currently, there is no method to predict tumor response to chemoradiotherapy. Stojanovic-Rundic et al.[79] evaluated whether p21 and p53 expressions could be reliable predictors of pathological response to chemoradiotherapy in patients

with locally advanced rectal cancer. Tumor regression was assessed according to Dvorak (tumor regression grade [TRG] scores) and Wheeler (rectal cancer regression grade [RCRG] scores) classification systems. Locally advanced rectal cancer patients with immune expression of p21 had a significantly higher percentage of complete regression than patients with low expression of p21. In contrast, correlations between p53 expression and histopathological, as well as regression, grades were not found. Thus, the results suggested that p53 expression did not predict pathological response to preoperative chemoradiotherapy, but p21 expression did. In general, it can be assumed that the evaluation of several markers will identify a certain group of patients with a better response to radiotherapy.

It should be emphasized that the most crucial role of the p53 gene in radiosensitivity is quite apparent. Nevertheless, today it is necessary to conduct research to correct mutational changes in the gene, increase the sensitivity of cancer cells against the background of T53 activation in them, and radioprotection of healthy tissues against the background of p53 deactivation in them. Thus, the TP53 gene is an essential marker for determining cell fate before radiotherapy.

Cancer stem cells and radioresistance

Currently, there is an increased interest in investigating CSCs, which are the cause of neoplastic phenotypic and functional heterogeneity. CSCs are neoplastic cells with an indefinite potential for self-renewal and, therefore, oncogenic capacity. Recent investigations report that a fraction of these neoplastic cells are considered CSCs, which explains the continuous resistance to the treatment and tumoral recurrence.[80]

The oncogenic capability of CSCs (Image 2) is due to the accumulation of mutations throughout their life, indefinite proliferation, resistance to apoptosis, evasion of anti-growth signaling, expression of telomerase activity, immune destruction, and increased cell motility.[81] If CSCs survive after IR, they are able to cause tumor recurrence (Image 3). It is well known that CSCs mediate radiation resistance of tumors through tumor-specific factors such as the number of CSCs before treatment and repopulation or reoxygenation during fractionated radiotherapy. Recent clinical evidence suggests that stem cell-associated surface markers can be directly used as predictors of radiocurability of tumors with comparable risk factors such as histology and size.[82]

Mesenchymal stem cells (MSCs) have been investigated for use in treating cancers as they can both preferentially home in on tumors and become incorporated into their stroma. This process increases after radiation therapy. A study by de Araújo Farias et al.[83] showed that in vitro, MSCs, when activated with a low dose of radiation, were a source of antitumor cytokines that decrease the proliferative activity of tumor cells, producing a potent cytotoxic synergistic effect on them. In vivo administration of unirradiated mesenchymal cells together with radiation led to an increased efficacy of radiotherapy. The authors concluded that IR and MSCs have a synergistic effect when they are applied together for tumor treatment.

