Editorial

The role of c-Src in lung cancer, its metastasis and anti-cancer therapy

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Cancer constitutes the second most common cause of death in developed countries, with deaths due to lung cancer being the most frequent. Of all cancer deaths 90% are caused by the metastasis of the primary tumour to distant sites in the body, displaying a diverse set of clinical features. The vast majority of primary lung cancers are carcinomas derived from epithelial cells[1].

Metastasis is a multi-step process, which leads to the establishment of a secondary tumour. Before they metastasize, the cancer cells of the primary tumour lose their capacity to adhere to their extracellular matrix (ECM) and to each other, and they penetrate the basement membrane of the epithelial tissue invade the surrounding stroma of connective tissue and intravasate into blood and lymph vessels from which they extravasate into a new environment where they can seed and grow[1,2]. This whole process is accompanied by a variety of changes in gene expression and function, such as, for example, the loss of epithelial markers and gain of mesenchymal markers, a process called epithelial-mesenchymal transi-tion[1,3].

Cell adhesion molecules play an important role in carcinogenesis, mainly in tumour growth and metastasis[4]. Normally, adhesive interactions are critical for the proliferation, survival and function of all cells, as cells unable to adhere and spread may undergo programmed cell death[5]. The ECM plays a dual role. On the one hand, it provides the scaffold for the organization of cells, and on the other, it controls cellular behaviour[2]. The heterodimeric cell surface receptors, the integrins, are the major family of receptors mediating cell adhesion to the ECM[5]. Interaction of integrins with their extracellular ligands results in cell adhesion and spread, through both the regulation of actin cytoskeleton organization and the cell-adhesion-mediated transduction of important signalling pathways that affect cell movement and cell proliferation in both normal and cancer cells[2]. The binding of integrins to their ECM ligands leads to activation of the primers and clustering to the sites of focal adhesions. Focal adhesions are structures through which integrins link the outside matrix to intracellular cytoskeletal complexes[5]. As integrins lack intrinsic enzymatic activity, they mediate regulation of the actin cytoskeleton organization and signalling transduction through the recruitment of several kinases, signalling molecules and cytoskeletal proteins into their cytoplasmic tail[5,6]. Focal adhesion kinase (FAK) and c-Src tyrosine kinases are the first molecules to be recruited at the sites of focal adhesions subsequent to the activation and clustering of integrins[6]. The

activation of integrins leads to the autophosphorylation of FAK to its tyrosine residue 397, creating a binding site for the Src family kinases, such as Src and Fyn, which in turn phosphorylate FAK in other tyrosine residues, creating SH2 binding sites for other molecules, such as the adaptor protein Grb2[5]. The Src-FAK complex phosphorylates and recruits other FAK-binding molecules, such as paxilin, p^{130CAS} and tensin[5].

Many mammalian cells, including epithelial cells, depend on their adhesion to their ECM for their survival. If detached, these cell types undergo apoptosis, a phenomenon called "anoikis" from the Greek word for homelessness. Anoikis is a mechanism that eliminates cells displaced from their natural environment. Tumour cells, however, are well known for their ability to grow in the absence of contact with the ECM. The observation that Src and Ras are activated in cells escaping from cell-detachment-mediated anoikis, might constitute evidence that these oncogenes provide a constitutive signal normally originated by integrins[5].

THE SRC FAMILY OF TYROSINE KINASES (SFKS)

c-Src is the cellular homologue of its viral counterpart v-Src, a constitutively active mutant of Src recognized for its ability to transform cells. c-Src was the first protooncogene to be identified in the human genome. It is strongly conserved and potentially capable of inducing cell transformation[7]. c-Src belongs to a family of non-receptor tyrosine kinases named the Src family of tyrosine kinases (SFKs), which have key roles in the regulation of signal transduction via a diverse set of receptors. The family comprises 8 members, named p55Blk, p56Fgr, p59Fyn, p61Hck, p56Lck, p56Lyn, p60Src**,** and p60Yes. Among these, Blk, Fgr, Hck, Lck and Lyn are preferentially expressed in haematopoietic cells, while Src, Fyn and Yes are expressed more generally[7,8].

