Transcription Protein STAT1: Biology and Relation to Cancer

(STAT1 / IFNs / biology / aberrations in cancer)

L. ADÁMKOVÁ, K. SOUČKOVÁ, J. KOVAŘÍK

Department of Experimental Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic

Abstract. Cell homeostasis is controlled and regulated by multiple signalling proteins that operate almost in all cellular compartments. Their common task is to process regulatory signals from both the extracellular and intracellular spaces by triggering a cascade of intracellular events leading to modulation of downstream gene activity. One of the important signalling pathways is represented by the STAT multigene family comprising seven members. In general, various STATs act as potent transcription factors delivering signals of diverse polypeptide ligands (i.e. cytokines and growth factors) into the nucleus. This review summarizes some up-to-date data on the role of STAT1 in maintaining cellular homeostasis with the emphasis on its role in the control of cell growth, proliferation, apoptosis, and immune reactions. Part of the review deals with expression and posttranslational abnormalities of this molecule identified in a variety of human pathological conditions including cancer. The direct or indirect involvement of STAT1 in the process of malignant transformation is highlighted in view of these molecular perturbances that may contribute to oncogenesis and that may be potentially used as novel targets for anticancer therapy.

Introduction

Signal transducer and activator of transcription proteins (STATs) were introduced as transducing factors of various polypeptide ligands with the ability to act as transcription factors modulating the expression of effector genes that are at least partially under their control. These proteins are localized in an inactive form in the cytoplasm. Their activation as a consequence of ligand-mediated receptor activation is provided by Janus tyrosine kinases (JAKs) that are constitutively associated with appropriate receptors (Takeda and Akira, 2000). The ligand signal results in the dimerization or oligomerization of the receptor and subsequent activation of JAKs. Once it has become activated, the JAK kinase protein phosphorylates tyrosine residues in the cytoplasmic tail of the receptor to provide docking sites for the recruitment of molecules recognizing the phosphotyrosine binding domain (Briscoe et al., 1996). These receptor tyrosine motives are recognized by the SH2 domains of STATs, thereby mediating the recruitment of STAT to the receptor complex and its phosphorylation on a conserved tyrosine by activated JAKs. In some STATs, serine residues are also phosphorylated, influencing the magnitude of transcription (Kovarik et al., 2001). Interestingly, recent studies have revealed the importance of STAT1 serine 727 phosphorylation in mediating an apoptotic pathway in cells (Janjua et al., 2002). Upon release from the receptor, activated STATs dimerize, move into the nucleus, where they bind to specific DNA-response elements in the promoters of target genes and thereby induce specific gene expression programmes (Seidel et al., 1995; Bromberg, 2001). The STAT activation process is transient and its deactivation takes place both at the nuclear and cytoplasmic levels.

The nuclear tyrosine phosphatase seems to be a principal enzyme that causes STAT dephosphorylation in the nucleus and provides signal for STAT export to the cytoplasm (Aoki and Matsuda, 2002; ten Hoeve et al., 2002). The negative regulators of both activated STATs and JAK kinases operate at the cytoplasmic and/or receptor sites. They comprise several phosphatases such as SH2-containing phosphatase-1 and protein-tyrosine phosphatase-1B (You et al., 1999; Aoki and Matsuda, 2002), protein inhibitors of activated STATs (PIAS), which interact directly with STATs and block their DNA-binding activity (Shuai, 2000), as well as suppressor of cytokine signalling (SOCS) family of proteins that bind to receptors and JAK family kinases to inhibit STAT activation and also act as STAT-induced STAT inhibitors (Naka et al., 1999; Shuai, 1999; Starr and Hilton, 1999; Wormald et al., 2006b).

Studies employing mouse models harbouring a null allele for the particular STAT gene disclosed the role of individual STATs in normal physiology and pathophysiology (Akira, 1999). Up to now, there have been seven members of the STAT superfamily identified bearing similar protein domains. Yet, some individual STATs possess unique functional properties exerting sometimes even antagonistic effects on cellular functions.

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Corresponding author: Jan Kovařík, Department of Experimental Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, Brno, Czech Republic. E-mail: kovarik@mou.cz

Abbreviations: IFN – interferon, JAK – Janus kinase, STATs – signal transducers and activators of transcription.

Thus, for example STAT1 and STAT3 exhibit opposite effects on the cellular proliferation and apoptosis (Bromberg and Darnell, 2000; Battle and Frank, 2002; Stephanou and Latchman, 2005). It has been proposed that differences in physiological properties of some STATs are due to their preferential activation by certain ligands, their binding to and activating specific genes, and/or due to distinct functional domains. The transcription capacity of STATs is modulated by posttranslational phosphorylation at different tyrosine and serine residues in a polypeptide chain. In addition, a wide variety of factors interacting with STATs exemplified by SOCSs, NF-κB, SMADs, c-Jun, Sp1, etc. may significantly influence the functional specificity of individual STATs.

This review briefly summarizes the most important data on the STAT1 functions in cell biology and discusses its perturbations that often occur in cancer and might contribute to oncogenesis and/or to impaired response of tumour cells to cytokine-based therapy. STAT1 was chosen since in contrast to most other members of this family it exerts predominantly growth inhibitory and pro-apoptotic activity.

