Molecular Pathways: Interferon/Stat1 Pathway: Role in the Tumor Resistance to Genotoxic Stress and Aggressive Growth

Nikolai N. Khodarev1, Bernard Roizman2, and Ralph R. Weichselbaum1

Abstract

STAT1 is activated by IFNs and other cell signals. Following activation, STAT1 is translocated to the nuclei and activates transcription of IFN-stimulated genes. Although the activation of STAT1 by IFNs is classically associated with antiviral defense and tumor-suppressive functions, emerging data indicate that expression of the STAT1 pathway confers cellular resistance to DNA-damaging agents and mediates aggressive tumor growth. Recent advances in the development of Janus-activated kinase/Stat inhibitors and peptide inhibitors specific for individual Stat proteins may provide new insights into the controversial functions of this pathway. Clin Cancer Res; 18(11); 1–7. ©2012 AACR.

Background

STAT1 is a cytoplasmic protein that, upon awakening from dormancy by signaling from IFNs and other factors, activates numerous genes designed, in principle, to defend the host (1–3). Activation of STAT1 occurs initially with binding of type I or type II IFNs to cognate receptors, which leads to phosphorylation-dependent activation of Janus-activated kinases (Jak1, Jak2, and Tyk2). These kinases, in turn, phosphorylate STAT1 at Tyr701. This step can be inhibited by Jak2 or Jak1/2 inhibitors recently tested in phase II and III clinical trials (see Fig. 1A; ref. 4). Jak inhibitors suppress different Stat proteins; however, tofacitinib (CP-690,550) is more selective for Stat1 than Stat3 (5). Phosphorylated STAT1 forms homodimers [gamma-IFN activation factor (GAF)] that are translocated to the nuclei by importin-alpha5 and beta1 (6, 7) and activate the transcription of IFN stimulated genes (ISG) by binding to the gamma-IFN–activated sequence (GAS; ref. 3). The peptide inhibitor H2-2, derived from the N-domain of Stat1, selectively blocks homodimerization of Stat1, but not Stat3, and suppresses activation of Stat1-dependent genes (Fig. 1; ref. 8). Upon activation by type I IFNs (IFN-alpha and/or -beta), STAT1 forms heterodimers with STAT2 and IRF9 (p48), thereby forming the protein complex known as IFN-stimulated transcription factor gamma 3 (ISGF3; ref. 9). ISGF3 is then transported to the nuclei by importins alpha3, alpha5, and alpha7 and binds to IFN-stimulated response elements (ISRE) in promoters of ISGs. Fludarabine traditionally used in the treatment of hematologic malignancies selectively suppresses Stat1. This drug binds to the SH2-phosphotyrosine binding pocket of Stat1, thereby blocking Stat1 interaction with IFN receptors, Tyr701 phosphorylation, and homodimerization (see Fig. 1; ref. 10). Recent data show that phosphorylation of Stat1 at the Ser708 position by inhibitor of I kappa B kinase-alpha (IKKalpha) can shift the balance between the formation of GAF and ISGF3 (Fig. 1A; ref. 11). The number of ISGs has been estimated to range from 600 to 2,000 (12). These genes determine the biologic consequences of STAT1 signaling. IFN-induced STAT1 signaling primarily activates genes that mediate immune functions, antiviral and/or antipathogen functions, suppression of cell proliferation, and the induction of apoptosis. As a result, STAT1 signaling is generally considered to be a prodeath and tumor-suppressive pathway. STAT1 signaling is also thought to play a key role in immune surveillance of tumors (13). However, this benign view of STAT1 signaling has been shattered by emerging evidence that upregulation of STAT1 causes activation of subsets of ISGs that are associated with increased resistance of tumor cells to genotoxic stress and/or tumor growth.

