Src as a potential therapeutic target in non-small-cell lung cancer

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Lung cancer is the most common cause of cancer-related death, with non-small-cell lung cancer (NSCLC) accounting for 80%–85% of all cases. Although survival rates are reasonably good for patients diagnosed with very early disease, the majority of patients present with advanced disease. For these patients, palliation and improvements in quality of life are the primary goals of therapy. Although chemotherapeutic agents remain the cornerstone of first-line therapy, these agents have limited use in patients who have relapsed and have metastatic disease. Therefore, new strategies are required to improve survival and quality of life in this setting. With the substantial advances in our understanding of tumour biology, it has been possible to identify signalling pathways involved in mediating tumour growth and progression. These pathways offer targets for new biological agents such as small molecule inhibitors and monoclonal antibodies. One such target is Src, a tyrosine kinase that is involved in multiple aspects of tumorigenesis including proliferation, migration and angiogenesis. Increased levels of Src expression have been found in a range of cancers, especially breast, colorectal, prostate and lung. Preliminary preclinical data and pharmacodynamic data suggest that Src inhibition is a viable therapeutic option in the treatment of advanced NSCLC.

Key words: non-small cell lung cancer, Src, targeted therapy, tyrosine kinase

introduction

Lung cancer will cause an estimated 31% of cancer deaths in men and 26% of cancer deaths in women in the United States in 2007 [1]. Although lung cancer mortality rates have been falling for men since the 1990s, rates in women are continuing to rise. In recent years, more women have died of lung cancer than breast and colorectal cancer combined [1]. The situation in Europe is similar, with lung cancer being the most common cause of death from cancer in men, although breast cancer is responsible for more deaths in women [2]. Non-small-cell lung cancer (NSCLC) accounts for ~80%–85% of cases, with the remaining 15%–20% of patients presenting with small-cell lung cancer. Current first-line treatments for patients with NSCLC include chemotherapy with a platinum agent in combination with a taxane or other cytotoxic agent such as gemcitabine or vinorelbine. Many patients with advanced NSCLC, however, have only a partial response to initial chemotherapy, and even those who do respond will subsequently progress. Weekly docetaxel is one of the three standard second-line therapies for patients who fail to respond to, or who are unable to tolerate, platinum-based regimens. Pemetrexed has recently demonstrated clinically equivalent efficacy to docetaxel, but with significantly fewer side effects. In addition, the epidermal growth factor receptor (EGFR) inhibitor erlotinib has also been approved for the treatment of locally advanced or metastatic NSCLC, after failure of one or two chemotherapy regimens. Nonetheless, the prognosis for patients with locally advanced or metastatic NSCLC remains poor. Indeed, the 5-year survival rate for all stages of lung cancer combined is 15% [3] with recent survival gains with chemotherapy and radiotherapy measured in terms of months. Therefore, as with other cancers, specific targeting of aberrant signalling or metabolic processes involved in tumorigenesis has become a new focus of NSCLC therapy. The cellular tyrosine kinase, Src, offers a particularly promising molecular target for anticancer therapy, as inhibition of Src leads to inhibition of multiple signalling pathways. Src tyrosine family kinases are key regulators of cellular proliferation, survival, motility and invasiveness [4]. This review will discuss the role of Src in the development and maintenance of NSCLC and its potential role in targeted therapy for this disease.

the role of Src in tumorigenic and metastatic processes

Aberrant Src activation has been implicated in the development of numerous human cancers, including cancer of the lung, prostate, pancreas, breast and colon [5]. Src is a member of the...
Src family of tyrosine kinases, which consists of nine known members: Src, Yes, Fgr, Yrk, Fyn, Lyn, Hck, Lck and Blk. These proteins are nonreceptor tyrosine kinases that are localised within the cytosol and transduce signals between cell surface proteins, other intracellular proteins and transcription factors [6]. Under physiologic conditions, Src is normally maintained in an inactive state—via phosphorylation of an amino acid near the C-terminus of the protein [7]. Dephosphorylation of this amino acid changes the conformation of Src and results in the autophosphorylation of another tyrosine residue within the activation loop of the protein. The protein is then fully active and able to interact with other proteins.

Increased Src activity up-regulates a number of signalling cascades associated with tumour development and progression leading to increased cell growth, migration and invasion (Figure 1). In epithelial tumours, the levels of expression or activation generally correlate with disease progression [8]. In addition, Src activity is higher in metastatic tissue compared with primary tumours and cells with limited metastatic potential [9]. Src mediates epithelial-mesenchymal transition (EMT), which changes the tumour tissue architecture and enables metastatic progression [10]. C-Met-mediated activation of Src causes down-regulation of E-cadherin, an event that is critical for EMT and tumour invasion [10].

**Src signalling in NSCLC**

Increased expression of Src has been reported in 60%–80% of adenocarcinomas and bronchioloalveolar cancers and in 50% of squamous cell carcinomas isolated from patients with NSCLC [11]. In addition, high levels of Src kinase activity have been reported in NSCLC, particularly adenocarcinomas, with the degree of kinase activity correlating with tumour size [12]. In a study of 60 cancer cell lines, the NSCLC lines had the highest median Src activity [13]. Furthermore, the mitogenic effects of both nicotine [14] and asbestos [15] in NSCLC cells are likely to involve the activation of Src.