STAT1

STAT1 was the first member of the multigene family discovered as a principal target of both type I and type II interferon (IFN) activation (Schindler et al., 1992; Darnell et al., 1994; Ihle and Kerr, 1995; Stark et al., 1998). Most, if not all, normal functions of STAT1 are closely related to the biological effects of IFNs since its utilization is notably specific to IFN ligands *in vivo*. The significant progress towards our understanding of the STAT1 role in cellular physiology enabled construction of either STAT -/- mice expressing a truncated (i.e. functionally impaired protein) (Meraz et al., 1996) or mice lacking STAT1 expression (Durbin et al., 2000). These authors showed that STAT1-deficient mice have lost responsiveness to both types of IFNs and acquired enhanced susceptibility to bacterial and viral pathogens. Through these experiments, the primary physiological role of STAT1 in mediating the antiviral and immune effects of IFNs, initially suggested by Darnell et al. (1994), was confirmed. It has been proposed that the mechanism of STAT1 function in the immune defence involves the induction of immune effector genes, e.g. MHC, IRF1, FcγRI, etc. In addition, recent reports on the role of STAT1 in the maintenance of immunological self-tolerance (Nishibori et al., 2004) as well as findings that STAT1 controls joint inflammation in the model systems (de Hooge et al., 2004) suggest that this transcription factor operates in broader levels of immune system homeostasis than so far recognized.

Both STAT1 and STAT2, as the almost exclusive mediators of IFN-α and IFN-γ biological effects, delineate an important molecular mechanism that controls cell growth and apoptosis. Several data gathered from STAT1-deficient cells *in vitro* (Durbin et al., 1996; Meraz et al., 1996) as well as from mice lacking the STAT1 gene (Bromberg et al., 1996; Kumar et al., 1997; Ramana et al., 2000) brought clear evidence that STAT1 activation by IFN ligands executes the anti-proliferative and pro-apoptotic events. Data presented by Lee et al. (2000b) have demonstrated that lymphocyte survival, proliferation and response to death stimuli also require transcriptionally active STAT1, but its activation in this case seems to be only partially dependent on IFN signalling. The observation that independently of IFNs, STAT1 can be activated by IL-4 and is required for the growth inhibitory effects of this cytokine (Chang et al., 2000) as well as data showing the importance of STAT1 in bone formation (Takayanagi et al., 2002) indicate that it influences a broad range of physiological processes in dependence on the activating ligands and tissue systems.

Although the accurate molecular mechanisms operating downstream of the STAT1 activation are not well understood, the endpoint of its role in cellular physiology consists in modulation of STAT1-dependent gene transcription. Thus, for example its immune, growth and apoptosis regulatory activities associate mostly with the transcriptional modification of IRF-1, MHC, FcγRI, Fas and FasL TRAIL, cyclin-dependent kinase inhibitors, p21waf1 and caspase genes (Battle and Frank, 2002; Ivashkiv and Hu, 2004). Recent analyses employing DNA microarray (Chip-chip) technique brought new insight into the accurate determination of STAT1-DNA binding sites. These studies, although being at the early stage, showed that STAT1 binds to many sites on chromosome 22 and revealed many new candidate target genes not previously associated with IFN-responsive genes that may be induced only in certain cell types and under specific conditions. In addition, microarray approaches also disclosed novel mechanisms for the regulation of STAT1-binding site selection (Martone et al., 2003; Hartman et al., 2005; Wormald et al., 2006a).

STAT1 in cancer

The discovery that transcriptionally active STAT1 is required for IFN-α and IFN-γ to exert growth inhibition of cultured cells (Muller et al., 1993; Shuai et al., 1993; Bromberg et al., 1996) has led to the assumption that inadequate function of STAT1 might result in cell growth deregulation and disturbed immune functions, i.e. disorders that are pertinent to malignancy. The potential involvement of STAT1 in cancer was supported by several observations reporting on STAT1 inappropriate activation and even loss of its expression in malignant cells derived from different histological types of tumours such as breast cancer, head and neck

cancer, melanoma, leukaemia, and lymphoma (Bowman et al., 2000; Levy and Gilliland, 2000; Buettner et al., 2002; Boudny et al., 2003; Kovarik et al., 2003, Kovarik et al., 2005). However, the convincing evidence on the STAT1 role in malignant tumours was brought by studies employing STAT1 knockout mice. Although no increased spontaneous malignancy was observed in STAT1-deficient mice, they manifested heightened susceptibility to both chemically induced and transplanted tumours compared to control, STAT1-expressing animals (Kaplan et al., 1998; Lee et al., 2000a; Lee et al., 2000b). These data sustained the idea that STAT1 might function as a tumour suppressor (Durbin et al., 1996). Subsequent studies revealed that STAT1 involvement in oncogenesis is more complex and that only a part of its tumour growth-suppressing activity is attributable to the loss of IFN direct anti-proliferative effects due to STAT1 deficiency.