SFKs have a molecular weight ranging from 52 to 62 kDa and contain six distinct conserved domains[9]. Briefly, beginning from the N-terminal these are: an SH4 domain that facilitates attachment of SFKs to the membrane, a unique domain responsible for specific interaction of the enzyme with particular receptors and protein targets, the very important SH3 and SH2 regulatory domains that recognize and bind to partners containing motifs rich in proline and phosphorylated tyrosines respectively, and a kinase domain. All SFKs contain within their molecule two conserved tyrosines, at the sites 416 and 527, within the kinase domain and the C-terminal respectively, which participate in the regulation of enzyme activity[9,10]. In the inactive form of c-Src, the Tyr-527 residue is phosphorylated. This event facilitates intramolecular interactions within the molecule of c-Src that result in the creation of a rigid "closed" conformation of the enzyme. This phosphorylation is performed by the kinase Csk and it is indicative that c-Src is inactive[9]. In order to be activated, c-Src has to be dephop-shorylated at Tyr-527 and hence to take its "open" conformation. The binding of c-Src with a molecular partner containing phosphorylated tyrosines and/or proline-rich motifs can promote a transient change in c-Src conformation that makes it possibly accessible to phosphatases[10]. This "open" conformation permits the phosphorylation of Tyr-416, resulting in the full activation of c-Src. The constitutive activation of c-Src caused by the substitution of Tyr-527 by another amino acid residue and the fact that the region containing Tyr-527 is absent in the constitutively active v-Src, suggest that this tyrosine is very important for the regulation of c-Src activity[9]. Thus, c-Src activity can be induced by a diverse set of events including: a) tyrosine phosphorylation/dephosphorylation, b) binding of Src to partners containing phosphorylated tyrosines or proline rich motifs, and c) mutation[10].

The SFKs are dormant during the cell cycle and are transiently activated in response to a diverse set of stimuli. The ligation of receptors for growth factors, cytokines, antigens and antibodies, G-protein-coupled recap-tors, adhesion receptors, oxidative stress and mitosis can all lead to the activation of SFKs[11]. A considerable body of evidence indicates the involvement of c-Src in several processes such as gene transcription, cell adhesion, migration, proliferation, apoptosis and differentiation of normal cells[9]. In recent years, SFKs have also been implicated in cancer by the observation that both their protein levels, and to a greater degree enzymatic kinase activity, have been shown to be elevated in certain human neoplastic tissues compared to adjacent normal tissues. The levels appear to increase with the stage of disease. Elevated c-Src activity has to date been detected in breast, colon, pancreatic, neural, ovarian, oesophageal, gastric and lung cancers and melanoma, with a variety of different effects. There are several possible explanations for the elevated activation of c-Src in human tumours. As an example, receptors such as epidermal growth factor receptor (EGFR) and hepatocyte growth factor receptor (HGFR), known to be active in the progression of cancer, can activate c-Src, while c-Src can reinforce the activation of these receptors so that the receptor-c-Src association works instrumentally in malignant transformation. Increased

c-Src activation might be caused by the dephosphorylation of the regulatory Tyr-527 caused by: i) insufficient Csk activity, ii) increased activity of c-Src phosphatases, and iii) c-Src interaction with viral or cellular proteins. Active mutants of c-Src have not been identified in human cancer cells, with the exception of a small subset of colon cancer. c-Src plays central role in multiple signalling pathways that are necessary, or sufficient, to produce the metastatic phenotype. Elevated c-Src activity can promote an increase in the growth rate of cells, reduce adhesion between cells, mediate survival of cancer cells from apoptotic cell death and enhance angiogenesis[12].

SRC IN LUNG CANCER

As described above, c-Src has lately been implicated in the development of several types of cancer, including lung cancer.

There are two main histological groups of lung cancer, named small-cell (SCLC) and non-small-cell (NSCLC) lung cancer, with differences in morphology, the tendency to metastasise, hormone secretion and responsiveness to chemotherapy and radiotherapy[13]. SCLC is thought to be of neuroendocrine origin; it grows and spreads quickly, and accounts for approximately 20-25% of all cases of lung cancer. NSCLC represents the remaining 75% of cases and is subdivided in the following types: adenocarcinoma (35-40%), squamous cell carcinoma (25-30%) and large-cell carcinoma (10-15%).

