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Hitting the right spot: Mechanism of action of OPB-31121, a novel and potent inhibitor of the Signal Transducer and Activator of Transcription 3 (STAT3)

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ABSTRACT

STAT3 is a key element in many oncogenic pathways and, like other transcription factors, is an attractive target for development of novel anticancer drugs. However, interfering with STAT3 functions has been a difficult task and very few small molecule inhibitors have made their way to the clinic. OPB-31121, an anticancer compound currently in clinical trials, has been reported to affect STAT3 signaling, although its mechanism of action has not been unequivocally demonstrated. In this study, we used a combined computational and experimental approach to investigate the molecular target and the mode of interaction of OPB-31121 with STAT3. In parallel, similar studies were performed with known STAT3 inhibitors (STAT3i) to validate our approach. Computational docking and molecular dynamics simulation (MDS) showed that OPB-31121 interacted with high affinity with the SH2 domain of STAT3. Interestingly, there was no overlap of the OPB-31121 binding site with those of the other STAT3i. Computational predictions were confirmed by *in vitro* binding assays and competition experiments along with site-directed mutagenesis of critical residues in the STAT3 SH2 domain. Isothermal titration calorimetry experiments demonstrated the remarkably high affinity of OPB-31121 for STAT3 with K_d (10 nM) 2–3 orders lower than other STAT3i. Notably, a similar ranking of the potency of the compounds was observed in terms of inhibition of STAT3 phosphorylation, cancer cell proliferation and clonogenicity. These results suggest that the high affinity and efficacy of OPB-31121 might be related to the unique features and mode of interaction of OPB-31121 with STAT3. These unique characteristics make OPB-31121 a promising candidate for further development and an interesting lead for designing new, more effective STAT3i.

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1. Introduction

Signal Transducers and Activators of Transcription (STATs) are a family of latent cytoplasmic proteins that once activated regulate many aspects of cell growth, survival and differentiation (Levy and Darnell, 2002; Yu et al., 2009). The main function attributed to STAT proteins is to act as signal transducers and transcription factors with the ability to transmit signals from the cell membrane to the nucleus (Levy and Darnell, 2002; Yu et al., 2009). However, recent studies have revealed a far more complex picture with a range of novel and diverse functions associated with STAT signaling both in the nucleus and other cell compartments (Sehgal, 2008; Xu et al., 2007; Yu et al., 2014). The STAT family includes seven members (STAT1, 2, 3, 4, 5a, 5b, and 6) that share extensive structural homology (Yu et al., 2009). The main structural motifs of STAT proteins are the N-terminal domain (NTD), coiled-coil domain (CCD), DNA-binding domain (DBD), Src Homology 2 domain (SH2) and C-terminal domain (CTD). The NTD and CCD are required for nuclear translocation and protein–protein interaction, respectively (Levy and Darnell, 2002; Lim and Cao, 2006). The DBD is necessary for the recognition of specific DNA sequence elements and binding to gene promoters. The SH2 domain is the most conserved domain of the family and is required for formation of STAT3 dimers upon phosphorylation of specific tyrosine residues in the CTD of STAT proteins (Lim and Cao, 2006; Zhong et al., 1994). In the case of STAT3 the key event is the phosphorylation of tyrosine 705 (pY705). This promotes the interaction between the SH2 domains of distinct monomers and has been considered the main pathway of activation of STAT3 signaling to the nucleus. pY705 is induced by binding of cytokines and growth factors to the respective receptors and consequent activation of the receptor-associated tyrosine kinases, like Janus Kinases (JAK) (Yu et al., 2009). Other non-receptor associated kinases, such as Src, also activate nuclear STAT3 signaling through the phosphorylation of Y705. Furthermore, in addition to Y705 phosphorylation, STAT3 is phosphorylated at serine 727 (pS727) by various serine protein kinases (Zhang et al., 1995). This modification has been reported to enhance the STAT3 transcriptional activity (Wen et al., 1995) and, more recently, to control mitochondrial localization and function of STAT3 (Gough et al., 2009; Wegrzyn et al., 2009). Acetylation and methylation by protein acetyltransferases and methyltransferases play also relevant roles in controlling STAT3 functions in normal and pathological conditions (Kim et al., 2013a; Lee et al., 2012; Yu et al., 2014; Yuan et al., 2005). Furthermore, un-phosphorylated STAT3, present both in the cytoplasm and nucleus, form dimers and has biological activity as transcription factor and signal transducer independent of its phosphorylation status (Liu et al., 2005; Sehgal, 2008; Timofeeva et al., 2012; Yang et al., 2007).