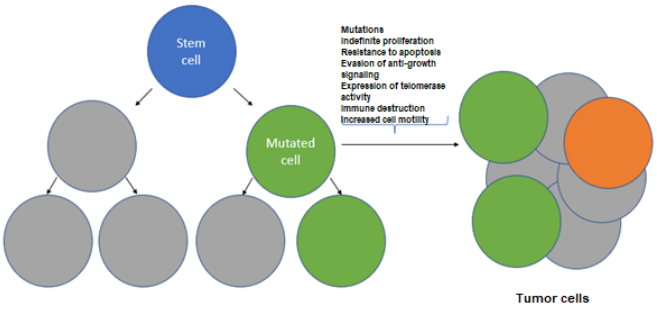

***Image 2**. Oncogenic capability of cancer stem cells (CSCs)*

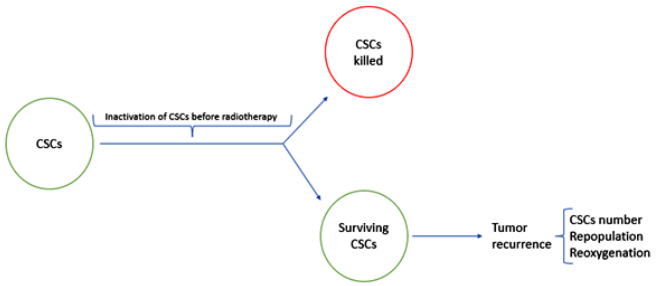

***Image 3.** CSCs surviving after IR and tumor recurrence.*

The NOTCH signaling pathway is critical in tissue development and is involved in malignant transformation. In preclinical lung cancer models, NOTCH inhibition has been shown to improve response to radiotherapy by targeting tumor stem cells. Giuranno et al.[84] showed that NOTCH signaling is active in both primary human models and in murine airway epithelial stem cell models, and, in combination with radiation inhibition of NOTCH, provokes a decrease in S phase and an increase in G1-phase arrest. NOTCH inhibition in irradiated lung basal stem cells resulted in more potent activation of DNA damage checkpoint kinases pATM and pCHK2 and led to an increase in the level of residual 53BP1 foci in irradiated lung basal stem cells, reducing their ability to self-renew.

Zamulaeva et al.[85] evaluated the prognostic significance of the proportion of CSCs in cervical scrapings from 38 patients with cervical cancer treatment and after irradiation at a total dose of 10 Gy. The results were assessed by the degree of tumor regression at 3-6 months after treatment. CSCs were identified as cells with the CD44+CD24low immunophenotype using flow cytometry. The proportion of CSCs in patients with complete tumor regression decreased by an average of 2.2±1.1% after irradiation, while in patients with partial regression, this indicator increased by an average of 3.3±2.3% (P=0.03). Multiple regression analysis revealed two independent indicators that affect tumor regression: the stage of the disease and the change in the proportion of CSCs after the first irradiation sessions. The proportion of CSCs before treatment had no prognostic value.

There is evidence that radiation-induced cells do not die immediately.[86] Contact of surviving tumor and non-tumor cells with the internal and external environment through

various signaling pathways and/or gene expression leads to the production of various chemokines, cytokines, growth factors, and protein hormones. The mechanism of how these close cellular interactions influence the tumor response to radiation is currently an important area of research. MSCs play an important role in non-tumor cells. Recent studies have shown that MSCs have an inhibitory effect on HCC, suggesting that they have potential as a novel therapeutic agent.[87,88] Adipose tissue-derived mesenchymal stem cells (AT-MSCs) are one of the most promising types of MSCs that can be easily obtained using minimally invasive procedures and can differentiate into numerous cell lines.[89] Wu et al.[90] showed that AT-MSCs can enhance the inhibitory effect of radiotherapy on reducing the growth, migration, and invasion of HCC cells in both in vitro and in vivo experiments. RNA-sequencing analysis revealed a noticeable interferon-induced transmembrane 1 (IFITM1)-induced tumor gene signature. Gain and loss mechanistic studies indicated that the mechanism was associated with decreased expression of signal transducer and transcription activator 3 (STAT3) and matrix metallopeptidases (MMPs) and increased expression of P53 and caspases. These data suggest that AT-MSCs can enhance the therapeutic effects of radiotherapy in HCC.

Compared to regularly proliferating cancer cells, CSCs have unique gene profiles and intracellular constitution, and also express specific membrane markers. Current therapeutic strategies targeting CSCs include CSC surface/intrinsic markers or signaling pathways, as well as CSC metabolism or the microenvironment, using antibodies, aptamers, peptide ligands, small molecules, or RNA-based therapeutics.[91-93] Among these strategies, therapeutic antibodies targeting CSC surface markers and small molecule inhibitors targeting CSC signaling pathways have already been investigated in clinical trials.

CD147 has been reported to be associated with CSC characteristics such as epithelial-mesenchymal transition (EMT) [94] and resistance to chemoradiotherapy.[95,96] Metuximab, the anti-CD147 drug, has been successfully used to prevent tumor recurrence after liver transplantation or radiofrequency ablation in patients with advanced HCC.[97,98] Fan et al.[99] demonstrated that anti-CD147 HAb18IgG sensitizes pancreatic cancer cells to chemoradiotherapy by reducing colony and sphere formation in a dose-dependent manner. In addition, HAb18IgG reduced the pancreatic CSC subpopulation and the expression of stem cell transcription factors OCT4, SOX2, and NANOG. Mechanically, HAb18IgG inhibited CSCs by blocking CD44s-pSTAT3 signaling. These results led the authors to suggest a promising therapeutic role for anti-CD147 HAb18IgG in suppressing pancreatic tumor initiation and overcoming relapses after chemoradiotherapy through direct targeting of CSCs.

