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REVIEW

Targeting IFN/STAT1 Pathway as a Promising Strategy to Overcome Radioresistance

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Abstract: The interferon (IFN)-mediated activation of the Janus kinase (JAK)-signal transducer and activator of transcription 1 (STAT1) signaling is crucial for cell sensitivity to ionizing radiation. Several preclinical studies have reported that the IFN/STAT1 pathway mediates radioresistance in the tumor microenvironment by shielding the immune responses and activating survival signaling pathways. This review focuses on the oncogenic function of the IFN/STAT1 pathway, emphasizing the major signaling pathway in radiation sensitization. Furthermore, it highlights the possibility of mediatory roles of the IFN/STAT1 pathway as a prognostic therapeutic target in the modulation of resistance to radiotherapy and chemotherapy. MicroRNA involved in the regulation of the IFN/STAT1 pathway is also discussed. A better understanding of radiation-induced IFN/STAT1 signaling will open new opportunities for the development of novel therapeutic strategies, as well as define new approaches to enhance radio-immunotherapy efficacy in the treatment of various types of cancers.

Keywords: interferon-gamma signaling, signal transducer and activator of transcription 1, tumor radioresistance, IFN/STAT1, microRNA

Introduction

Radiation therapy (RT) is a widely applied modern technique in cancer therapy, which directly induces DNA damage and indirectly removes cancer cells by inducing cytotoxic pathways.[1] Recently, the preclinical data suggest that the generation of anti-tumor immune response may also play an important role in the effectiveness of RT.[2] RT combined with immunotherapy shows a synergic potentiation to enhance systemic antitumor immunity.[3] However, the radioresistance of tumor cells, as well as the specific radioprotection of normal tissues, are the main challenges of RT and need to be addressed in treatments aimed at preventing tumor recurrence. A recent study reported intrinsic tumor cell radioresistance, genotoxic stress, and tumor microenvironment (TME) factors as the main obstructions of effective RT.[4]

Interferons (IFNs) are a group of pleiotropic cytokines in the tumor-suppressive network which have been extensively studied in the context of viral infections, antitumor immune responses,[5] and RT alone or with immunotherapy.[6] As the master transcription factor of IFN-related intracellular signaling, signal transducer and activator of transcriptions (STATs), has been widely explored in cancer progression. STAT1 and STAT2 are the first STAT proteins identified in the IFN signal transduction pathways that have an essential role in suppressing tumor cell proliferation, differentiation, apoptosis and angiogenesis. Similarly, in the STATs family, STAT3

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and STAT5 are the important players in the cancer development, whereas STAT1 is predominantly viewed as a tumor suppressor.[7,8] However, the bulk of evidence indicated that aberrant STAT1 activation triggers radioresistant properties in several cancers, including human head and neck squamous cell carcinoma,[9] myeloma,[10] renal cell carcinoma,[11] and breast cancer.[12] Moreover, the suppression of STAT1 expression reversed tumor radioresistance and malignant progression in renal cell carcinoma[11] and nasopharyngeal cancer.[13] In comparison with other STAT isoforms, STAT1 is the main transcription factor that acts as a critical control point of the phenomenon of therapeutic resistance to ionizing radiation (IR) in different types of cancer cells. Thus, understanding the mechanisms of the interaction between the IFN/STAT1 pathway and cancer cells could lead to the development of highly tailored radio-immunotherapy.

In this review, we summarized the current knowledge of radioresistance mediated by the IFN/STAT1 pathway, with a particular focus on the oncogenic function of STAT1 signaling and its interaction with tumor cells in the TME. Furthermore, this review aimed to explore the possible roles of the IFN/STAT1 pathway as a prognostic therapeutic target in radioresistance.

The Canonical IFN/STAT1 Signaling Pathway in Tumor Radioresistance

IFN/STAT1 pathway-induced inherent and/or acquired resistance to the cytotoxic effects of RT might impede the effectiveness of cancer treatment. A literature review showed that the IFN/STAT1 pathway in the TME has different functions and regulates several proto-oncogenes, such as inducible nitric oxide synthase (iNOS), JUN, FOS, and cyclooxygenase 2 (COX-2).[7,14,15] RT has been proposed to enhance innate and adaptive antitumor immunity, as well as increase the expression levels of IFNs in the TME. Figure 1 presents the RT-activated IFN/Janus kinase (JAK)/STAT1 pathway in detail. Specifically, Type I IFN (IFN-α and IFN-β) binds to interferon Type I receptors (IFNAR1 and IFNAR2), thereby activating the downstream signaling components, JAK1 and tyrosine kinase 2. These kinases phosphorylate STAT1 and STAT2 in the cytoplasm and assemble a heterotrimeric transcription factor complex known as the interferon-stimulated gene factor 3 (ISGF3) protein complexes. ISGF3 is an essential mediator of IFN signaling comprised interferon regulatory factor 9, STAT1, STAT2, and STAT3.[16] ISGF3 translocates into the nucleus to bind interferon-stimulated response elements and activate the expression of interferon-stimulated genes (ISGs) transcription.[17,18] Type II IFN (IFN-γ) binds to a specific receptor, the interferon Type II receptor (IFNGR), and then participates in phosphorylation-dependent activation of JAK1 and JAK2. The phosphorylated form of STAT1 is a homodimer able to translocate into the nucleus, where it activates the transcription of ISGs by binding to the interferon-γ-activated sequence.[17,19] As a result of Type I/II IFN signaling, ISGs represent a diverse group of genes involved in many cellular functions, such as transcription, apoptosis, and cell cycle regulation.[17]