Clinical–Translational Advances

Radioresistance and the STAT1 pathway

The term radioresistance is usually applied to the restoration of wild-type sensitivity to radiation in model organisms that have DNA repair defects. Clinically, the term refers to tumors that are not cured by local radiotherapy. Radiation treatment failure is, at least in part, ascribed to intrinsic radioresistance of tumor cells (14). To identify genes that mediate tumor radioresistance, xenografts of a radiosensitive cell line SCC-61 were subjected to repeated cycles of...
A, IFN/Stat1 pathway. Ruxolitinib (Jakafi; Incyte Corporation), Jak1/2 inhibitor, U.S. Food and Drug Administration approved for intermediate and/or high-risk myelofibrosis. TG10138-Jak2 inhibitor, phase I and II trials. P, phosphate groups. See explanations in text. B, tumor cells (TC) sensitive to cytotoxic factors of microenvironment (blue) are committed to death because of the secretion of cytotoxic ligands by host cells (T, T lymphocytes; B, B lymphocytes; TAM, tumor-associated macrophages; FB, fibroblasts; DC, dendritic cells). A small fraction of TCs constitutively express Stat1/ISGs (red) and are resistant to the cytotoxic environment and genotoxic therapy. The overall population of tumor cells is relatively radio- and/or chemosensitive. C, constitutive expressors of Stat1/ISGs can activate the prosurvival secretome, including IL-6 and IL-8 (41). The prosurvival secretome can enhance selective advantages of surrounding tumor cells. D, elimination of Stat1L, IFN-sensitive cells; initial stages of enrichment of tumor population by tumor clones resistant to cytotoxic microenvironment and genotoxic stress. E, final selection of tumor clones resistant to cytotoxic microenvironment and genotoxic therapy (Stat1H,I F NR phenotype, see ref. 35).

Figure 1. A, IFN/Stat1 pathway, and B-E, Stat1/ISG-driven selection of radio- and/or chemoresistant tumor clones by tumor microenvironment. A, IFN/Stat1 pathway. Ruxolitinib (Jakafi; Incyte Corporation), Jak1/2 inhibitor, U.S. Food and Drug Administration approved for intermediate and/or high-risk myelofibrosis. TG10138-Jak2 inhibitor, phase I and II trials. P, phosphate groups. See explanations in text. B, tumor cells (TC) sensitive to cytotoxic factors of microenvironment (blue) are committed to death because of the secretion of cytotoxic ligands by host cells (T, T lymphocytes; B, B lymphocytes; TAM, tumor-associated macrophages; FB, fibroblasts; DC, dendritic cells). A small fraction of TCs constitutively express Stat1/ISGs (red) and are resistant to the cytotoxic environment and genotoxic therapy. The overall population of tumor cells is relatively radio- and/or chemosensitive. C, constitutive expressors of Stat1/ISGs can activate the prosurvival secretome, including IL-6 and IL-8 (41). The prosurvival secretome can enhance selective advantages of surrounding tumor cells. D, elimination of Stat1L, IFN-sensitive cells; initial stages of enrichment of tumor population by tumor clones resistant to cytotoxic microenvironment and genotoxic stress. E, final selection of tumor clones resistant to cytotoxic microenvironment and genotoxic therapy (Stat1H,I F NR phenotype, see ref. 35).
radiation, which resulted in the selection of a radioresistant tumor designated nu61 (15). Expressional profiling of nu61 and SCC61 tumors identified a set of 49 genes that were overexpressed in nu61 cells. Of these 49 initially identified genes, 31 are currently identified as ISGs, including STAT1 (Supplementary Table S1). This set of genes was designated as the IFN-related DNA damage signature (IRDS; ref. 16). Further experiments revealed that members of the IRDS are upregulated by fractionated ionizing radiation both in vitro and in xenografted tumor models of head and neck, breast, and colon cancers (17). These results were recently confirmed for breast, glioma, and prostate cancer cell lines (18, 19) and indicate that ionizing radiation leads to the induction of the IFN/Stat1 pathway in tumor cells. STAT1 seems to have a dominant role in the expression of IRDS genes. Enforced expression of STAT1 in radiosensitive SCC61 rendered overexpressing clones radioresistant, both in short-term and clonogenic assays (17). Concurrently, overexpression of STAT1 led to the upregulation of IRDS genes (16). Stable knock-down of STAT1 led to the radiosensitization of SCC61 and nu61 tumors and downregulation of genes in the IRDS (16, 17, 20). Consistent with these results, suppression of STAT1 by fludarabine led to the radiosensitization of renal cell carcinoma cell lines (21). Zhan and colleagues showed that increased expression of STAT1 promotes radioresistance of breast cancer stem-like cells cultured as mammospheres (22). Fryknäs and colleagues showed that a myeloma cell line overexpressing STAT1 and ISGs was significantly more radioresistant relative to the parental cell line exhibiting a low expression of STAT1 (23). Taken together, these studies show that constitutive expression of STAT1 confers a radioresistant phenotype to tumor cells.