Alterations of the STAT3 signaling are associated with different human diseases (O'Shea and Plenge, 2012). STAT3 is over-expressed and activated in many human cancers and promotes cell proliferation, survival, tumor angiogenesis and immune-evasion (Sansone and Bromberg, 2012; Yu et al., 2009). Activation of the JAK/STAT3 pathway has been shown to contribute to tumor initiation and progression in various cancer models (Yu et al., 2014, 2009). Recently, activation of STAT3 has been associated with promotion and maintenance of cancer stem-like cells (CSC), tumorigenicity and metastatic capability in many human cancers, including prostate cancer (Kroon et al., 2013; Marotta et al., 2011; Schroeder et al., 2014; Yu et al., 2014). In many cancers activation of STAT3 is associated with advanced disease, metastasis and clinical progression (Sansone and Bromberg, 2012; Yu et al., 2009). The JAK/STAT3 pathway contributes also to reduced response to treatment promoting survival and development of resistance after treatment with kinase inhibitors or, in prostate cancer, after androgen deprivation therapy (Lee et al., 2014; Schroeder et al., 2014; Sos et al., 2014). We have shown recently that activation of the JAK/STAT3 pathway contributes the establishment of immune-tolerance and chemoresistance in a prostate cancer mouse model through the secretion of immunosuppressive cytokines in the tumor microenvironment (Toso et al., 2014).

Over-activity of STAT3 in human cancers is frequently the result of deregulation of upstream pathways leading to activation of cytokine and growth factor receptor associated tyrosine kinases, like JAK family kinases (Grivennikov and Karin, 2008; Sansone and Bromberg, 2012; Yu et al., 2014). Alternative pathways controlling transcriptional and non-transcriptional functions of STAT3 may have also important roles in abnormal activation of STAT3 signaling in cancer (Meier and Larner, 2014; Yu et al., 2014). In prostate cancer STAT3 has been reported to induce cell transformation and tumor development in the absence of pY705 (Qin et al., 2008). The oncogenic effect of STAT3 in this system depended on pS727 and transcriptional dependent and independent functions of STAT3 (Qin et al., 2008). Acetylation and methylation are also crucial for the role of STAT3 in the acquisition of cancer stem cell-like phenotype and tumor progression (Kim et al., 2013a; Su et al., 2011).

Because of its central role in multiple oncogenic pathways and its diverse functions, STAT3 is an attractive target for development of anticancer drugs and great effort has been devoted over the last decade to the discovery of small molecule inhibitors (Debnath et al., 2012; Miklossy et al., 2013; Yu et al., 2009). Inhibitors of STAT3 can be classified as direct and indirect inhibitors (Benekli et al., 2009; Debnath et al., 2012). Indirect inhibitors interfere with cytokine and growth factor receptors or upstream kinases that phosphorylate STAT3. Conversely, direct inhibitors interact with the STAT3 protein and are expected to interfere with its multiple functions (Debnath et al., 2012). Direct inhibitors can be divided based on targeted protein domain, e.g. the NTD, DBD or SH2 domain. Due to its critical involvement in STAT3 activation, the SH2 domain has been seen as the most attractive site and SH2-targeting compounds constitute the largest class of direct STAT3i (Debnath et al., 2012).

Genetic knockout, knockdown and small molecule inhibitors of STAT3 have been shown to prevent tumor development and growth in preclinical models (Chan et al., 2004; Kortylewski et al., 2005; Yu et al., 2009). However, despite the preclinical evidence that STAT3 would be an ideal target for cancer therapy, effective strategies to inhibit STAT3 in the clinic are still lacking

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