IFNs coordinate tumor-immune system interactions by activating the downstream JAK/STAT1 pathway.[7] Any abrogation of Type I IFN signaling in cancer cells enhanced tumor response to IR.[20,21] In antigen-presenting cells, IFNs upregulate the major histocompatibility complex class I molecules in STAT1-dependent manner, which effectively promotes the presentation of tumor antigens, and is therefore essential for generating adaptive immune responses.[20] Besides, the IFN-γ secreted by T-cells can upregulate the expression of some angiogenic chemokine in tumor-associated macrophages (TAMs), such as CXCL9, CXCL10, and CXCL11.[22] However, a wide array of these ISGs is now considered to be tumor promoters and is used to determine the biological consequence of the IFN/STAT1 pathway. Emerging data indicate that Type I IFN can protect cancer cells from $CD8^+$ T-cell-mediated toxicity after RT by upregulation of the immune checkpoints and downregulation of anti-apoptotic and MHC class I molecules.[23,24] Recently, serpin family B member 9 (SERPINB9) transcription factor is introduced as a key mediator of enhanced susceptibility to T cell-mediated killing after radiation.[21] Activation of Type I IFN signaling and induction of SERPINB9 protects cancer cells from $CD8^+$ T cell-mediated cytotoxicity in a dose-dependent fashion indicating that IFN signaling has a paradoxical effect on tumor control after RT.[21] In the TME of radioresistant cells, most STAT1 is phosphorylated and initiates multiple gene expression. These genes mediate diverse cellular functions, such as immune modulation, the regulation of cell proliferation, and cell apoptosis control. Interestingly, STAT1 has a dual function in the TME of radioresistant cells; it can promote the phenotype of radioresistant cancer cells and tumor metastasis. Previous study indicated that STAT1 downregulates the expression of the p53 inhibitor murine double minute 2 (MDM2) oncogene and acts as p53 co-activator.[25] MDM2 is the negative

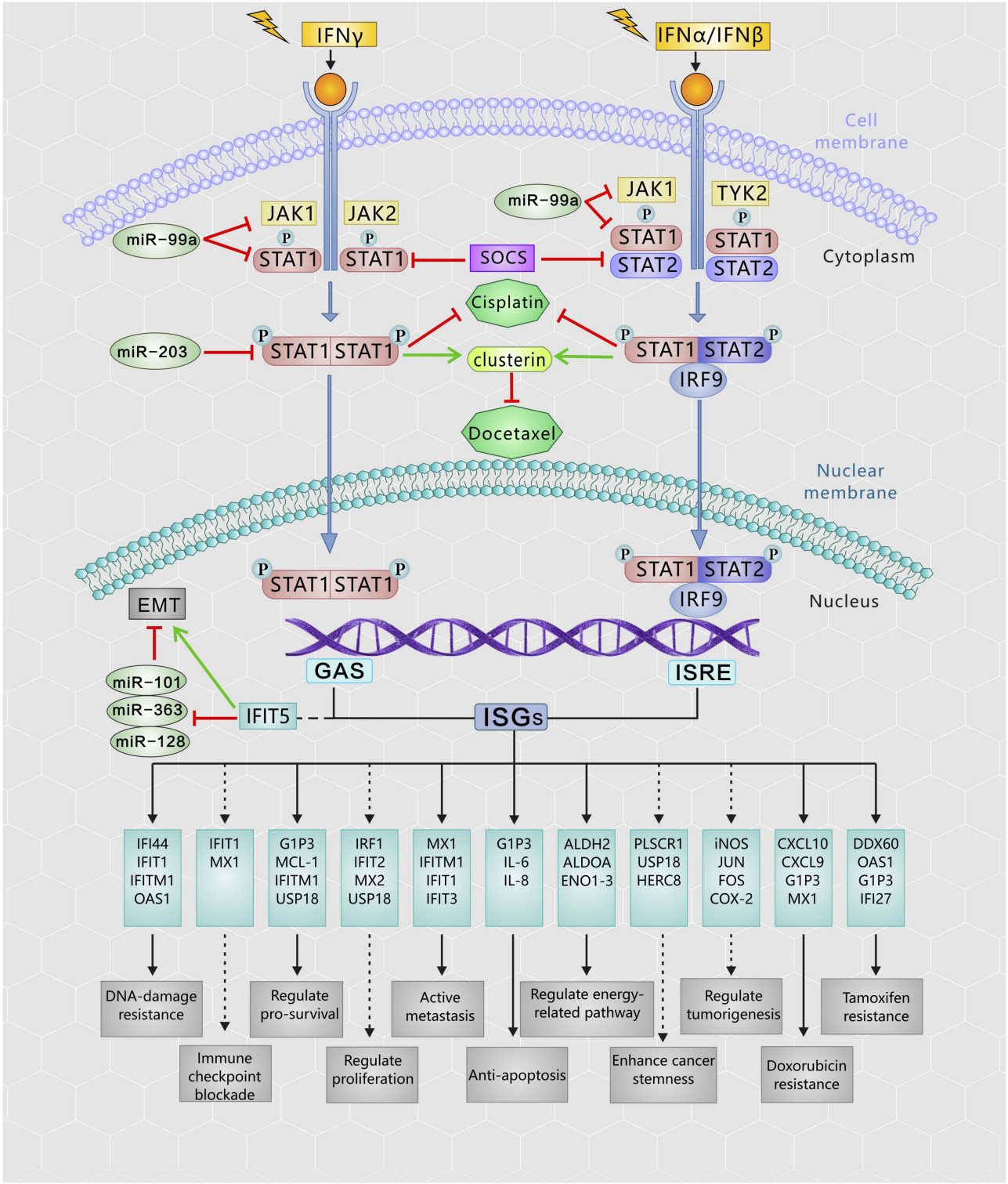

Figure 1 The RT activates the IFNs-JAK/STAT1 pathway in cancer cells. After exposure to the IR, Type I and II IFN bind to their respective receptors to induce a number of signal transduction cascades, then the JAK kinase and the STAT1 being recruited to the receptor and phosphorylated in cytoplasm. The phosphorylated STAT1 triggers the heterodimers (ISGF3) or homodimers, and migrate to the nucleus. These complex bind to ISRE or interferon-gamma-activated sequence (GAS) gene promoter elements, respectively drives the downstream IFN-regulated genes expression and perform cellular functions, which are associated with radioresistance. Phosphorylated STAT1 also contributes to develop cisplatin resistance, as well as upregulate clustering in docetaxel-resistant cells. The miRNAs and SOCS proteins functions as the major regulators to control of the IFNs/STAT1 pathway.