To evaluate the clinical significance of these in vitro observations, expressional databases from a variety of different cancers were investigated. Of the 5 types of human cancers analyzed, 37% of head and neck, 48% of lung, 29% of prostate, 46% of breast cancers, and 50% of high-grade gliomas constitutively expressed ISGs. Other investigators obtained similar results showing constitutive expression of ISGs in samples of breast, lung, ovarian, and cervical cancers (24–26). An IRDS-based 7-gene classifier was applied to a data set from 295 patients with early-stage breast cancer (NKI295). The 243 patients who received adjuvant radiotherapy were analyzed for local–regional failure using Kaplan–Maier survival statistics. Results showed that IRDS-positive patients suffered a 30% to 40% local regional failure rate at 10 years compared with IRDS-negative patients (16). Further analyses with independent databases are necessary to evaluate the clinical validity of these studies, but taken together, these data indicate that in several model systems and clinical samples, constitutive expression of the ISGs might lead to radioresistance of tumor cells and that targeting of this pathway can sensitize tumors to ionizing radiation.

**Chemoresistance and the STAT1 pathway**

Rickardson and colleagues used a panel of 10 cell lines and 66 drugs representing antimetabolites, alkylating agents, topoisomerase inhibitors, protease inhibitors, and tubulin-binding agents to determine the correlation between gene expression patterns and resistance to different drugs. STAT1 was one of the top genes whose expression positively correlated with resistance to doxorubicin and topoisomerase-II inhibitors (27). Upregulation of STAT1 in doxorubicin-resistant cell lines was accompanied by the upregulation of ISGs that overlapped, in part, with the IRDS genes (28). STAT1-dependent resistance to doxorubicin was associated with increased resistance to ionizing radiation (23). Roberts and colleagues investigated the sensitivity of 14 ovarian cancer cell lines to 4 platinum compounds and found that upregulation of STAT1, IRF9, IFTM1, and HLA-C were associated with resistance to cisplatin and AMD473 (29). Recently, the role of STAT1 in the resistance of ovarian cancer cells to cisplatin has been confirmed by other investigators (30). The STAT1 pathway was also upregulated in the docetaxel-resistant prostate cancer cell line DU145-DR compared with parental DU145 cells. Knock-down of STAT1 led to resensitization of DU145-DR to docetaxel (31). A combination of bromodeoxyuridine and distamycin A induced cellular senescence, activation of STAT1, and upregulation of ISGs (32). Luszczek and colleagues used a combination of DNA-methyltransferase I and histone deacetylase (HDAC) inhibitors (HDACi) to induce DNA damage in small cell lung cancer cell lines and found that resistant cell lines overexpressed STAT1 and ISGs (33). These results suggest that STAT1 overexpression is associated with resistance of tumor cells to antitumor drugs.

**Coevolution of resistance to IFNs and genotoxic stress in tumor cells determined by the tumor microenvironment**

Kita and colleagues showed that chronic exposure of human fibroblasts to IFN-α and IFN-β rendered cells radioresistant (34). Similar data were obtained for SCC61 after chronic exposure to IFN-α and IFN-γ (17). In both cases, chronic exposure to IFNs led to the constitutive upregulation of ISGs or/and STAT1. These results suggested that during tumor development, IFNs can select tumor clones resistant to a cytotoxic microenvironment and concurrently to genotoxic therapy. B16F1 melanoma clones obtained by serial passages through immunocompetent mice showed that clones with high constitutive expression of STAT1 and STAT1-dependent genes (STAT1H genotype) were significantly more resistant to IFN-γ compared with tumor clones that did not express STAT1 and ISGs (STAT1L genotype). STAT1H clones were also more resistant to ionizing radiation and doxorubicin (35). It was possible to conclude that the population of B16F1 cells contained preexisting STAT1H variants prior to in vivo passage, which is consistent with the previous data about the clonal heterogeneity of B16F1 melanomas (36). STAT1H clones, resistant to cytotoxic and genotoxic stress, were preferentially selected by the lung microenvironment and were more proficient at colonizing the lungs. It is possible to suggest that upregulation of the IFN/STAT1 pathway in vivo, triggered by interaction of tumor clones with a microenvironment (13), may induce
negative selection against STAT1\textsuperscript{L} clones, thereby selecting for preexisting STAT1\textsuperscript{HI} clones (Fig. 1). Sronach and colleagues (30) determined that cisplatin-resistant cells are selected from the initially heterogeneous population and overexpress the IFN/STAT1 pathway through activation of STAT1 by constitutive expression of HDAC4. Taken together, these data suggest that tumor clones with Stat1-dependent resistance to a cytotoxic microenvironment may have selective advantages to promote the development of an aggressive radio- and/or chemoresistant tumor (Fig. 1).