Src may stimulate tumorigenesis in NSCLC in a variety of ways. Schematic Src-signalling pathways are shown in Figure 2. One of the most well-described Src-mediated pathways involves signal transducer and activator of transcription (STAT)-3 and focal adhesion kinase (FAK), both of which are involved in tumour survival [16, 17]. STATs are transcription factors that mediate expression of genes involved in cell cycle progression and apoptosis. FAK is a tyrosine kinase involved in integrin signalling, and elevated FAK levels have been associated with increased cell motility, invasion and proliferation in cancer cells [18]. Src-mediated constitutive STAT-3 activity has been found in multiple NSCLC lines [16]. Studies show that activation of STAT-3 and FAK by Src is required for anchorage-dependent and -independent cell growth in a range of human tumours including NSCLC [16, 17]. In addition, in NSCLC, stimulation of STAT-3 by epidermal growth factor (EGF), interleukin 6 and hepatocyte-derived growth factor all require Src activity [16]. Another growth factor involved in Src-STAT-3 signalling is prostaglandin E2, which activates Src, and results in growth of lung cancer cells [19]. Src also activates the VEGF pathway via STAT-3 [20] and in response to hypoxia in human lung adenocarcinoma cells, thus increasing the blood supply to the oxygen-starved tumour [21].

In human lung tumour cells, Src activity is also associated with inhibition of anoikis—a form of cell death induced by the detachment of adherent cells from their substratum [22]. Following detachment from the primary tumour, Src activity is increased in adenocarcinoma cells. It is thought that up-regulation of Src is able to compensate for the loss of survival signals from the cell matrix. When Src is inhibited, the detached cells undergo anoikis.

**Src interaction with EGFR**

Src offers a particularly promising molecular target for anticancer therapy, as inhibition of Src leads to inhibition of
Src activation of EGFR is found in a subset of NSCLC tumours that are dependent on EGFR for survival, and selective inhibition of EGFR has demonstrated some success in the treatment of NSCLC.

Studies in nude mice show that Src and EGFR work synergistically giving rise to a particularly aggressive phenotype [27]. Tumours in nude mice inoculated with Src/EGFR overexpressing fibroblasts were significantly larger than those inoculated with fibroblasts overexpressing either Src or EGFR alone [27]. Src is also required for both EGF- and LPA-induced growth in a time- and dose-dependent manner [44]. AZD0530 has been shown to be active in preclinical models of CML and solid tumours. In multiple NSCLC lines, AZD0530 blocked cell growth and prevents tumour invasion in NSCLC cells treated with dasatinib show decreased cell growth, invasion [32, 33] (Figure 3). In EGFR-dependent NSCLC cell lines, treatment with dasatinib results in apoptosis [32]. In the clinical setting, initial pharmacodynamic data have demonstrated that patients with solid tumours exposed to dasatinib show substantially inhibited Src activity [42]. Furthermore, no dose-limiting toxicity was observed in a dose-escalation study in patients with solid tumours [43].

AZD0530 is an orally active dual Src/Abl inhibitor that has been shown to be active in preclinical models of CML and solid tumours. In multiple NSCLC lines, AZD0530 blocked cell growth in a time- and dose-dependent manner [44]. AZD0530 is an orally active dual Src/Abl inhibitor that has been shown to be active in preclinical models of CML and solid tumours. In multiple NSCLC lines, AZD0530 blocked cell growth in a time- and dose-dependent manner [44].
Regarding cardiovascular safety. In all, 16 of 131 patients (12%) experienced serious cardiovascular adverse events.

M475271 is an orally available inhibitor of Src kinase that reduces cellular proliferation and VEGF-mediated neovascularisation in lung adenocarcinoma cell lines [50]. In addition, in M475271-treated natural killer cell-depleted mice, subcutaneous tumours showed retarded growth and lung metastases were inhibited.

The results from these preliminary studies support Src inhibition as a valid strategy for the treatment of solid tumours, and the results of ongoing single agent and combination trials of Src inhibitors in NSCLC are eagerly awaited. Further data from prospective phase III trials will be required to confirm the efficacy of these agents in improving clinical outcomes in patients with NSCLC.

Conclusions

Aberrant Src expression and activity occur in many NSCLC tumours, but the clinical significance of this oncogene in the development and proliferation of tumours remains to be quantified. It is clear that the interaction of Src with factors such as FAK and VEGF helps to promote tumour growth and metastasis. In addition, synergy between Src and other tyrosine kinases such as EGFR appears to be an important mechanism for stimulating tumour growth. Preclinical studies suggest that Src inhibition may play a role in treating selected patients with NSCLC. There may also be a role for anti-Src therapy in combination with other targeted treatments. For example, dual attack with Src inhibitors in combination with EGFR inhibitors may prevent the development of EGFR inhibitor-resistant clones. The heterogeneity of NSCLC means that defining predictors of prognosis and selecting appropriate patients for treatment present a clear challenge in this new era of targeted therapy.

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References