Abbreviations: EMT, epithelial-to-mesenchymal transition; GAS, interferon-gamma-activated sequence; IR, ionizing radiation; IRF9, interferon regulatory factor 9; ISGF3, interferon-stimulated gene factor 3; ISRE, interferon-stimulated response element; IFN, interferon; JAK, Janus kinase; miRNA, microRNA; RT, radiation therapy; SOCS, suppressor of cytokine signaling; STAT1, signal transducer and activation of transcription 1.

regulator of p53 tumor suppressor gene, that p53-MDM2 pathway represents well-known targets for RT.[26] Contrarily, it can also promote apoptosis of cancer cells.[27] The "Friend" or "Foe" function of STAT1 signaling in the IFN/STAT1 pathway is illustrated in Figure 2. The STAT1 signaling induced by IR does not play a "Foe" role. Thus, STAT1 may directly participate in DNA damage repair.

Extrinsic Determinants in Tumor Radioresistance

Regulation of Immunosuppressive Factors

Recent studies have highlighted that sustained IFNs expression may induce many inhibitory factors that stimulate tumor growth in the STAT1-dependent pathway, especially by triggering programmed death-ligand 1 (PD-L1).[28,29] PD-L1 is a co-signaling molecule expressed on tumor cells or other infiltrating immune cells, which can initiate T-cells apoptosis with the engagement of programmed death 1 expressed on cytotoxic T-lymphocytes.[30] PD-L1 expressed in several human hematopoietic tumor cell lines also confers resistance to natural killer cell-mediated lysis. In IFN-γ-induced JAK/STAT1 signaling pathway, the silencing of JAK1 and JAK2 was shown to reverse the resistant phenotype of tumor cells.[31] Thus, these modifications in the TME balance of tumor cells can rapidly promote resistance to RT. It is clear that combination therapy of RT with anti-PD-L1 is one of the modern treatment methods to overcome resistance to checkpoint inhibition in RT, which may lead to the downregulation of genes involved in antitumor responses, T-cell exhaustion, antigen presentation, and chemokine production in TME.[21,32] For example, the upregulation of PD-L1 in stage II melanoma was determined to be a mechanism involved in the resistance to RT.[32] In a PD-L1-dependent manner, this resistance programming can be orchestrated by Type II IFN/STAT1 signaling due to the activation of ISGs, as interferon-induced protein with tetratricopeptide repeats (IFITs) and interferon-induced GTP-binding protein (MX1) families.[32] IFNGR and IFNAR knockout experiments

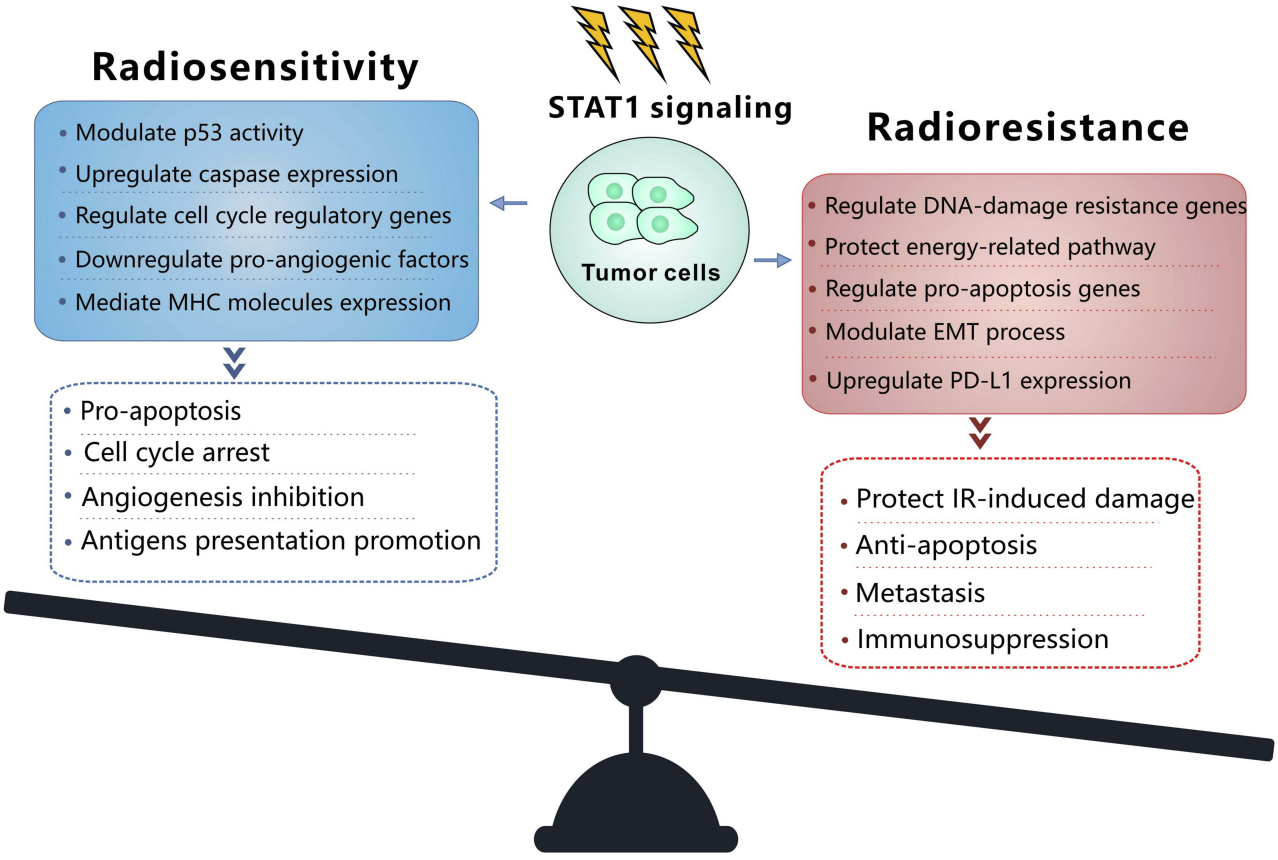

Figure 2 Model of balance between the mechanism and the cellular function of the STAT1 signaling to promote radiosensitivity and radioresistance. In the radioresistant cancer cells, IR activates STAT1 signaling and trigger the function of radioresistance or radiosensitivity.