**Potential mechanisms of STAT1 prosurvival signaling**

**Upstream signaling.** STAT1 signaling is associated with IFNs, yet many other signals also activate the STAT1 pathway. For example, the STAT1 pathway is activated in the context of EGF receptor (EGFR), interleukin-6 (IL-6), platelet-derived growth factor (PDGF), and erythropoietin signaling (1, 37, 38). Many cytokines including IL-2, IL-3, IL-12, and IL-22 use the JAK-STAT axis for signal transduction (39). STAT1 signaling can be activated by hypoxia (40), which is consistent with the fact that hypoxic tumors are radioresistant. These data suggest that activation of the STAT1 pathway has a broader biologic significance for resistance to cancer therapeutics than IFN signaling. It is possible that IFNs and ionizing radiation may act in concert with other factors in the tumor microenvironment, such as EGF, PDGF, and IL-6, to induce radioresistance. Indeed radioresistant nu61 produced more IL-6 and IL-8 than the parental radiosensitive SCC61 cell line, and ionizing radiation further increased production of IL-6 and IL-8 in both cell lines in a Stat1-dependent manner (41).

Constitutive Jak/Stat signaling and fusion proteins with tyrosine kinase activity are associated with different forms of hematologic neoplasias (Fig. 2). TEL-JAK2 fusion induces T-cell leukemia and atypical chronic myelogenous leukemia. The TEL-JAK2 fusion also leads to the constitutive activation of STAT1, STAT3, and STAT5 (42). TEL--PDGF receptor-β (PDGFR\textbeta) fusion is an oncogene associated with myeloproliferative neoplasia. This fusion leads to the activation of the STAT1 pathway with proteins overlapping with the IRDS (43). Clonal analysis of oncogenic Jak2V617F mutants revealed that constitutive activation of the STAT1 pathway is associated with essential thrombocytopenia (44). These data indicate that constitutive activation of the Stat1 pathway may be oncogenic, which is in agreement with our data (15).

**Modifications of STAT1.** Multiple protein modifications have been described for STAT1 (see ref. 7 for a review). These modifications include phosphorylation, ubiquitination, SUMOylation, ISGylation, acetylation, and methylation (Fig. 2). Each of these modifications can alter the transcriptional activity of STAT1 and potentially change the pattern of activated genes. Important recent findings were described by Stark and colleagues, who found that unphosphorylated STAT1 (U-STAT1) can transcriptionally activate a fraction of STAT1-dependent genes, which overlap with the IRDS and are potentially involved in cellular resistance to genotoxic stress and prosurvival functions (45, 46). Importantly, U-STAT1 occupies only a fraction of the promoters that bind phosphorylated STAT1 (P-STAT1) after IFN-γ stimulation (47). Unphosphorylated Stat2 and Stat3 activated transcription of subsets of downstream genes, only partially overlapping with genes activated by phosphorylated Stats (46, 48). If prosurvival ISGs are preferentially activated by U-Stat1, then the equilibrium between P-STAT1 and U-STAT1 can be of critical importance to affect the prosurvival functions of STAT1. One hypothesis is that this equilibrium is maintained by the activity of the kinases phosphorylating STAT1 in Y701 and modifications leading to the dephosphorylation of nuclear STAT1 (see Fig. 2). Acetylation of STAT1 by histone acetyltransferase CBP in the K410 and K413 positions leads to the dissociation of the DNA-binding domain from the promoter sequences and subsequent dephosphorylation of STAT1 by protein phosphatase PTPN2 (TCP45; see Fig. 2; ref. 49). Similarly, SUMOylation of STAT1 in the K703 position can prevent phosphorylation in the Y701 position and affect nucleo-cytoplasmic distribution of STAT1 (50). SUMOylation leads to a change in the pattern and time course of downstream gene activation, consistent with U-STAT1 data (45, 51). Therefore, upregulation of U-Stat1 and modifications leading to dephosphorylation can be associated with transient activation of subsets.
of prosurvival ISGs. Another mechanism may be connected with constitutively phosphorylated Stat1. STAT1 has been shown to bind HDAC4, which is constitutively expressed in cisplatin-resistant ovarian cancer cells, thereby triggering Y701 phosphorylation of STAT1, its nuclear–cytoplasmic transport, and subsequent constitutive activation of ISGs (30). Similarly, constitutive phosphorylation of STAT1 due to constitutively active fusion tyrosine kinases and Jak2V617F has been described in some hematologic malignancies (42, 44). These data indicate 2 pathways through which STAT1 can control expression of prosurvival genes. Namely, U-STAT1 provides activation of a small fraction of STAT1-dependent genes (45, 46, 52), which are enriched by genes that are protective against genotoxic stress or have prosurvival functions. Another pathway may be connected with the constitutive activation of STAT1 and operates through the selection of STAT1-resistant clones (see Fig. 1).

