Clinical Cancer Research

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

Nikolai N. Khodarev[1], Bernard Roizman[2], and Ralph R. Weichselbaum[1]

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); 3015–21. ©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-α5 and β1 (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-α and/or -β), 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 α3, α5, and α7 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 homo- and/or heterodimerization (see Fig. 1; ref. 10). Recent data show that phosphorylation of Stat1 at the Ser708 position by inhibitor of IκB kinase-ε (IKKε) 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.

Authors' Affiliations: [1]Department of Radiation and Cellular Oncology and [2]Marjorie B. Kovler Viral Oncology Laboratory, The University of Chicago, Chicago, Illinois

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Ralph R. Weichselbaum, Department of Radiation and Cellular Oncology, The University of Chicago, 5758 S. Maryland Avenue, MC 9006, Chicago, IL 60637. Phone: 773-702-0817; Fax: 773-702-1968; E-mail: rrw@radonc.uchicago.edu

doi: 10.1158/1078-0432.CCR-11-3225

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

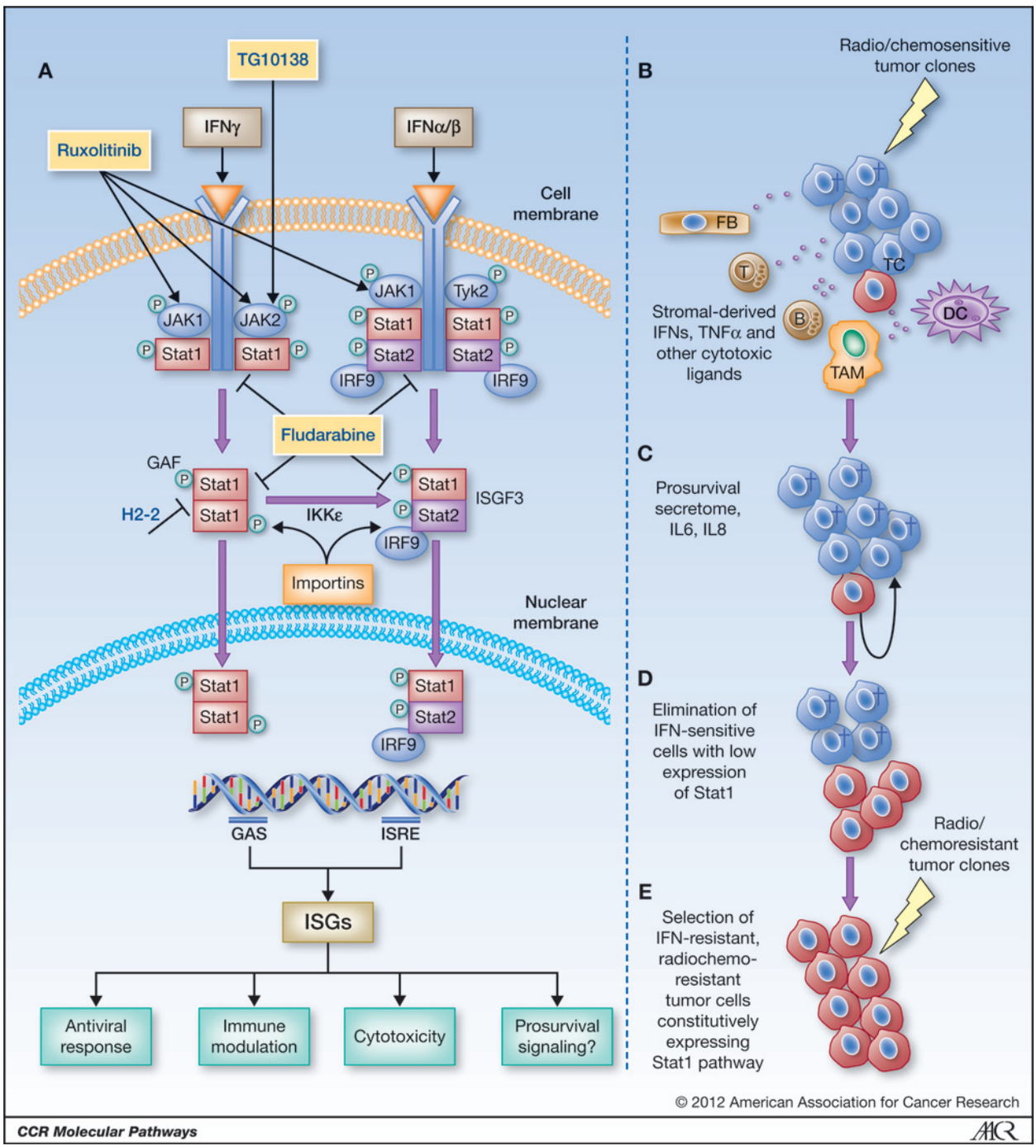

Figure 1. A, IFN/Stat1 pathway, and B–E, Stat1/ISG-driven selection of radio- and/or chemoresistant tumor clones by tumor microenvironment. A, IFN/Stat1 pathway. Ruxolitinib (Jakafi; Incyte Corporation), Jak1/2 inhibitor, U.S. Food and Drug Administration approved for intermediate and/or high-risk myelofibrosis. 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 $Stat1^{L}$, 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 ($Stat1^{H}$, IFN^{R} 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, proteasome 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, IFITM1, 