Abbreviations: EMT, epithelial-to-mesenchymal transition; IR, ionizing radiation; MHC, major histocompatibility complex; PD-L1, programmed death ligand-1; STAT1, signal transducer and activation of transcription 1.

showed that IFNs were basic for melanoma cells to maintain both PD-L1-dependent and PD-L1-independent resistance.[32] In both tumor cells and stromal cells, IFN/STAT1 pathway stimulated the indoleamine-2,3-dioxygenase (IDO), resulting in progressive immune dysfunction and tumorigenesis. In TME, elevated IDO activity may lead to an impaired T-cells activation. For example, human dendritic cells can mediate IDO-dependent inhibition of T-cells proliferation in the model of mixed leukocyte reaction.[33] Moreover, in vitro study demonstrated that IDO-transfected fibrosarcoma cells suppressed T-cell proliferation.[34] Blocking of IDO expression may improve the effectiveness of antitumor therapy, such as RT.[35] Therefore, the results of these studies clearly show that the IFN/STAT1 pathway promotes the development of radioresistance in cancer cells by inducing immunosuppressive factors in the TME.

Regulation of Immunosuppressive Cells

The presence of radioresistant immunosuppressive cells in TME such as myeloid-derived suppressor cells (MDSCs), regulatory T-cells (Tregs), and TAMs with the M2 phenotype are also the reduced the efficiency of the effective RT.[4,36] Through suppressing the adaptive T-cell-mediated antitumor immunity, these cells can promote tumors to immune tolerance.[37] Multiple studies have noted that IFN/STAT1 pathway plays a role in the recruitment and activation of immune inhibitory cells. In this view, IFNs are critical for the formation of a tumor radioresistant environment. The MDSCs in breast cancer are a well-evaluated example of Type II IFN/STAT1 pathway's role that active the immunosuppressive cells and enhance the suppressive function of MDSCs by inducing the expression of pro-inflammatory tumor necrosis factor α, transforming growth factor β, and interleukin-13, STAT1 signaling can recruit the MDSCs in inflamed TME.[38] Furthermore, Type II IFN was shown to induce a higher level of iNOS, arginase I, NO production, and arginase activity in both MDSCs and the TAM, which are a response to immune inhibition and T-cell deletion in several cancer models.[39] Similarly, in vivo study shows that Tregs with STAT1 deficiency failed to control allograft rejection.[40] In a mice model, the absence of certain STAT1 transcription factors reduced the frequency of MC38 tumor-associated interleukin (IL)-10^{+} Tregs in the colon carcinoma cells.[41] Furthermore, IDO expression in melanoma cells enhanced the recruitment of Tregs to evade immune recognition.[42] Overall, the ability of the IFN/STAT1 pathway to regulate T-cell function displays a high environmental dependence and variation among tumor types and factors. However, the study findings presented indicate that the IFN/STAT1 pathway is a potential target to improve the effect of cancer RT by recruiting immune inhibitory cells and inhibiting effector T-cells. The regulation of Tregs by the IFN/STAT1 pathway is controversial, however, and more research is needed to elucidate the role of Tregs function in the TME of radioresistant cells.

Enhancement of Radioresistance in Cancer Stem Cells

Cancer stem cells (CSCs), with strong self-renewal capability, have been demonstrated to contribute to tumorigenesis, therapy resistance, and also metastasis, which could result in RT failure.[43] Recently, studies have indicated a novel function of STAT1 associated with CSCs. The upregulation of active STAT1 and many ISGs, such as PLSCR1, USP18, and HERC8, is involved in the resistance of CSCs to RT.[44,45] A detailed overview of the ISGs associated with tumor radioresistance in the IFN/STAT1 pathway is illustrated in Figure 1 and Table 1. These ISGs, together with STAT1, activate the canonical Type I IFN signaling pathway in cooperation with CD95 in the stemness of CSCs.[44] The knockdown of STAT1 is considered one of the best treatment methods for radioresistant cancer cells, which is achieved by reducing the non-apoptotic activities of CD95.[44] In addition, higher apoptosis rates were observed in irradiated $CD24^{-/low}/CD44^{+}$ breast CSCs that treated with STAT1 inhibitor. These cells show that STAT1 inhibited irradiation-induced apoptosis in cancer-initiating cells, resulting in resistance of the breast cancer cells to RT.[45] After 4 Gy irradiation, the radioresistant glioma stem cells expressed the highly unregulated STAT1 with some DNA metabolism-related genes.[46] Based on these findings, one potential link between STAT1 and radioresistance is presumably through the increased stemness of CSCs, which is mediated by STAT1-activated genes. Targeting the Type I IFN/STAT1 pathway in CSCs may have a broad therapeutic application to rescue cancer radioresistance.

Intrinsic Determinants in Tumor Radioresistance

Many in vivo and in vitro studies have demonstrated the critical roles of STAT1 in the induction of cell apoptosis.[9–12] The "Friend" role of STAT1 in triggering apoptosis is modulated transcriptionally by anti-proliferation and pro-apoptotic signaling. Caspases, FAS, and TRAIL are the most well-known examples of genes controlled by STAT1 in these types of