In 1992, Mazurenko et al, studying the expression of c-Src in the two histological groups of lung cancer, observed an elevated expression in 100% of the examined SCLC and 60% of NSCLC. This finding was quite unexpected, considering the differences in the metastatic and morphological properties of these two groups of cancers and the differences in expression of other oncogenes. In addition they indicated that c-Src was most frequently expressed in adenocarcinomas (80%) and less frequently in squamous cell carcinomas (50%) with a correlation between c-Src expression and the level of differentiation of the squamous cell carcinoma[14]. Later, in 2003, Masaki et al. provided evidence that c-Src might play a role in the malignant transformation of lung carcinomas. They showed that both protein levels and the activity of c-Src are elevated in malignant lung tissues, and preferentially in adenocarcinomas, compared to the surrounding normal tissues. Another important finding was the increase of c-Src kinase activity with the size of the tumour in adenocarcinoma, while the increase in kinase activity was higher than the elevation of the c-Src expression[15].

The activation of c-Src in tumour cells may induce signalling pathways that affect cell growth and survival, accounting for tumour mass formation, and a decrease in cell-cell and cell-ECM adhesion, facilitating tumour invasion and motility through the reorganization of the actin cytoskeleton[16]. Several signalling pathways that participate in the regulation of all these events have been elucidated. The activation of the FAK-Src complex downstream to the activation of integrins results in the recruitment of substrates such as CAS, paxillin, and p190RhoGAP, which have a central role in the reorganization of the actin cytoskeleton and migration[17]. FAK activity has been found to be increased in cancer cell lines and tissue lysates extracted from patients with metastatic cancer and it has also been found to regulate cancer cell migration as a response to ECM stimuli[18,19]. In adenocarcinoma, lung cancer cells stimulated by fibronectin, invasion and migration by FAK is achieved by FAK-Src interaction, through the ERK1/2 and PI3K/Akt signalling pathways which result in proteolytic cleavage of adhesions and ECM degradation through the activation of calpain and Metalloproteinase-9 (MMP-9) and RhoA/MMP-9 expression[19]. c-Src also regulates the proliferation of cancer cells induced by growth factor (GF) receptors. It has been shown in NSLC that c-Src and janus-activated kinase (JAK) proteins are involved in the activation of signal transducer and activator of transcription-3 (STAT-3), which in turn is involved in the increased expression of targets such as Bcl-xL, cyclin D1 and survivin implicated in increased cell survival, proliferation and tumour growth[20]. One of the best described signalling pathways through which Src stimulates tumorigenesis in NSCLC involves STAT-3 and FAK, both of which are implicated in tumour survival. In lung cancer the Src-STAT-3 signalling can be induced by several factors, including the epidermal growth factor (EGF), interleukin 6, hepatocyte growth factor (HGF) and prostaglandin E2. The role of EGFR in lung tumorigenesis has been well studied, as mutations of this receptor are commonly found in NSCLC and also it has been tested for use in molecular-targeted therapy[21]. As described above c-Src acts synergistically with EGFR through mutual phosphorylation and activation, and EGFR-c-Src interaction and mediated signalling may play an important role in the development of cancer. A number of studies in NSCLC with constitutively active EGFR mutations have revealed the importance of c-Src in EGFR-mediated signalling that results in cancer cell survival[22]. Moreover, c-Src has been lately implicated in adenocarcinoma lung cell migration

induced by prostagladin E2 through the EP4 receptor-β-Arrestin1-c-Src signalling pathway[23].

Because of their central role in multiple signalling pathways, Src kinases are very attractive as molecular drug targets in lung cancer[24]. Some of the Src inhibitors that are being investigated in clinical trials are dasatinib, saracatinib (AZD0530), bosutinib[25]. Preliminary data regarding those inhibitors suggest that they are well-tolerated at clinically meaningful drug concentrations[26].