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 ($STAT1^H$ genotype) were significantly more resistant to IFN-γ compared with tumor clones that did not express STAT1 and ISGs ($STAT1^L$ genotype). $STAT1^H$ clones were also more resistant to ionizing radiation and doxorubicin (35). It was possible to conclude that the population of B16F1 cells contained preexisting $STAT1^H$ variants prior to *in vivo* passage, which is consistent with the previous data about the clonal heterogeneity of B16F1 melanomas (36). $STAT1^H$ 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 $STAT1^{L}$ clones, thereby selecting for preexisting $STAT1^{H}$ clones (Fig. 1). Stronach 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 (PDGFRβ) 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 thrombocythemia (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-

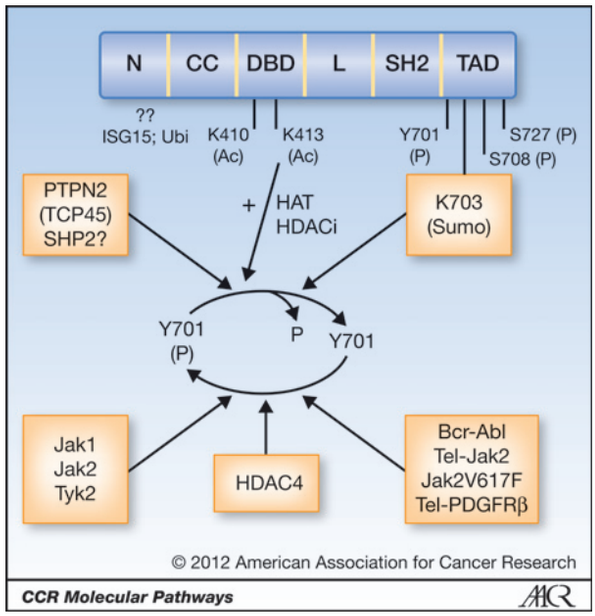

Figure 2. Stat1 protein modifications and balance of phosphorylation and/or dephosphorylation of Tyr701. Modifications are described for different domains of Stat1 (N, *N*-terminal domain; CC, coil-coiled domain; DBD. DNA-binding domain; L, linker; SH2, Src homology 2 domain; TAD, *trans*-activation domain). Sizes of domains are not scaled. P, sites of phosphorylation; Ac, sites of acetylation. Exact positions for ubiquitination and ISGylation are not described yet. Inducible tyrosine kinases (Jak1, Jak2, and Tyk2), as well as constitutively active Jak2V617F and NRTKs, phosphorylate Tyr701; TCP45 is responsible for dephosphorylation. Sumoylation in K703 and acetylation in K410/K413 shift the balance toward dephosphorylation of Tyr701; Stat1 deacetylation by HDACs shifts the balance toward Tyr701 phosphorylation. S727 phosphorylation activates transcription, whereas S708 phosphorylation suppresses formation of GAF and shifts signaling from type II to type I IFNs.