A study by Konířová et al.[100] was focused on evaluating the response to IR of neural stem cells derived from mouse brains and grown in vitro. Under IR, neural stem cells expressed high mRNA levels of the stemness markers nestin and Sox2, and also showed high expression of Mki67 and Mcm2, markers associated with cell proliferation. The data obtained showed increased transcriptional activity of p53 targets, including Gadd45a, and proliferation arrest after irradiation. Moreover, most of the cells did not undergo apoptosis after irradiation, but stopped proliferation and started the differentiation program. The induction of differentiation and the demonstrated ability of irradiated cells to differentiate into neurons may represent a mechanism by which damaged neural stem cells circumvent the effects of cumulative DNA damage.

Dose-dependent radiation damage to intestinal stem cells (ISCs) is the main cause of radiation-induced gastrointestinal syndrome (RIGS). Self-renewal and proliferation of ISCs and thus maintenance of homeostasis and repair of the intestinal epithelium is primarily dependent on Wnt-β-catenin signaling.[101,102]

Bhanja et al.[103] demonstrated that a small molecular agent BCN057 (3-[(Furan-2-ylmethyl)-amino]-2-(7-methoxy-2-oxo-1,2-dihydro-quinolin-3-yl)-6-methyl-imidazo[1,2-a] pyridin-1-ium) activates canonical Wnt-β-catenin signaling, mitigates RIGS, and improves survival when applied 24 h after a lethal dose of radiation exposure. In an ex vivo crypt organoid model developed from human and mouse intestinal epithelium, BCN057 was shown to rescue ISC from radiation toxicity and induce epithelial repair with activation of Wnt-β-catenin signaling. However, BCN057 did not show any radioprotective effect in tumor tissue. Thus, BCN057 may be a potential emollient against RIGS and may be useful in abdominal radiotherapy.

Damage to heart, lung, and bone marrow (BM) tissues is one of the most important side effects of radiation therapy for breast cancer, which limits the success of tumor treatment.[104-106] Several studies have demonstrated the protective effects of radio-detoxified (gamma irradiation-fragmented) lipopolysaccharide (RD-LPS), also called tolerin,[107] in reducing radiation-induced tissue damage.[108] A study by Hegyesi et al.[109] examined the effects of RD-LPS in a model of cardiotoxicity. The authors focused on endothelial progenitor cells (EPCs) and BM cell-derived small extracellular vesicles (sEVs) as potential biomarkers of the effect of RD-LPS. The effects of local irradiation were studied in a model of cardiac injury in mice with chest irradiation. The researchers found increased mortality after irradiation at a dose of 16Gy. Treatment with RD-LPS significantly extended survival. Using flow cytometry, it was shown that with the introduction of RD-LPS, the number of BM-EPCs increased in the bone marrow and, in particular, in the bloodstream. In addition, mass spectrometric analysis showed that RD-LPS altered the proteomic composition of sEVs derived from BM cells. Treatment with RD-LPS increased the expression of interferon-induced transmembrane protein-3 (IFITM3) in BM cells and in BM cell-derived sEVs. In conclusion, it was noted that treatment with RD-LPS induced an increase in the number of circulating EPCs in parallel with a decrease in radiation-related mortality.