Table 1 STAT1-Activated Genes in Tumor Radioresistance

Name	Radiotherapy	Total Dose (Gy)	Function	Drug Resistance	Cancer	References
IFNStimulated Genes						
CXCL10	γ-RAD	4 or 8	Antigen presentation	DOXO	M	[10]
IFI27	γ-RAD	4 or 8	Apoptosis regulation	DOXO	M	[10]
	X-ray	64		TCT	BC	[67]
IFI44	X-ray	40	Proliferative regulation	DOXO	SCC	[12]
IFI44L	γ-RAD	4 or 8	Tumor cell survival promotion	DOXO	M	[10]
IFI6	γ-RAD	4 or 8	Apoptosis regulation	DOXO	M	[10]
	X-ray	64		TCT	BC	[67]
IFIT1	γ-RAD	4 or 8	Anti-viral response	DOXO	M	[10]
	X-ray	20			SCC	[82]
	X-ray	20			MM	[83]
IFIT3	X-ray	20	Anti-viral response	DOXO	SCC	[82]
					MM	[83]
IFITM1	X-ray	40	Anti-viral response	DOXO	SCC	[12]
		20			SCC	[82]
		20			MM	[83]
IRF7	γ-RAD	4 or 8	Transcriptional activation	DOXO	M	[10]
ISG15	X-ray	40	Protein degradation	DOXO	SCC	[12]
MCL-1	X-ray	40	Apoptosis regulation	DOXO	SCC	[12]
MX1	γ-RAD	4 or 8	Anti-viral response	DOXO	M	[10]
	X-ray	40			SCC	[12]
		20			SCC	[82]
		20			MM	[83]
OAS1	X-ray	64	Anti-viral response	TCT	BC	[67]
OASL	X-ray	20	Anti-viral response	DOXO	SCC	[82]
USP18	X-ray	40	Protein modification	DOXO	SCC	[12]
Tumor-Associated Genes						
JUN	γ-RAD	64	Proliferation regulation	–	NPC	[84]
FOS	γ-RAD	64	Proliferation regulation	–	NPC	[84]
Glycolysis Pathway-Related Genes						
ALDH1A1	X-ray	30	Oxidation of HAc to acetate	–	SCC	[62]
ALDH2	X-ray	30	Oxidation of HAc to acetate	–	SCC	[62]
ALDOA	X-ray	30	Oxidation of GAPD	–	SCC	[62]
ENO1	X-ray	30	Dehydration of PCK	–	SCC	[62]
ENO2	X-ray	30	Dehydration o of PCK	–	SCC	[62]
ENO3	X-ray	30	Dehydration of PCK	–	SCC	[62]
GAPDH	X-ray	30	Oxidation of GAPDH	–	SCC	[62]
GPI	X-ray	30	Conversion of GPI	–	SCC	[62]
LDHA	X-ray	30	Reduction of pyr to lactate	–	SCC	[62]
LDHAL6B	X-ray	30	Reduction of pyr to lactate	–	SCC	[62]
LDHB	X-ray	30	Reduction of pyr to lactate	–	SCC	[62]
PGAM1	X-ray	30	Phosphoryl transferring	–	SCC	[62]
PGK1	X-ray	30	Phosphoryl transferring	–	SCC	[62]
PGM1	X-ray	30	Interconversion of G1P and G6PD	–	SCC	[62]
PKM2	X-ray	30	Phosphoryl transferring	–	SCC	[62]
TPI1	X-ray	30	Interconversion of DHAP	–	SCC	[62]

(*Continued*)

Table 1 (Continued).

Name	Radiotherapy	Total Dose (Gy)	Function	Drug Resistance	Cancer	References
Oxidative Phosphorylation-Related Genes						
ATP5A1	X-ray	30	Synthesis of ATP from ADP	–	SCC	[62]
ATP5O	X-ray	30	Synthesis of ATP from ADP	–	SCC	[62]
ATP5B	X-ray	30	Synthesis of ATP from ADP	–	SCC	[62]
COX4I1	X-ray	30	Oxidation of cyc	–	SCC	[62]
SDHA	X-ray	30	Oxidation of NADH	–	SCC	[62]
MDH2	X-ray	30	Oxidation of MDH	–	SCC	[62]

Abbreviations: ALDH1A1, aldehyde dehydrogenase 1 family member A1; ALDH2, aldehyde dehydrogenase 2; ALDOA, fructose-bisphosphate aldolase A; ATP5A1, ATP synthase, H+ transporting, mitochondrial F1complex, alpha subunit 1, cardiac muscle; ATP5PO, ATP Synthase Peripheral Stalk Subunit OSCP; ATP5B, ATP synthase subunit beta; BC, breast cancer; COX4I1, cytochrome c oxidase subunit IV isoform 1; CXCL10, chemokine ligand 10; DOXO, doxorubicin; ENO1, enolase-1; ENO2, enolase-2; ENO3, enolase-3; FOS, FOS proto-oncogene, AP-1 transcription factor subunit; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPI, glucose phosphate isomerase; Gy, gray; IFNs, interferons; IFI27, interferon alpha inducible protein 27; IFI44, interferon-induced protein 44; IFI44L, interferon-induced protein 44 like; IIFI6, interferon alpha inducible protein 6; IFIT1, interferon-induced protein with tetratricopeptide repeats 1; IFIT3, interferon-induced protein with tetratricopeptide repeats 3; IFITM1, interferon-induced transmembrane protein 1; IRF7, interferon regulatory factor 7; ISG15, interferon-stimulated gene product 15; JUN, Jun proto-oncogene, AP-1 transcription factor subunit; LDHA, lactate dehydrogenase A; LDHAL6B, lactate dehydrogenase A-like 6B; LDHB, lactate dehydrogenase B; M, myeloma; MCL-1, myeloid cell leukemia-1; MDH2, malate dehydrogenase 2; MM, metastatic melanoma; MX1, MX dynamin like GTPase 1; NPC, nasopharyngeal carcinoma; PGAM1, phosphoglycerate mutase 1; PGK1, phosphoglycerate kinase 1; OAS1, 2′-5′-Oligoadenylate synthetase 1; OASL, 2′-5′-Oligoadenylate synthetase like; PGM1, phosphoglucomutase 1; PKM2, pyruvate kinase muscle isozyme M2; γ-RAD, gamma radiation; SCC, squamous cell carcinoma; SDHA, succinate dehydrogenase complex, subunit A; TCT, tamoxifen; TPI1, triose phosphate isomerase 1; USP18, ubiquitin-specific peptidase 18.

signaling.[27] However, as mentioned above, high levels of constitutively active STAT1 are indispensable to support cell survival in several different types of cancer after RT. Table 1 presents a wide spectrum of multifunctional genes upregulated by STAT1 in different radioresistant tumor cells. Most of these radioresistance-associated genes are involved in anti-viral responses, DNA damage-induced apoptosis, and regulation of proliferation in the resistance to both X- and γ-radiation therapies. The activation of STAT1 by radiation may lead to tumor adaptation by directly affecting cellular signaling of the tumor cells.[17] The cellular signaling triggered by IR occurs at two distinct sites: the nucleus and the cytoplasm. Intrinsic tumor radioresistance depends on the balance between DNA damage and DNA repair in the nucleus following irradiation.[47] However, cytoplasmic signaling events, such as proliferation, anti-apoptosis, and pro-survival reaction, are equally important as DNA-related activities in the nucleus of radioresistant tumors. Therefore, the nuclear and cytoplasmic signaling pathways triggered by IR are described separately in this article in more detail.