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 radioprotection (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

Grant Support

This work was supported by the Ludwig Center for Metastasis Research, the Center for Radiation Therapy, the Chicago Tumor Institute, Dr. Lloyd Old, Mr. and Mrs. Vincent Foglia and the Foglia foundation, Lung Cancer Research Foundation, the Cancer Research Foundation, and NIH Grants R01 CA111423 and PO1-CA71933.

Received January 4, 2012; revised February 20, 2012; accepted March 9, 2012; published OnlineFirst May 21, 2012.

References

1. Silvennoinen O, Schindler C, Schlessinger J, Levy DE. Ras-independent growth factor signaling by transcription factor tyrosine phosphorylation. Science 1993;261:1736–9.
2. Schindler C, Shuai K, Prezioso VR, Darnell JE Jr. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 1992;257:809–13.
3. Dale TC, Imam AM, Kerr IM, Stark GR. Rapid activation by interferon alpha of a latent DNA-binding protein present in the cytoplasm of untreated cells. Proc Natl Acad Sci U S A 1989;86:1203–7.
4. Stein BL, Crispino JD, Moliterno AR. Janus kinase inhibitors: an update on the progress and promise of targeted therapy in the myeloproliferative neoplasms. Curr Opin Oncol 2011;23:609–16.

5. Rosengren S, Corr M, Firestein GS, Boyle DL. The JAK inhibitor CP-690,550 (tofacitinib) inhibits TNF-induced chemokine expression in fibroblast-like synoviocytes: autocrine role of type I interferon. Ann Rheum Dis 2012;71:440–7.
6. Reich NC. STAT dynamics. Cytokine Growth Factor Rev 2007;18: 511–8.
7. Najjar I, Fagard R. STAT1 and pathogens, not a friendly relationship. Biochimie 2010;92:425–44.
8. Wang H, Yang Y, Sharma N, Tarasova NI, Timofeeva OA, Winkler-Pickett RT, et al. STAT1 activation regulates proliferation and differentiation of renal progenitors. Cell Signal 2010;22:1717–26.
9. Levy DE, Kessler DS, Pine R, Darnell JE Jr. Cytoplasmic activation of ISGF3, the positive regulator of interferon-alpha-stimulated transcription, reconstituted in vitro. Genes Dev 1989;3: 1362–71.
10. Sikorski K, Czerwoniec A, Bujnicki JM, Wesoly J, Bluyssen HA. STAT1 as a novel therapeutical target in pro-atherogenic signal integration of IFNg, TLR4 and IL-6 in vascular disease. Cytokine Growth Factor Rev 2011;22:211–9.
11. Ng SL, Friedman BA, Schmid S, Gertz J, Myers RM, Tenoever BR, et al. IkB kinase epsilon (IKK(epsilon)) regulates the balance between type I and type II interferon responses. Proc Natl Acad Sci U S A 2011;108:21170–5.
12. Samarajiwa SA, Forster S, Auchettl K, Hertzog PJ. INTERFEROME: the database of interferon regulated genes. Nucleic Acids Res 2009; 37[Database issue]:D852–7.
13. Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol 2006;6:836–48.
14. Lee JM, Bernstein A. p53 mutations increase resistance to ionizing radiation. Proc Natl Acad Sci U S A 1993;90:5742–6.
15. Khodarev NN, Beckett M, Labay E, Darga T, Roizman B, Weichselbaum RR. STAT1 is overexpressed in tumors selected for radioresistance and confers protection from radiation in transduced sensitive cells. Proc Natl Acad Sci U S A 2004;101:1714–9.