Evidence shows that tissue stem cells with accumulated DNA damage can lead to cancer.[110,111] IR-related DNA damage can be accumulated in the pools of tissue stem cells.[112-114] High-dose-rate radiation has been found to recruit Lgr5+ colonic stem cells, while low-dose-rate radiation does not.[115] Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) was first identified as a molecular marker on stem cells that could

develop into tumors as cells of origin in cancer.[116] The small and large intestines contain Lgr5+ stem cells in the bottom of crypts. ISCs expressing Lgr5 are cycling stem cells necessary for maintaining tissue in a steady state. Otsuka et al.[117] compared the effects of high-dose-rate (30 Gy/h) and low-dose-rate (0.003 Gy/h) radiation on the replenishment of colonic Lgr5+ stem cells. In Lgr5+ stem cells irradiated with high dose rates, pathways associated with DNA damage response, cell growth, cell differentiation, and cell death were found to be upregulated. In Lgr5+ stem cells irradiated with low dose rates, pathways associated with apical junctions and extracellular signaling were upregulated. High-dose-rate radiation-induced a considerable reduction in cell numbers in the colonic crypts and a dramatic increase in mitosis, which may stimulate the replenishment of the stem cell pool and the accumulation of genetic mutations in tissue stem cells.

CSCs exhibit a range of genetic and cellular adaptations that confer radioresistance. Among the mechanisms, one should take into account efficient DNA repair, the role of the CSC microenvironment and hypoxia,[118] and resistance to apoptosis via activation of the Akt pathway.[119] Cell-cycle phase also determines radiosensitivity, with cells most radiosensitive in the G2-M phase.[120] In addition, microRNAs are well known to play a critical role in the cellular response to IR.[121-125] Griñán-Lisón et al.[126]studied how IR affects the expression of miRNAs associated with stemness in various molecular subtypes of breast cancer (BC). Irradiation of BC cells at doses of 2, 4, or 6Gy affected their phenotype, functional characteristics, pluripotency gene expression, and oncogenic capacity in vivo. The effect of IR on the expression of eight miRNAs associated with stemness and radioresistance (miR-210, miR-10b, miR-182, miR-142, miR-221, miR-21, miR-93, miR-15b) varied depending on subpopulations of cell lines and clinical and pathological features of BC patients. The authors concluded that miRNAs related to BC stem cell subpopulations could provide a valuable method to predict and monitor tumor radio-response depending on the molecular BC subtype.

Malignant tumor cells, including laryngeal cancer cells, mainly obtain energy via the glycolysis of glucose, even under aerobic conditions. This aerobic glycolysis is called the Warburg effect,[127,128] and is believed to be involved in the development of cancer radioresistance.[128-130] Glucose transporter-1 (GLUT-1), localized on the cell membrane and acting as a channel protein for glucose uptake by cancer cells, is a key regulator of the Warburg effect.[128,131] GLUT-1 is expressed at high levels in radioresistant laryngeal cancer.[132-134] Inhibition of GLUT-1 using GLUT-1 small interfering RNA (siRNA) may enhance the radiosensitivity of laryngeal cancer cells.[135] Zhong et al.[136] created the CD133+-Hep-2R cell line and used in vitro and in vivo models of laryngeal cancer to test the radiosensitizing effect of GLUT-1 siRNA on CD133+-Hep-2R cells, exploring the cellular mechanisms underlying radiosensitivity enhancement, using RT-PCR, Western blotting, CCK-8 assay, colony formation assay, and Transwell assay in vitro and in a xenograft tumor model in nude mice. Transfection with GLUT-1 siRNA through inhibition of GLUT-1 expression led to inhibition of proliferation and invasive ability of CD133+-Hep-2R cells, which caused cell-cycle redistribution (a higher proportion of cells in the G0/G1 phase and a lower ratio in the S and G2/M phases). It was also found that suppressing RAD51 and DNA-PKcs expression increased the apoptosis rate and reduced DNA repair capability. Hence, GLUT-1 siRNA can enhance the radiosensitivity of CD133+-Hep-2R cells by inducing a redistribution of cell-cycle phases, inhibiting DNA repair capability, and increasing apoptosis.

Stem cells and the molecules they produce are summarized in Table 3.

Table 3.

Stem cells and the molecules they produce.