Nuclear Events: The Balance Between DNA Damage Resistance and Apoptosis

IR leads to double-strand DNA breaks. Thus, protecting DNA from radiation-induced damage is one of the most important mechanisms tumor cells employ to develop RT resistance. Emerging evidence has shown that the constitutive expression of STAT1 and ISGs was associated with tumor DNA-damage resistance. In this regard, previous study reported that IFI27, IFI35, BST2, MX1, ISG15, and THBS1 were highly expressed in small cell lung cancer cells. These ISGs activated resistance to epigenetic modulators and other DNA damaging agents.[48] Furthermore, there is a set of STAT1-induced genes, termed interferon-related DNA damage resistance signature (IRDS) genes, including IFI44, IFIT1, IFITM1, OAS1, ISG15, and MX1.[12] These IRDS genes were derived from a primary breast cancer gene-expression profile that was more broadly associated with resistance to X-ray-induced DNA damage and doxorubicin.[12] It should be noted that IRDS (+) breast cancer patients have a high rate of local-regional failure after adjuvant RT, which highlights the significance of IRDS in predicting recurrence after RT.[12] The overexpression of the IRDS gene in glioblastoma multiforme (GBM) patients is related to poor survival outcome and RT response.[49] Therefore, the balance of the STAT1-dependent genes involved in resistance to DNA damage is a critical determinant for the biological function of the IFN/STAT1 pathway in radioresistant cancer cells (the "Foe" role of STAT1). The purpose of these ISGs is mediated by the following factors: i) Different TME: the strong predictive value of IFI44, OAS1, ISG15, and MX1 in GBM patients was highly subtype-dependent and that the expression level of these ISGs was different in vitro and in vivo.[49,50] ii) Manner of RT delivery: in breast cancer, prostate cancer, and gliosarcoma patients treated with multi-fraction

radiation treatment (5 × 2 Gy) and a single-dose radiation treatment had different expression levels of both STAT1 and ISGs.[50] iii) Modifications of STAT1: Numerous epigenetics and protein modifications can alter the transcriptional activity of STAT1 and potentially change the pattern of IFN/STAT1 pathway genes.[17] Several studies have reported that the unphosphorylated STAT1 prolong the expression of ISGs, such as DDX60, G1P2, IFI27, IFI44, IFIT1, OAS1, OAS3, and PLSCR1. Most of their ISGs are upregulated in DNA damage-resistant cancer cells.[51] In the contrary, acetylation of STAT1 counteracts IFN-induced STAT1 phosphorylation, nuclear translocation, and DNA binding.[52] Moreover, acetylation of STAT1 also confers susceptibility to apoptosis by mediating p65-binding and suppressing anti-apoptotic NF-kB target genes in melanoma cells.[53] Liu and colleagues demonstrate that Type 1 IFN signaling stimulates the translocation of USP12 from cytoplasm to the nucleus, that blocks the acetylation effect of CREB-binding protein (CBP) in nucleus.[54] Therefore, in radioresistant cancer cells, aberrant IFNs signaling reduces the acetylation of STAT1 and inhibits the dephosphorylation of STAT1, leading to the high expression of STAT1.[53,54] Consequently, balance between dynamic phosphorylation and acetylation of IFN-dependent STAT1 signaling regulates cancer response to RT. The interactions of STAT1 and ISGs' roles in radiation-induced cell death are still unknown in various cancers. Hence, further research is needed for better understanding of the delicate regulation of STAT1-mediated acetylation and IFN signaling to overcome radioresistance in cancer.

Cytoplasmic Events: Pro-Survival Signaling

In contrast to nuclear signaling, radiation-induced cytoplasmic signaling events are much more likely to prompt a pro-survival response in radioresistant cancer cells.[47] The efficacy of STAT1 to cause cell death and growth arrest depends on the transcriptional activation of pro-apoptotic genes and the inhibition of anti-apoptotic genes.-[27] In STAT1-overexpressing nu61 cancer cells, an impaired response to RT was observed.[55] The expression of growth factor IL-6 and IL-8 in nu61 cells was higher relative to radiosensitive cells and knockdown of STAT1 led to the downregulation of these cytokines.[55] Thus, a high level of STAT1 may be more likely to perform anti-apoptotic functions in radioresistant tumors (Figure 2). One hypothesis for this phenomenon is that under certain conditions, an RT-activated IFN/STAT1 pathway with pro-survival activity might antagonize apoptotic activity. For example, two IFN-induced genes, G1P3 with pro-survival activity and TRAIL with pro-apoptotic activity, antagonize each other in human myeloma cells.[56] The other important pro-survival genes regulated by STAT1 signaling MCL-1,[57] IFITM1,[58] and USP18.[59] Moreover, the dual function of STAT1 signaling depends on the cellular mechanisms, such as enzymes activation, glycolysis, oxidative phosphorylation, and mucin 1 oncoprotein, which is mainly expressed in human breast cancers, cooperatively worked with STAT1, indicating that STAT1-dependent genes in the context of STAT1 could predict a poor prognosis in breast cancers.[60] In colon cancer cells, the cancer-upregulated gene 2 increased STAT1 activity to enhance cancer cell survival and migration.[61] Likewise, in human head and neck squamous cell carcinoma cells, the active form of STAT1 was reported as a main control point in the X-ray suppression of the energy-related pathway.[62] Briefly, STAT1, by upregulating many metabolism-associated genes, is involved in tumor glycolysis, oxidative phosphorylation, and the citrate cycle pathway (Table 1).[62] As a result, the constitutive expression of STAT1 and STAT1-dependent genes may promote a switch from blocking the cytotoxic response to pro-tumor survival in radioresistant cancer cells.