**Downstream IFN-stimulated genes.** ISGs included in the IRDs have been reported to be associated with response to genotoxic stress, radio- and/or chemoresistant tumor phenotypes and oncogenesis (see Supplementary Table S1). They represent several functional groups (see Supplementary Fig. S1). The most abundant group is represented by genes involved in antiviral defense and regulated by type I IFNs (53). One of these genes is IFITM1 (leu13, 9-27, Fragilis). Recently IFITM1 was shown to be involved in cellular defense against the West Nile and Dengue viruses (54). Several laboratories have also reported the involvement of IFITM1 in the regulation of early development (55), colorectal cancer progression (56), and radioresistance (34). The RNA-dependent protein kinase PRKR is a key cellular enzyme of anti–HSV-1 defense (57). PRKR was recently reported to confer radioresistance through the activation of NF-κB and AKT1 (58). IFI6 (G1P3) suppresses replication of yellow fever virus YFV (59). IFI6 was also recently recognized as an antiapoptotic protein associated with tamoxifen-resistant aggressive breast cancers (60). USP18 (UBP43) is involved in the regulation of the stability of antiviral proteins, modified by ISG15. This protein also suppresses drug and IFN-α-induced apoptosis, perhaps by the described mechanism of binding to the IFNAR2 and displacing JAK1 (61, 62). USP18 is also involved in the regulation of EGFR expression through transcriptional modulation of miR-7 (63). Another member of IRDS, MCL1, which is an IFN-inducible member of the BCL-2 family, is considered an important antiapoptotic oncogene (64). The association of a constitutively active STAT1 with overexpression of MCL1 was identified in Wilms tumors and is considered an important factor in oncogenesis and apoptosis resistance (8, 65). These examples illustrate that a subset of genes described as ISGs indeed show stress-protective and prosurvival functions. These data also show that some genes, which are involved in antiviral defense, can simultaneously have radio- and chemoprotective functions and are associated with oncogenesis.

**Conclusion**

STAT1 has been extensively investigated in the IFN-signaling pathway in the context of its antiviral actions. Recently, it has been shown that this signaling pathway mediates tumor cell resistance to ionizing radiation and chemotherapy and connotes a poor response to cytotoxic treatments in patients who overexpress genes in this pathway. The mechanisms of STAT1’s stress-protective and oncogenic functions are still largely unknown. However, taking into consideration that STAT1 signaling is one of the primary mechanisms of tumor cell elimination in early stages of tumor development (13), we hypothesize that tumor clones that escape this selection have common STAT1-related properties responsible for their aggressive phenotype and resistance to genotoxic stress. Decoding the molecular properties leading to this aggressive treatment-resistant phenotype may lead to the discovery of new therapeutic targets and prognostic tools.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Development of methodology: N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Writing, review, and/or revision of the manuscript: N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): N.N. Khodarev, B. Roizman, R.R. Weichselbaum

Study supervision: N.N. Khodarev, B. Roizman, R.R. Weichselbaum

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