EUROPEAN JOURNAL OF CANCER 42 (2006) 2668–2670

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Editorial Comment

JAK/STAT signalling pathway in colorectal cancer: A new biological target with therapeutic implications

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The Janus family of tyrosine kinases (JAK) and the signal transducer and activator of transcription (STAT) family are critical components of diverse signal transduction pathways that are actively involved in cellular survival, proliferation, differentiation, and apoptosis. Accumulating evidence also supports a role for STAT proteins in oncogenesis. STATs transduce signals emanating from the large haematopoietin subfamily of cytokines, including the interferon, the gp130, the γC and the single chain families of receptors.[1] STATs can also be activated by receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), colony stimulating factor-1 receptor (CSF-1R), and platelet derived growth factor receptor (PDGFR). Several members of the G-protein-coupled receptors have also been shown to signal through STATs.[2]

The binding of a cytokine to its receptor induces the receptor's oligomerisation which in turn triggers the activation of JAKs by either auto- or transphosphorylation. There are four members of the JAK family in mammals, Jak1, Jak2, Jak3 and Tyk2. Jak1, Jak2 and Tyk2 are expressed ubiquitously, whereas the expression of Jak3 is restricted to cells of the myeloid and lymphoid lineages.[3] The activated JAKs phosphorylate the receptors on specific tyrosine sites, which generate docking sites for the recruitment of cytoplasmic monomeric STAT proteins *via* their SH2 (Src homology 2) domains. Subsequently, the recruited STATs are phosphorylated by activated JAKs and dimerise *via* reciprocal phosphotyrosine-SH2 domain interactions. STAT 3 is also regulated by phosphorylation on its serine residue 727.[4] This phosphorylation can be inhibitory and the kinase responsible is not clearly identified. The dimers translocate to the nucleus, where they bind to specific DNA response elements which regulate gene expression.[5] STAT signalling is thought to be terminated by dephosphorylation through nuclear tyrosine phosphatases and/or through proteolytic degradation. Moreover, few phosphatases such as SHP1 are known to dephosphorylate JAK/STAT proteins and SHP1 remains one of the phosphatases identified which downregulates STAT3.[6] Seven members of the STAT family encoded in distinct genes have been identified in mammalian cells: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT 6. STAT1 and STAT3 exhibit two isoforms each resulting from alternative splicing[5] whereas STAT5a and STAT5b are encoded by distinct genes. STAT proteins have a well-defined structure including a DNA-binding domain, a conserved NH_2-terminal domain, a COOH-terminal transactivation domain, and an SH2 domain. Concerning STAT signalling pathway, it is modulated by interacted molecules: among them, the SOCS protein and PIAS protein have been described to play a crucial role in the regulation of STAT signalling pathway.[7] The SOCS protein belongs to a group of cytokine-inducible genes that have been shown to inhibit STAT signalling by binding to JAKS. Some of the SOCS protein can also be regulated by STATS themselves, providing that STAT can regulate its own phosphorylation status.[8] For instance, SOCS-3 has been demonstrated as a negative regulator of STAT3 and STAT5 activation. As for PIAS proteins, they generally play a role by decreasing DNA activation via STAT DNA-binding activity.[9] The overexpression of PIAS1 and PIAS3 represents a specific nuclear inhibition of STAT1 and STAT3 and by consequence prevents the activation of STAT-dependent gene transcription.

The mechanism underlying the EGFR-induced STAT activation is thought to involve the kinases of the JAK family and/or the non-receptor tyrosine kinase Src.[10,11] At least three different family members (STAT1, STAT3 and STAT5) are activated by EGF.[7,12]

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.07.006

The contribution of specific STAT family members to the control of normal cellular processes has been partially elucidated based on studies of homozygous deletion or by tissue-specific, conditional knockout of each STAT family member in mice.[5,11] STAT1-deficient mice show defective innate immune responses to viruses and bacteria because of the absence of interferon signalling, and Stat2 knockout mice exhibit defective responses to interferon α/β. Targeted disruption of the Stat4 or Stat6 gene in mice indicates that these STATs are required for IL-12- or IL-4-induced proliferation of activated T lymphocytes, respectively. STAT5a knockout experiments demonstrated that the product of this gene mediates prolactin signalling along with mammary gland development, whereas disruption of STAT5b abrogates sexually dimorphic liver gene regulation and is associated with a loss of the characteristic male body growth rates. STAT5a/b double knockouts show, in addition to impaired breast development, defects in haematopoiesis. Unlike STAT1, STAT3 deficient mice display a very early embryonic lethality, indicating a critical role in normal development. Tissue-specific gene deletions have contributed to elucidate the critical role of STAT3 in the regulation of epithelial cell apoptosis, involution in the postlactating mammary gland, skin remodelling, keratinocyte migration, macrophage inactivation, and down-regulation of inflammatory cytokines in T-helper cell responses.[7]

In normal cells, STAT-mediated gene regulation is both transient and tightly regulated, whereas constitutive activation of STATs, in particular STAT3 and STAT5, is associated with permanent changes in the expression of genes that control fundamental cellular processes subverted in oncogenesis. As summarised by Bowman et al.,[11] four major findings have identified STATs as critical mediators in development and progression of human tumours: (1) STATs are selectively activated by oncogenic tyrosine kinase signalling pathways; (2) dominant-negative STAT mutants block STAT-dependent transcription and transformation induced by activated TKs; (3) constitutively-activated mutants of STATs can induce some aspects of cell transformation; and (4) inappropriate activation of STATs in oncogenesis leads to induction of genes involved in the control of cell proliferation and survival.