MicroRNA: A Potential Radioresistance Regulator

MicroRNAs (miRNAs) are a class of small non-coding RNA approximately 22 nucleotides in length, known as critical post-transcriptional regulators, which regulate gene expression by binding to 3′ untranslated regions of the target mRNA.[63] Recently, mechanistic studies from a range of tumor-bearing mouse models demonstrated some miRNAs involved in the STAT1-activated cellular process that were associated with the IR response by targeting the IFN/STAT1 signaling pathway. The miRNAs in Table 2 act as tumor-promoter and tumor-suppressor miRNAs by modulating the oncogenic function of STAT1 in different tumor contexts. Consequently, it shows the complexity of the IFN/STAT1-miRNAs regulatory network in cancer cells to stimulate the STAT1 positive feedback loop and STAT1-dependent gene expression. Remarkably, the balance between IFN/STAT1 and tumor-promoting miRNAs (oncomiRNAs) promotes the epithelial-to-mesenchymal transition process, increasing radioresistance and perhaps, distant metastasis of tumors.[64] Besides that, the feedback

Table 2 MicroRNA Regulate the IFNs/STAT1 Signaling Pathway in Cancer

Name	Function	Role on INFs/JAK/STAT1	Cancer	References
Tumor Suppressor miRNAs				
miR-15A miR-16-1	Inhibit immunosuppressive B cells accumulation and repress of tumor growth	Inhibit activation of STAT1	CRC	[85]
miR-99a	Induce apoptosis and inhibit proliferation	Downregulate the phosphorylation levels of STAT1	Rb	[65]
miR-203	Inhibit cell proliferation and migration and increase sensitivity to apoptosis	Inhibit activation of STAT1	GBM	[66]
miR-375	Inhibit PD-L1 expression	Inhibit JAK2/STAT1 signaling	HNSCC	[86]
miR-944	Inhibit tumor cell growth and proliferation	Downregulate expression of STAT1	LAC	[87]
oncomiRNAs*				
miR-9	Promotes endothelial cell migration and angiogenesis	Activate oncogenic function of STAT1	NSCLC, M, PC, GBM, CRC	[88]
miR-19b miR-30b	Negative regulation of SOCS1 and SOCS3	Regulate constant overexpression of STAT1	MPM	[89]
miR-30c miR-2909	Contributes to cell growth	Activate oncogenic function of STAT1	PCa	[90]
miR-101 miR-128 miR-363	Interaction with IFIT5 and reduce cancer metastasis	Regulate IFN-γ-JAK/STAT1 induced EMT	PCa	[64]
miR-221 miR-222	Regulate IRDS expression contribute to radioresistance	Regulate STAT1 tyrosine phosphorylation	Glioma	[17, 91]

Note: *Micro-RNAs are involved in tumorigenesis.

Abbreviations: CRC, colorectal cancer; EMT, epithelial-to-mesenchymal transition; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; JAK, Janus kinase; IFIT5, interferon-induced tetratricopeptide repeat 5; IFN, interferon; IFN-γ, interferon-γ; IRDS, interferon-related DNA damage resistance signature; LAC, lung adenocarcinoma; LLC1, Lewis lung carcinoma; M, myeloma; miR; micro-RNA; MPM, malignant pleural mesothelioma; NSCLC, non-small cell lung cancer; PC, pancreatic cancer; PCa, prostate cancer; PD-L1, programmed-death ligand 1; RB, retinoblastoma; RCC, renal cell carcinoma; STAT1, signal transducer and activator of transcription 1; SOCS1, suppressor of cytokine signaling 1; SOCS3, suppressor of cytokine signaling 3; SOCS5, suppressor of cytokine signaling 5.

relationship between STAT1-targeted miRNAs, such as miR-99a,[65] miR-203[66] and STAT1-dependent genes, is likely to regulate STAT1 signaling at the TME level (Figure 1). Thus, blocking oncomiRNAs or activating tumor suppressor miRNAs may be effective ways to improve tumor radiosensitivity (Table 2).

IFN/STAT1 Signaling Pathway in Drug Resistance

Similar to that observed in RT, chronic exposure to both Type I and Type II IFNs may also lead to the STAT1-mediated mechanism of drug resistance. A correlation between STAT1 expression and cisplatin resistance in human ovarian cancer cells has been reported in many in vivo and in vitro studies. As shown in Table 1, cisplatin-, tamoxifen- or doxorubicin-resistant cancer cells demonstrated the increased expression of different ISGs, including DDX60, OAS1, G1P3, and IFI27.[67] The STAT1-dependent association with histone deacetylase (HDAC)-4 axis enhances the acquired platinum resistance in ovarian cancer cells, and also has been linked to the failure of etoposide in lung cancer cells.[68,69] Also, in a prostate cancer cell line, DU145-DR, STAT1 and its subsequent regulation of clustering genes, was essential for docetaxel resistance.[70] Moreover, prolonged STAT1 signaling can mediate tumor cell resistance to both RT and chemotherapy. As mentioned above, cancers with high expressions of IRDS genes are cross-resistant to doxorubicin and RT (Figure 1).[12] Intriguingly, combination therapy of Type II IFN with doxorubicin in MDA-MB 435 breast cancer cells activated STAT1 and enhanced doxorubicin-induced apoptosis.[71] Therefore, tumor cells, with the participation of IR, may develop another resistant mechanism mediated by the upregulation of STAT1 and/or STAT1-induced genes, resulting in an impaired anti-tumor IFN/STAT1 signaling response. The role of STAT1 signaling response in combined RT and chemotherapy is complex and driven by the expression of many subsets of ISGs in different cancer. Further explorations are required to unveil how STAT1-mediates

cross-resistance to RT and chemotherapy and to confirm that targeting STAT1 may be an effective therapeutic technique to improve the effectiveness of anti-cancer treatment.

Future Direction

Constitutively activated STATs, particularly STAT1, STAT3, and STAT5 have been found in a variety of human tumors. Many preclinical trials aim to improve the effectiveness of anti-tumor therapy by stimulating the anti-neoplastic function of STAT1 and inhibiting the oncogenic function of STAT3 and STAT5.[7,72] Despite extensive studies on STATs activation and its role in RT, still STAT1 and STAT3 represent an attractive target in the modern era of radio-immunotherapy.[8]