Stem cells	Molecules produced by stem cells	Source
MSCs	TRAIL and DKK3	de Araújo Farias et al. (2015) [83]
Basal airway stem cells	NOTCH	Giuranno et al. (2019) [84]
Cervical CSCs	CD44, CD24, and CD45	Zamulaeva et al. (2019) [85]
AT-MSCs	IFITM1	Wu et al. (2019) [90]
Pancreatic CSCs	CD147	Fan et al. (2019) [99]
Neural stem cells	Sox2, MKi67 and MCM2	Konířová et al. (2019) [100]
Intestinal stem cells	Wnt-β-catenin	Bhanja et al. (2019) [103]
Bone marrow-derived endothelial progenitor cells	IFITM3	Hegyesi et al. (2019) [109]
Intestinal stem cells	Lgr5	Otsuka et al. (2017) [117]

Cellular senescence, cancer and cellular radiosensitivity

Cellular senescence is an extremely stable form of cell-cycle arrest and constitutes a strong natural tumor suppressor mechanism. Senescent cells have rather heterogeneous phenotypes and can exhibit both antitumor and tumor-promoting features. Data on the role of cellular senescence in cancer and radioresistance are sometimes ambiguous and even contradictory. Studies published in the past decade have demonstrated that malignant and non-malignant cells with lastingly persistent senescence can acquire pro-tumorigenic properties in certain conditions.

B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1) is a polycomb group protein that regulates cell proliferation and is upregulated in various human cancer types, suggesting a potential role of Bmi-1 as an oncogene [137-140] that can induce anti-senescence in tumor cells. A study by Ye et al.[141] investigated the response of U87 glioma cells to radiation exposure and the role of Bmi-1 in the response following radiotherapy. It was found that X-ray radiation inhibits U87 cell proliferation by inducing senescence rather

than apoptosis. Following radiation exposure, the expression of Bmi-1 was upregulated, particularly when a dose of ≥6 Gy was administered. Bmi-1 may be significant in increasing the radioresistance of glioma cells by enabling cell senescence. Overexpression of Bmi-1 may reduce the expression of p16 and p19Arf, which induce anti-senescence in tumor cells.[142]

SHP-1, a cytosolic protein tyrosine phosphatase, can play either negative or positive roles in regulating signal transduction pathways and is differentially expressed in a number of cancer cell lines,[143-145] having different roles and mechanisms in regulating cell cycle and cell proliferation in different types of tumors. A study by Sun et al.[146] aimed to assess the role of SHP-1 in the radioresistance and senescence of nasopharyngeal carcinoma (NPC) cells. SHP-1 downregulation increased senescence, radiosensitivity, and a higher proportion of cells in G0/G1. Furthermore, SHP-1 overexpression resulted in radioresistance, inhibition of cellular senescence, and cell-cycle arrest in the S phase. Thus, SHP-1 had a critical role in radioresistance, cell- cycle progression, and senescence of NPC cells.

Although cellular senescence is a normal consequence of aging, there is increasing evidence showing that the radiation-induced senescence in both tumor and adjacent normal tissues contributes to tumor recurrence, metastasis, and resistance to therapy, while chronic senescent cells in the normal tissue and organ are a source of many late damaging effects. There is a growing body of evidence suggesting that senescence is associated with the disruption of the tissue microenvironment and the development of a pro-oncogenic environment.[147] Cellular senescence is characterized by irreversible cell-cycle arrest in response to various stress stimuli, resistance to apoptosis and senescent-associated secretory phenotype (SASP). SASP is a phenotype associated with senescent cells wherein those cells secrete a complex mixture containing hundreds of proteins, including pro-inflammatory cytokines/chemokines, immune modulators, tissue-damaging proteases, factors that can adversely affect stem and progenitor cell function, homeostatic factors, ceramides, bradykinins, and growth factors.[148-151] Cancer cells can be equally induced to cellular senescence through a variety of stress and damage signals, including irradiation. Senescent cells exhibit apoptosis resistance, metabolic activity, and secretion of pro-inflammatory and proliferative molecules. The effect of the SASP is highly dependent on context and cell type and is variable during the different stages of cancer progression.[152,153] Acute induction of cellular senescence is considered important for cancer prevention by stimulating the immune system to rapidly eliminate the genetically unstable cells, while chronic cellular senescence due to persistent stress signals (ROS, chronic inflammation) and the accumulation of dysfunctional senescent cells cannot be removed by immune cells; chronic cellular senescence creates a tumor-promoting environment through a secretion of SASP, including IL-1 alpha/beta, IL-6/8, MMPs, VEGF, TGF-beta, HFH, etc. Foregoing factors contribute to the increase in tumor radioresistance.[152,154]