Dasatinib is an orally available Src/Abl inhibitor with significant antiproliferative activity against solid tumour cell lines[26]. Treatment of NSCLC cell lines carrying EGFR mutations with the SFKs tyrosine kinase inhibitor, dasatinib have resulted in increased apoptosis through the down-regulation of Akt and STAT-3 survival proteins. In turn, treatment of NSCLC without EGFR mutations with dasatinib caused arrest of cell cycle, inhibition of activated FAK and prevention of tumour cell invasion[22]. Another study indicated that treatment of NSCLC cells with glabridin inhibits lung cancer and endothelial cell migration and invasion, as well as angiogenesis *in vivo* by inhibiting the integrin/Src/FAK signalling pathway[27]. Bosutinib is a dual Src/Abl kinase inhibitor with activity against other SFKs[26]. It has recently been reported that SFKs are activated in 33% of cases of NSCLC, while treatment of NSCLC cell lines with bosutinib resulted in antiproliferative and proapoptotic effects, and especially in cell lines with an increased autophosphorylation level of activated Src[28]. Saracatinib is another inhibitor of Src and SFKs with activity against Abl and activated mutants of EGFR[26]. In one study, AZD0530 lowered the barriers to apoptosis in lung cancer cells by reducing the levels of Bcl-xL[24], while in another it demonstrated inhibitory effects on migration and invasion[26]. Due to the multifactorial role of Src in tumour progression and its implication in several signalling pathways, it appears that these molecules might work better in combination with other agents[26]. A synergistic inhibition of proliferation and increased apoptosis was demonstrated when dasatinib was combined with an experimental inhibitor of JAK kinase in the NSCLC cell line[20].

Following these promising preliminary results dasatinib, bosutinib and saracatinib have been entered into clinical trials. A phase I/II study of the Src inhibitor dasatinib, in combination with the EGFR inhibitor erlotinib, conducted with patients who had advanced NSCLC showed that the regime was well tolerated and resulted in disease control and inhibition of plasma angiogenesis markers[29]. A phase II study of dasatinib in NSCLC has been performed with 16 patients, of whom one had partial response with no evidence of recurrence for at least 18 months, 6 had stable disease and 9 had progressive disease[30].

To conclude, c-Src plays a central role in numerous signalling pathways that regulate important cellular functions, including proliferation, adhesion, migration and apoptosis. c-Src is overexpressed and highly activated in certain types of cancer, such as lung cancer. Further research could possibly establish c-Src as an important molecular target in anti-cancer therapy, since its inhibition results in the inhibition of several signalling pathways. In addition, it appears that c-Src expression and activity might have a possible use as indices inestimation of the prognosis and the evaluation of the effectiveness of treatment of lung cancer.

REFERENCES

1. Yilmaz M, Christofori G, Lehembre F. Distinct mechanisms of tumor invasion and metastasis. Trends in Molecular Medicine, 2007; Vol. 13, No 12.
2. Guo G, Giancotti FG. Integrin signaling during tumor progression. Nat Rev Mol Cell Biol, 2004;5:816-826.
3. Tzouvelekis A, Karameris A, Bouros D. Telomeres, telomerase and immortality: A common link between lung cancer and pulmonary fibrosis. Pneumon, 2007;20:312-317.
4. Papalimneou V, Charalambopoulos C. Adhesion molecules and lung cancer. Pneumon, 2001;14:109-117.
5. Kumar CC. Signaling by integrin receptors. Oncogene, 1998;17:1365-1373.
6. Mitra SK, Schlaepfer D. Integrin-regulated FAK-Src signaling in normal and cancer cells. Current opinion in Cell Biology, 2006;18:516-523.
7. Thomas SM, Brugges JS. Cellular functions regulated by Src family kinases. Ann Rev Cell Dev Biol, 1997;2:467-475.
8. Courtneidge SA. Role of Src in signal transduction pathways. Biochemical Society Transactions, 2002; Vol. 30, part 2.
9. Tatosyan AG, Mizenina OA. Kinases of the Src family: Structure and functions. Biochemistry, 1999;65:49-58.
10. Bjorge JD, Jakymiw A, Fujita JD. Selected glimpses into the activation and function of Src kinase. Oncogene 2000;19:5620-5635.
11. Lowell CA, Soriano P. Knockouts of Src-family kinases: stiff bones, wimpy T cells, and bad memories. Genes Dev, 1996;10:1845-1857.
12. Irby BR, Yeatman JT. Role of Src expression and activation in human cancer. Oncogene 2000;19:5636-5642.
13. Carney DN, Gazdar AF, Bepler G, et al. Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res, 1985;45:2913-23.
14. Mazurenko NN, Kogan AE, Zborovskaya BI, Kisseljov LF. Expression of $pp60^{c\text{-}src}$ in human small cell and non-small cell lung carcinomas. Eur J. Cancer, 1992;Vol. 28, No 2/3:372-377.
15. Masaki T, Igarashi K, Tokuda M, et al. $pp60^{c\text{-}src}$ activation in lung adenocarcinoma. European Journal of Cancer, 2003;39: 1447-1455.

Inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. J Clin Oncol, 2010;28:1387-1394.
16. Yeatman TJ. A renaissance for SRC. Nat Rev Cancer 2004;4:470-80.
17. Playford MP, Schaller MD. The interplay between Src and integrins in normal and tumor biology. Oncogene, 2004;23:7928-7946.
18. Cance WG, Harris JE, Iacocca MV, et al. Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: correlation with preinvasive and invasive phenotypes. Clin Cancer Res, 2000;6:2417-2423.
19. Meng XN, Jin Y, Yu Y, et al. Characterization of fibronectin-mediated FAK signaling pathways in lung cancer cell migration and invasion. British journal of cancer, 2009;101:327-334.
20. Byers LA, Sen B, Saigal B, et al. Reciprocal regulation of c-Src and STAT3 in non-small cell lung cancer. Clin Cancer Res 2009;15:6852-61. Epub 2009 Oct 27.
21. Giaccone G, Zucali PA. Src as a potential therapeutic target in non-small-cell lung cancer. Annals of Oncology, 2008;19:1219-1223.
22. Song L, Morris M, Bagui T, Lee Y F, Jove R, Haura BE. Dasatinib (BMS-354825) selectively induces apoptosis in lung cancer cells dependent on epidermal growth factor receptor signaling for survival. Cancer Res, 2006; 66:(11).
23. Kim JI, Lakshmikanthan V, Frilot N, Daaka Y. Prostaglandin E2 promotes lung cancer cell migration via EP4-betaArrestin1-c-Src signalsome. Mol Cancer Res 2010;8:569-77. Epub 2010 Mar 30. PubMed PMID: 20353998; PubMed Central PMCID: PMC2855782.
24. Lee D, Gautschi O. Clinical development of Src tyrosine kinase inhibitors in lung cancer. Clinical lung cancer, 2006;7:6:381-384.
25. Rothschild SI, Gautschi O, Haura EB, Johnson FM. Src inhibitors in lung cancer: current status and future directions. Clin Lung Cancer 2010;11(4):238-42. Review. Pub Med PMID: 20630825.
26. Aleshin A, Finn SR. Src: a century of scince brought to the clinic, 2010;18:8:599-607.
27. Tsai YM, Yang CJ, Hsu YL, et al. Glabridin inhibits migration, invasion, and angiogenesis of human non-small cell lung cancer A549 cells by inhibiting the FAK/Rho signaling pathway. Integr Cancer Ther, 2010; 1-9 (in press).
28. Zhang J, Kalyankrishna S, Wislez M, et al. SRC-family kinases are activated in non–small cell lung cancer and promote the survival of epidermal growth factor receptor–dependent cell lines. Am J Pathol, 2007;170:366–376.
29. Haura EB, Tanvetanyon T, Chiapori A. Phase I/II study of the Src inhibitor dasatinib in combination with erlotinib in advanced non-small-cell lung cancer. J Clin Oncol, 2010; 28: 1387-1394.
30. Johnson FM, Tang X, Tran H, et al. Phase II study of dasatinib in non-small lung cancer (NSCLC). J Clin Oncol, 2009.