Although STAT3 is of great interest and the deeply investigated STAT protein in cancer research, emerging findings suggest STAT1 as a critical mediator of oncogenic signaling and participate in the formation of radioresistant phenotype in some cancers, such as breast cancer,[12] head and neck cancer,[9] myeloma,[10] and renal cell carcinoma.[11] In these tumor cells with high expression of STAT1, targeting IFN/STAT1 pathway may lead to the discovery of new treatment strategies for targeted RT. Undoubtedly, the potential usefulness of STAT-targeted therapeutics in RT needs more investigation to unravel the relevant physiological pathway of the STAT protein family. In addition, the possibility of the combination of STATs inhibitors with other targeted therapy will be useful in the synthetic lethality approach of RT; which depends on the availability of a clinically relevant delivery system. While the mechanisms of cancer radioresistance have not been fully elucidated, the results of previous studies suggest that the outcome of IFN/STAT1 pathway effects in tumors after radiation depends on the intensity of the signal, microenvironment cues, and the tumor-specific context.[5] Therefore, strategies to overcome resistance in the context of the IFN/STAT1 pathway need to take into full account how to balance the pro-survival and cytotoxic aspects of STAT1 signaling in RT (Figure 2). IFN/STAT1 approaches to reduce tumor radioresistance have been derived from new findings and have not yet been used in clinical settings.[18] Treatments targeting STAT1 may be verified as radiosensitizing strategies in various pre-clinical settings. Future comprehensive molecular and cellular investigations need to focus on the roles of the IFN/STAT1 pathway in radioresistant cancer cells. IFN/STAT1 pathway blockers can be divided into the following: i) targeting the JAK/STAT1 signaling. Several JAK inhibitors have already received FDA approval for rheumatoid arthritis, including the upadacitinib,[73] baricitinib,[74] and tofacitinib.[75] Besides, fludarabine has been described as a radiosensitizer, by blocking STAT1 improves the efficacy of RT in many cancers.[76,77] Moreover, some preclinical studies have found that targeting IFN/STAT1 signaling effectively reduces the expression of PD-L1 in cancer cells. For example, SAR302503, a selected JAK2 inhibitor, has been reported to suppress STAT1 activation in radioresistant non-small cell lung cancer (NSCLC) cell lines, as well as abrogate tumor cell-intrinsic expression of PD-L1 in NSCLC cells.[78] Also, 15-deoxy-δ12, 14-prostaglandin J2, a peroxisome proliferator-activated receptor-gamma activator, can strongly downregulate Type II IFN-elicited PD-L1 expression through targeting the JAK/STAT1 signaling pathway in melanoma cells.[79] In this field, efficacy in appropriate delivery of IFN/STAT1 signaling inhibitor, biodistribution, selectivity and preliminary biosafety are the major challenges for pharmacologists; ii) targeting STAT1 modulation. As discussed above, acetylation of STAT1 inhibits IFN-dependent STAT1 phosphorylation and STAT1-dependent gene expression pattern.[68] The upregulation of the acetylation of Type II IFN/STAT1 pathways in cancer cells may improve the sensitivity to RT. Acetylation of STAT1 correlates with the dissociation of HDACs and interaction with CBP.[52] In cisplatin-resistant ovarian cancer cells, silencing of HDAC-4 increases the STAT1 acetylation levels, and restores cisplatin sensitivity.[68] Thus, targeting the phosphorylation-acetylation switch may be a promising strategy to regulate STAT1 signaling. (iii) targeting the STAT1-activated genes. As Table 1 shows, some STAT1-induced genes, especially ISGs, can not only mediate resistance to radiation but also mediate resistance to some anti-neoplastic drugs, such as doxorubicin and cisplatin. Certainly, approaches to target these genes in radioresistant cancer cells may have significant benefits in cancer patients undergoing RT. However, considering the original function of these drugs, such as anti-viral activity, warrant further investigation in the clinic. (iv) targeting the miRNAs. Suppressing or inhibiting oncomiRNAs is a potential method to impair the oncogenic function of the IFN/STAT1 pathway. Additionally, activating of tumor-suppressor miRNAs, may increase the activation state of STAT1 in radioresistant tumors and improve the tumor radiosensitivity. (v) targeting the STAT3 protein. It is well established that STAT1 and STAT3 can counteract each other's functions by competing for the same DNA binding sequence.[8]

However, positive association between levels of STAT1 and STAT3 has been found in breast cancer cells.[80] Through binding to the STAT1 promoter, STAT3 can significantly enhance STAT1 gene expression, as well as activates the expression of iNOS and COX-2.[80] Because the homology between STAT1 and STAT3, off-target STAT1 blockade by a STAT3 inhibitor may result in some benefit effects in radioresistant cancers.[8,81] For example, Honokiol, a novel blocker of STAT3 activation, has a great potential for the treatment of hepatocellular carcinoma, that also suppresses constitutive activation of JAK1 and JAK2.[16] Of note, the ratio of STAT1 and STAT3 expression constitutes important mechanisms in controlling their response to cytokines and growth factors.[72] Thus, targeting STAT3 therapy should take into full account about the balance state of STAT1 and STAT3, as well as reduce its toxic and adverse effects to achieve more stable efficacy.

Conclusions

In conclusion, this review highlights the mechanistic functions of the IFN/STAT1 pathway to confer radioresistance and radiosensitization in radio-immunotherapy, which require further comprehensive studies. The possibility of a radiation-induced IFN/STAT1 mechanism will open new opportunities for the development of novel therapeutic strategies, as well as define a new approach to enhance and radio-immunotherapy efficacy in the clinical treatment of various cancers. In the future, more investigations are needed into the interplay between the overexpression of STAT1 signaling and STAT1-dependent genes in different tumors. Meanwhile, laboratory investigations need to continue to elucidate the paradoxical effects of the IFN/STAT1 pathway response to RT.

Abbreviations

CBP, CREB-binding protein; CSCs, cancer stem cells; GBM, glioblastoma multiforme; HDAC, histone deacetylase; IDO, indoleamine-2,3-dioxygenase; IFNs, interferons; IFITs, interferon-induced protein with tetratricopeptide repeats; iNOS, inducible nitric oxide synthase; IRDS, interferon-related DNA damage resistance signature; IR, ionizing radiation; ISGs, interferon-stimulated genes; ISGF3, interferon-stimulated gene factor 3; JAK, Janus kinase; MDM2, murine double minute 2; MDSCs, myeloid-derived suppressor cells; miRNAs, microRNAs; MX1, interferon-induced GTP-binding protein; NSCLC, non-small cell lung cancer, PD-L1, programmed death-ligand 1; RT, radiation therapy; SERPINB9, serpin family B member 9; STATs, signal transducer and activator of transcriptions; TAMs, tumor-associated macrophages; TME, tumor microenvironment; Tregs, regulatory T-cells.

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Disclosure

The authors report no conflicts of interest in this work.

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