16. Weichselbaum RR, Ishwaran H, Yoon T, Nuyten DS, Baker SW, Khodarev N, et al. An interferon-related gene signature for DNA damage resistance is a predictive marker for chemotherapy and radiation for breast cancer. Proc Natl Acad Sci U S A 2008;105:18490–5.
17. Khodarev NN, Minn AJ, Efimova EV, Darga TE, Labay E, Beckett M, et al. Signal transducer and activator of transcription 1 regulates both cytotoxic and prosurvival functions in tumor cells. Cancer Res 2007;67:9214–20.
18. Tsai MH, Cook JA, Chandramouli GV, DeGraff W, Yan H, Zhao S, et al. Gene expression profiling of breast, prostate, and glioma cells following single versus fractionated doses of radiation. Cancer Res 2007;67:3845–52.
19. John-Aryankalayil M, Palayoor ST, Cerna D, Simone CB 2nd, Falduto MT, Magnuson SR, et al. Fractionated radiation therapy can induce a molecular profile for therapeutic targeting. Radiat Res 2010;174: 446–58.
20. Pitroda SP, Wakim BT, Sood RF, Beveridge MG, Beckett MA, MacDermed DM, et al. STAT1-dependent expression of energy metabolic pathways links tumour growth and radioresistance to the Warburg effect. BMC Med 2009;7:68.
21. Hui Z, Tretiakova M, Zhang Z, Li Y, Wang X, Zhu JX, et al. Radiosensitization by inhibiting STAT1 in renal cell carcinoma. Int J Radiat Oncol Biol Phys 2009;73:288–95.
22. Zhan JF, Chen LH, Yuan YW, Xie GZ, Sun AM, Liu Y, et al. STAT1 promotes radioresistance of CD44(+)/CD24(-/low) cells in breast cancer. Exp Biol Med (Maywood) 2011;236:418–22.
23. Fryknäs M, Dhar S, Oberg F, Rickardson L, Rydåker M, Göransson H, et al. STAT1 signaling is associated with acquired crossresistance to doxorubicin and radiation in myeloma cell lines. Int J Cancer 2007;120: 189–95.
24. Perou CM, Jeffrey SS, van de Rijn M, Rees CA, Eisen MB, Ross DT, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proc Natl Acad Sci U S A 1999;96: 9212–7.
25. Chung CH, Bernard PS, Perou CM. Molecular portraits and the family tree of cancer. Nat Genet 2002;32[Suppl]:533–40.
26. Rajkumar T, Sabitha K, Vijayalakshmi N, Shirley S, Bose MV, Gopal G, et al. Identification and validation of genes involved in cervical tumourigenesis. BMC Cancer 2011;11:80.
27. Rickardson L, Fryknäs M, Dhar S, Lövborg H, Gullbo J, Rydåker M, et al. Identification of molecular mechanisms for cellular drug resistance by combining drug activity and gene expression profiles. Br J Cancer 2005;93:483–92.
28. Rickardson L, Fryknäs M, Haglund C, Lövborg H, Nygren P, Gustafsson MG, et al. Screening of an annotated compound library for drug activity in a resistant myeloma cell line. Cancer Chemother Pharmacol 2006;58:749–58.
29. Roberts D, Schick J, Conway S, Biade S, Laub PB, Stevenson JP, et al. Identification of genes associated with platinum drug sensitivity and resistance in human ovarian cancer cells. Br J Cancer 2005;92: 1149–58.
30. Stronach EA, Alfraidi AM, Rama NR, Datler C, Studd JB, Agarwal R, et al. HDAC4-regulated STAT1 activation mediates platinum resistance in ovarian cancer. Cancer Res 2011;71:4412–22.
31. Patterson SG, Wei S, Chen X, Sallman DA, Gilvary DL, Zhong B, et al. Novel role of Stat1 in the development of docetaxel resistance in prostate tumor cells. Oncogene 2006;25:6113–22.