The role of STATs in oncogenesis involves up-regulation of genes encoding apoptosis inhibitors (*Bcl-x_1*, *Mcl-1*), cell cycle regulators (cyclins D1/D2, *c-Myc*), and inducers of angiogenesis (VEGF).[7] In recent years Stat3 has gained status as an oncogene,[11] which is consistently activated in transformed cell lines and increasingly found in activated form in human cancers.[13] Constitutive activation of Stat3 has been reported in malignant solid tumours (established tumour cell lines and/or clinical tumour samples) of the breast, head and neck, prostate, renal cell, melanoma, brain, ovarian, lung, pancreas, as well as in blood malignancies (leukaemia, lymphoma, and multiple myeloma).[7,11] STAT5 is also commonly found to be constitutively activated in haematological malignancies, especially leukaemias and lymphomas. The involvement and implication of STAT5 in lymphoma and leukaemia, when overexpressed, was for instance demonstrated in mice.[14,15] Schwaller et al.[16] have also demonstrated that STAT5 was essential for the development of myelo-and lymphoproliferation induced by the Tel-Jak2 translocation. RAR-STAT5 translocation was also described in human leukaemia by Maurer et al.[17] and at the first time by Gouilleux-Gruart et al.[18] in 1996.

Several different mechanisms account for the activation of STATs including overexpression or dysregulation of kinases (such as EGFR) or inhibition of negative regulators.

Very few studies have reported the abnormal expression or activation of STATs in colorectal cancer. STAT3 activation in colorectal carcinoma (CRC) was investigated in tumour cell lines and limited series of tumour samples in two recent publications.[19,20] Ma et al. showed that protein levels of phospho-STAT3 (activated form), cyclin D1 and *Bcl-x_L* were increased in 45 primary CRC samples as compared to adjacent normal mucosae.[19] A significant correlation was also demonstrated between the phosphorylation of STAT3 on tyrosine and cyclin D1 expression. Corvinus et al. reported that constitutive Stat3 activity is abundant in dedifferentiated cancer cells and in infiltrating lymphocytes of CRC samples (32 biopsies evaluated), but not in non-neoplastic colon epithelium.[20]

Tadlaoui Hbibi et al. report for the first time the combined detection of EGFR and STAT3 and their tyrosine phosphorylated forms in a large series of primary CRC samples (113 patients) and conclude that the combination of these factors results in a particularly aggressive profile.[21] The Tyrosine-992 and phosphorylated forms of the EGFR, the levels of tyrosine 416-phosphorylated p60c-src and the tyrosine 705 phosphorylated form of STAT3 were determined by tissue microarray in this 113 CRC patient population. A positive reactivity was observed for P-p60c-src in 76% of the cases, for EGFR in 76% of the cases, for STAT3 in 94% of the cases and for P-STAT3 in 71% of these clinical samples. Multivariate analysis showed that the detection of STAT3 and P-STAT3 was independently associated with vascular emboli ($p = 0.009$ and $p = 0.009$, respectively) and perineural invasion ($p = 0.01$ and $p = 0.01$, respectively). As previously observed by the same authors,[22] vascular emboli, lymph-node involvement, perineural invasion, synchronous metastases and tumour differentiation status were significantly associated with overall survival and these parameters thus appear to be of prognostic value for CRC outcome. Of note, the expression of the EGFR and STAT3 and the detection of their tyrosine phosphorylated forms did not constitute independent prognostic factors for overall survival. These results provide evidence that, as in a number of other solid tumours, STAT3 is constitutively activated in CRC suggesting that this transcription factor may play an important role in the tumourogenesis of CRC. Therefore, targeting STAT3 may also be an interesting therapeutic option in CRC. Indeed, inhibition of STAT signalling has repeatedly been demonstrated to result in growth inhibition and induction of apoptosis in tumour cells harbouring constitutive activation of STAT3 or STAT5.[7] Different strategies are currently under investigation in other solid tumours, i.e. the blocking of tyrosine kinase activity upstream of STAT pathways using selective TK inhibitors (Src or JAK or EGFR inhibitors),[23] or the use of more specific inhibitors of STAT signalling including antisense oligonucleotides or gene therapy with dominant-negative STATs.[7,24] Recently small phosphotyrosyl peptides were shown to effectively block STAT3-mediated DNA binding activity, gene regulation and cell transformation.[25] Targeting multiple sites in the signal transduction pathways (i.e. EGFR and JAK/STAT pathways) may be

an interesting therapeutic strategy to be explored in the future management of CRC,[26] likely in head and neck cancer as recently reported.[27]

Conflict of interest statement

None declared.

Acknowledgements

The authors are very grateful to Foundation BETTENCOURT-SCHUELLER and the Association Pour La Vie-Espoir Contre Le Cancer (AVEC) for providing its support for many years to enable this work to go through.

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