IR is known to induce stress-induced, premature senescence (SIPS) in both normal and cancer cell types after exposure to relatively high doses (10 Gy) of radiation.[155-159] IR-induced senescence, apoptosis resistance, and EMT are three major mechanisms by which radioresistance develops. In a study by Yu et al.,[160] acute IR exposure induced cancer cell senescence and apoptosis, but after long-term IR exposure, cancer cells exhibited radioresistance. The proliferation of radioresistant cells was retarded, and most cells were arrested in G0/G1 phase. The radioresistant cells simultaneously showed resistance to further IR-induced apoptosis, premature senescence, and EMT. Acute IR exposure steadily elevated CDC6 protein levels, one of a group of proteins known as the pre-replication complex, an essential regulator of DNA replication. The ectopic overexpression of CDC6 leads to DNA hyper-replication, DNA damage, and genomic instability.[161] CDC6 overexpression has been detected in a number of cancer types, and high levels of CDC6 correlated with poor prognosis in cancer patients[161,162] and radioresistance in cancer cells.[163] CDC6 ectopic overexpression in CNE2 cells resulted in apoptosis resistance, G0/G1 cell-cycle arrest, premature senescence, and EMT, similar to the characteristics of radioresistant CNE2-R cells. Targeting CDC6 with siRNA promoted IR-induced senescence, sensitized cancer cells to IR-induced apoptosis, and reversed EMT. Furthermore, CDC6 depletion synergistically repressed the growth of CNE2-R xenografts when combined with IR. The authors concluded that CDC6 is a novel radioresistance switch regulating senescence, apoptosis, and EMT.

Genes and proteins involved in cellular senescence and radiation response are presented in Table 4.

Table 4.

Genes/proteins involved in cellular senescence and radiation response.

Gene/ Protein	Action	Source
CDC6	DNA hyper-replication, DNA damage, and genomic instability	Borlado et al. (2008) [161]
Bmi-1	Reduction of p16 and p19Arf expression	Bruggeman et al. (2007) [142]
SHP-1	Downregulated: increased cellular senescence and radiosensitivity Overexpression: decreased cellular senescence and radiosensitivity	Sunet al. (2015) [146]

Conclusion

Radiotherapy has been identified as the most common cancer treatment over the past decades. Unfortunately, the efficacy of this treatment modality is low in several malignancies due to the resistance of cancer to radiation. Multiple mechanisms, including cell-cycle checkpoint function, DNA repair, and cell death pathways, modulate the radio-responsiveness of cancer cells. Recently, increasing interest has focused on the role of p53 in the regulation of cellular growth induced by intense oncogenic signals or replicative stress.[164] Upon stimulation, p53 regulates the

expression of a large number of target genes involved in cell-cycle arrest, DNA repair, senescence, and apoptosis.[165] Numerous studies have shown that p53 plays a critical role in maintaining genomic integrity through its role in DNA damage response.[166] Loss of p53 function promotes (directly and indirectly) chromosomal instability, inducing cells to enter either senescence or apoptosis.[167] A deep understanding of the mechanisms by which p53 is implicated in regulating cellular senescence is of great interest for the development of new therapeutic strategies.

Currently, there is an increased interest in investigating CSCs, which are the cause of neoplastic phenotypic and functional heterogeneity. Emerging evidence suggests that CSCs exhibit a range of genetic and cellular adaptations that confer radioresistance and play a critical role in tumor initiation, malignant progression, disease relapse, and distant metastasis.

Cellular senescence is an extremely stable form of cell-cycle arrest and constitutes a strong natural tumor suppressor mechanism. Studies published in the past decade have demonstrated that, in certain conditions and contexts, malignant and non-malignant cells with lastingly persistent senescence can acquire pro-tumorigenic properties. Senescent cells may have a role in oncogenesis mainly through the SASP, which produces an immunosuppressive environment. A rising number of studies point out that spontaneous senescence and therapy-induced senescence play a substantial role in cancer aggressiveness. Ambiguous and even controversial data on the role of cellular senescence in cancer and radioresistance require further research.

Competing Interests

The author declares that there is no conflict of interest.

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