32. Novakova Z, Hubackova S, Kosar M, Janderova-Rossmeislova L, Dobrovolna J, Vasicova P, et al. Cytokine expression and signaling in drug-induced cellular senescence. Oncogene 2010;29:273–84.
33. Luszczek W, Cheriyath V, Mekhail TM, Borden EC. Combinations of DNA methyltransferase and histone deacetylase inhibitors induce DNA damage in small cell lung cancer cells: correlation of resistance with IFN-stimulated gene expression. Mol Cancer Ther 2010;9:2309–21.
34. Kita K, Sugaya S, Zhai L, Wu YP, Wano C, Chigira S, et al. Involvement of LEU13 in interferon-induced refractoriness of human RSa cells to cell killing by X rays. Radiat Res 2003;160:302–8.
35. Khodarev NN, Roach P, Pitroda SP, Golden DW, Bhayani M, Shao MY, et al. STAT1 pathway mediates amplification of metastatic potential and resistance to therapy. PLoS ONE 2009;4:e5821.
36. Fidler IJ. The organ microenvironment and cancer metastasis. Differentiation 2002;70:498–505.
37. Ernst M, Jenkins BJ. Acquiring signalling specificity from the cytokine receptor gp130. Trends Genet 2004;20:23–32.
38. Halupa A, Bailey ML, Huang K, Iscove NN, Levy DE, Barber DL. A novel role for STAT1 in regulating murine erythropoiesis: deletion of STAT1 results in overall reduction of erythroid progenitors and alters their distribution. Blood 2005;105:552–61.
39. Haan C, Kreis S, Margue C, Behrmann I. Jaks and cytokine receptors—an intimate relationship. Biochem Pharmacol 2006;72:1538–46.
40. Terui K, Haga S, Enosawa S, Ohnuma N, Ozaki M. Hypoxia/re-oxygenation-induced, redox-dependent activation of STAT1 (signal transducer and activator of transcription 1) confers resistance to apoptotic cell death via hsp70 induction. Biochem J 2004;380:203–9.
41. Efimova EV, Liang H, Pitroda SP, Labay E, Darga TE, Levina V, et al. Radioresistance of Stat1 over-expressing tumour cells is associated with suppressed apoptotic response to cytotoxic agents and increased IL6-IL8 signalling. Int J Radiat Biol 2009;85:421–31.
42. Benekli M, Baumann H, Wetzler M. Targeting signal transducer and activator of transcription signaling pathway in leukemias. J Clin Oncol 2009;27:4422–32.
43. Dobbin E, Graham C, Freeburn RW, Unwin RD, Griffiths JR, Pierce A, et al. Proteomic analysis reveals a novel mechanism induced by the leukemic oncogene Tel/PDGFRb in stem cells: activation of the interferon response pathways. Stem Cell Res (Amst) 2010;5:226–43.
44. Chen E, Beer PA, Godfrey AL, Ortmann CA, Li J, Costa-Pereira AP, et al. Distinct clinical phenotypes associated with JAK2V617F reflect differential STAT1 signaling. Cancer Cell 2010;18:524–35.
45. Cheon H, Stark GR. Unphosphorylated STAT1 prolongs the expression of interferon-induced immune regulatory genes. Proc Natl Acad Sci U S A 2009;106:9373–8.
46. Cheon H, Yang J, Stark GR. The functions of signal transducers and activators of transcriptions 1 and 3 as cytokine-inducible proteins. J Interferon Cytokine Res 2011;31:33–40.
47. Robertson G, Hirst M, Bainbridge M, Bilenky M, Zhao Y, Zeng T, et al. Genome-wide profiles of STAT1 DNA association using chromatin