Kaplan et al. (1998) demonstrated that the elevated growth of tumour cells in STAT1 deficient, i.e. IFN-γ insensitive mice, is at least partly due to the absence of well-known IFN-γ effects on the tumour cell immunogenicity and/or host response to tumour antigens. Since IFN-γ represents a critical immune system component required for a tumour surveillance machinery, it is likely that impaired STAT1 function negatively affects the immunogenic phenotype of developing tumours. Thus, the impaired responsiveness to IFN-γ due to STAT1 dysfunction may cause selective growth advantage of some malignant cells at the early stage of tumour development, the process known as cancer immuno-editing (Shankaran et al., 2001; Ikeda et al., 2002). Several reports showing that some spontaneous human tumours are selectively unresponsive to IFN-γ due to perturbed STAT1 activation suggest that similarly to animal models, the STAT1-dependent tumour surveillance mechanism also operates in humans (Wong et al., 1997; Xia et al., 1998; Pansky et al., 2000; Widschwendter et al., 2002; Boudny et al., 2005; Timofeeva et al., 2006).

Discovery that transcriptionally active STAT1 is required for apoptosis in some cell types (Chin et al., 1996; Kumar et al., 1997; Su et al., 1997; Bromberg et al., 1998; Sahni et al., 2001) paved the way for revealing an additional mechanism by which dysfunctional STAT1 may contribute to cancer development and progression. It has been well documented that cancer cells are less susceptible to death stimuli and possess impaired balance between pro-apoptotic and pro-survival gene expression, the later being prevailing. The association of STAT1 deficiency with disorders in apoptosis was first drawn from STAT1-deficient mice studies. The increased susceptibility of these animals to carcinogen-induced oncogenesis is at least in part due to down-regulation of pro-apoptotic genes, whose expression is to some degree dependent on IFN-γ and transcriptionally active STAT1 (Kaplan et al., 1998). Moreover, reduced susceptibility to apoptosis was reported in lymphocytes derived from STAT1-deficient mice, and the correlation with depressed levels of caspases 1 and 11 was noted (Lee et al., 2000b). The data obtained from transgenic mice confirmed the pro-apoptotic role of STAT1 *in vivo* and impaired apoptotic pathways in STAT1-deficient systems (Kumar et al., 1997; Mascareno et al., 2001; Sahni et al., 2001). Several molecular mechanisms that may contribute to perturbed apoptosis due to loss and/or aberrant STAT1 signalling in diverse tumours have been proposed. The lack of transcriptionally active or otherwise altered STAT1 may downregulate expression of caspases, Fas, FasL TRAIL and p21/waf1 genes (Chin et al., 1996; Kumar et al., 1997; Xu et al., 1998; Huang et al., 2000; Lee et al., 2000c; Stephanou et al., 2000; Shin et al., 2001; Suk et al., 2001). Some other reports relate the STAT1 role in the apoptotic deregulation of the blockade of IFN-γ signalling and subsequent deficit in the inducibility of pro-apoptotic proteins, Bak and Bax (Ossina et al., 1997; Koshiji et al., 1998). Regardless that execution of apoptosis requires multiple signalling pathways and gene systems, data so far available show that STAT1 represents another protein that controls cellular apoptosis via STAT1/caspases interactions and/or through the constitutive regulation of caspase expression. Interestingly, there is experimental evidence that STAT1 may mediate constitutive expression of caspases independent of STAT1 tyrosine phosphorylation and dimerization (Chatterjee-Kishore et al., 2000). The findings that doxorubucin and cisplatin potentiate STAT1 phosphorylation, which in turn enhances drug-induced apoptosis, may suggest a novel mechanism through which some anticancer agents cause cell death (Thomas et al., 2004; Townsend et al., 2004). However, whether and to what extent STAT deregulation decreases or abolishes tumour cell apoptosis in clinical situations and what are the kinds of key molecular or functional damage that interfere with the pro-apoptotic function of STAT1 remains to be established.

Conclusion

The studies briefly outlined in this review highlight the importance of STAT1 in the physiological processes that control cell growth, proliferation, apoptosis, and functions of the immune system. The discovery that this molecule may act as a tumour suppressor via multiple mechanisms as well as observations that some oncogenic events are intimately associated with impairment of STAT1 function should direct future research. First, the precise understanding of dominant molecular changes that interfere with STAT1 normal function in cancer cells is highly needed for monitoring their occurrence and frequency in diverse human tumours as well as for clarifying their possible diagnostic and prognostic value. More should be learned about STAT1 co-activators and/or factors that are controlled by this

molecule to disclose new possibly altered pathways that might deregulate STAT1 function. It should also be established whether impaired STAT1 function occurring at the early stage of transformation may be suggestive for its direct involvement in generation of the malignant phenotype, or whether STAT1 defects are merely a consequence of complex genomic and extragenomic changes that gradually accumulate during tumour progression. Moreover, the important question whether STAT1, besides the nucleus, also functions in the extra-nuclear compartments still remains unanswered. The above reports proving on the experimental models that most cancers associate with STAT1 disturbances urgently call for exploration of STAT1 aberrations in human malignancies *in vivo* and their possible link with the tumour behaviour.

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