immunoprecipitation and massively parallel sequencing. Nat Methods 2007;4:651–7.
48. Testoni B, Völlenkle C, Guerrieri F, Gerbal-Chaloin S, Blandino G, Levrero M. Chromatin dynamics of gene activation and repression in response to interferon alpha (IFN(alpha)) reveal new roles for phosphorylated and unphosphorylated forms of the transcription factor STAT2. J Biol Chem 2011;286:20217–27.
49. Krämer OH, Knauer SK, Greiner G, Jandt E, Reichardt S, Gührs KH, et al. A phosphorylation-acetylation switch regulates STAT1 signaling. Genes Dev 2009;23:223–35.
50. Zimnik S, Gaestel M, Niedenthal R. Mutually exclusive STAT1 modifications identified by Ubc9/substrate dimerization-dependent SUMOylation. Nucleic Acids Res 2009;37:e30.
51. Ungureanu D, Vanhatupa S, Grönholm J, Palvimo JJ, Silvennoinen O. SUMO-1 conjugation selectively modulates STAT1-mediated gene responses. Blood 2005;106:224–6.
52. Chatterjee-Kishore M, Wright KL, Ting JP, Stark GR. How Stat1 mediates constitutive gene expression: a complex of unphosphorylated Stat1 and IRF1 supports transcription of the LMP2 gene. EMBO J 2000;19:4111–22.
53. Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001;14:778–809.
54. Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell 2009;139:1243–54.
55. Tanaka SS, Yamaguchi YL, Tsoi B, Lickert H, Tam PP. IFITM/Mil/fragilis family proteins IFITM1 and IFITM3 play distinct roles in mouse primordial germ cell homing and repulsion. Dev Cell 2005;9:745–56.
56. Andreu P, Colnot S, Godard C, Laurent-Puig P, Lamarque D, Kahn A, et al. Identification of the IFITM family as a new molecular marker in human colorectal tumors. Cancer Res 2006;66:1949–55.
57. Roizman B. HSV gene functions: what have we learned that could be generally applicable to its near and distant cousins? Acta Virol 1999;43:75–80.
58. von Holzen U, Pataer A, Raju U, Bocangel D, Vorburger SA, Liu Y, et al. The double-stranded RNA-activated protein kinase mediates radiation resistance in mouse embryo fibroblasts through nuclear factor kappaB and Akt activation. Clin Cancer Res 2007;13:6032–9.
59. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature 2011;472:481–5.
60. Cheriyath V, Kuhns MA, Jacobs BS, Evangelista P, Elson P, Downs-Kelly E, et al. G1P3, an interferon- and estrogen-induced survival protein contributes to hyperplasia, tamoxifen resistance and poor outcomes in breast cancer. Oncogene 2011 Sep 26 [Epub ahead of print].
61. Potu H, Sgorbissa A, Brancolini C. Identification of USP18 as an important regulator of the susceptibility to IFN-alpha and drug-induced apoptosis. Cancer Res 2010;70:655–65.
62. Malakhova OA, Kim KI, Luo JK, Zou W, Kumar KG, Fuchs SY, et al. UBP43 is a novel regulator of interferon signaling independent of its ISG15 isopeptidase activity. EMBO J 2006;25:2358–67.
63. Duex JE, Comeau L, Sorkin A, Purow B, Kefas B. USP18 regulates EGF receptor expression and cancer cell survival via microrna-7. J Biol Chem 2011;286:25377–86.
64. Craig RW. MCL1 provides a window on the role of the BCL2 family in cell proliferation, differentiation and tumorigenesis. Leukemia 2002;16:444–54.
65. Timofeeva OA, Plisov S, Evseev AA, Peng S, Jose-Kampfner M, Lovvorn HN, et al. Serine-phosphorylated STAT1 is a prosurvival factor in Wilms' tumor pathogenesis. Oncogene 2006;25:7555–64.