# Src, chemoresistance and epithelial to mesenchymal transition: are they related?

Ami N. Shah[a,b] and Gary E. Gallick[b]

**The Src family of nonreceptor tyrosine kinases regulates numerous cellular processes, including proliferation, differentiation, migration, survival and angiogenesis. In solid tumors, Src is frequently aberrantly active, and promotes tumor progression and metastasis. Although multiple Src functions may contribute to metastasis, recently Src has been shown to play a role in epithelial to mesenchymal transition. Increased Src activity promotes this process and inhibition of Src suppresses epithelial to mesenchymal transition. Although the molecular events causing epithelial to mesenchymal transition are becoming well defined, the processes in tumor cells that trigger the onset of this phenotype remain unclear. Recent studies have associated epithelial to mesenchymal transition with the development of chemoresistance. Src has also been shown to be involved in chemoresistance of cancer cells. The activation of Src in chemoresistant cells is related to an increase in motility, invasiveness and detachment, all phenotypes characteristic both of Src activation and of epithelial to mesenchymal transition. This review focuses on upregulation of Src in cancer as it relates to chemoresistance and epithelial to mesenchymal transition.** ***Anti-Cancer Drugs*** **18:371–375 © 2007 Lippincott Williams & Wilkins.**

**Anti-Cancer Drugs** 2007, **18**:371–375

**Keywords: chemoresistance, epithelial to mesenchymal transition, metastasis, Src, tumor progression**

Departments of [a]Surgical Oncology and [b]Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Correspondence to Professor Gary E. Gallick, PhD, Department of Cancer Biology, Box 173, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030-4009, USA
Tel: +1 713 563 4919; fax: +1 713 563 5489;
e-mail: ggallick@mdanderson.org

Sponsorship: This research was supported in part by NIH U54 CA 090810 (G.E.G.), NIH T32 CA 09599 (A.N.S.) and The Eleanor B. Pillsbury Fellowship–University of Illinois Hospital (A.N.S.).

**Received** 15 August 2006 **Revised form accepted** 24 October 2006

## Introduction

The Src family of nonreceptor tyrosine kinases has been extensively studied for its key roles in regulating signal transduction pathways that control basic cellular processes, such as cell proliferation, motility, adhesion and survival [1]. Aberrant expression and/or activity of Src is observed in numerous human tumors [2], and the recent development of Src-selective small-molecule inhibitors has led to an increasing number of clinical trials that are testing the efficacy of Src inhibitors as single agents and in combination with chemotherapy and other signal transduction inhibitors in solid and hematologic malignancies. Although these studies are in their infancy, recent advances in the field suggest that abnormal expression or activation of Src may play critical roles in affecting chemoresistance, a major problem in conventional cancer therapy. This review will first provide a basic overview on Src, describe the evidence that increased expression and activity of Src leads to chemoresistance in advanced tumors, and then delineate possible mechanisms by which Src affects resistance including activation of survival pathways such as phosphatidylinositol-3-kinase/Akt, Abl and epithelial to mesenchymal transition (EMT). We will also review evidence of Src tyrosine kinase inhibitors in reversing chemoresistance.

The Src family is comprised of nine nonreceptor tyrosine kinases (Blk, Fgr, Fyn, Hck, Lck, Lyn, Src, Yes and Yrk) of closely related structure, all of which are expressed in a tissue-specific manner. Nearly a century ago, Peyton Rous first described the Rous sarcoma virus, a viral transmissible vector that caused sarcomas in chickens [3], and now known to harbor the viral Src gene (v-Src). The study of the Rous sarcoma virus led to many new discoveries in the field of oncogenesis, including that expression of v-Src in a number of types of normal cells was sufficient for malignant transformation. In 1976, Bishop and Varmus [4] demonstrated that the proto-oncogene c-Src was the progenitor of v-Src, the first member of the Src family of kinases and the first proto-oncogene to be identified.

All Src family kinases have a common overall structure and mode of regulation. Src family kinases are composed of a N-terminal region that is always myristolated, a C-terminal regulatory domain, a poorly conserved unique domain and four Src homology domains. Src homology 4 (SH4) domain is present at the N-terminus as a localization signal, the SH3 domain is involved in intermolecular binding through conserved proline-rich motifs, the SH2 domain binds phosphorylated tyrosines embedded in conserved amino acid sequences and the SH1 domain, the catalytic or kinase domain [2]. In the closed, inactive state of human c-Src, the C-terminal autoinhibitory tyrosine, Y530, is phosphorylated and binds to the SH2 domain with SH3 bound to the kinase

domain. Phosphorylation of Src on Y530 is catalyzed by two known kinases, C-terminal Src kinase and the C-terminal Src kinase homologue kinase [5]. In contrast, oncogenic v-Src is constitutively active through a loss in the C-terminal regulatory domain, thus the v-Src protein cannot form a closed complex and is constitutively activated.

## Src and cancer

Deregulation of Src activity has been implicated in cancer progression through increased proliferation, increased motility and invasiveness, resistance to apoptosis, and increased angiogenesis [2]. Increased Src kinase activity has been found in many epithelial cancers including breast, colon, ovarian, bladder, gastric, lung and pancreatic cancer [6–11]. Src dysregulation is proposed to occur by several different mechanisms, some of which occur simultaneously in tumor cells.

First, several protein tyrosine phosphatases (PTPs), including PTPα, PTP1B and Shp *2*, have been implicated in the activation of Src through the dephosphorylation of Y530. Many of these phosphatases have been demonstrated to be aberrantly activated in various human cancers, leading to dephosphorylation of SrcY530 resulting in Src activation. Rare activating mutations of c-Src have been identified in a small number of cases of colon cancer and endometrial cancer that result in a loss of the negative regulatory role of Y530 [12–14]; however, many additional studies have not found such mutations. Rather, association of Src with aberrantly expressed or mutated growth factor receptors, focal adhesion kinases and other binding partners appears to be the primary mechanisms by which Src is activated in tumors.

A common mechanism of Src activation is through constitutive activation of growth factor receptor pathways. Almost any growth factor can be shown to upregulate Src tyrosine kinase activity leading to an increase in Src-mediated activities of growth, migration and invasion, especially in tumor cells. Many tumors have been noted to have an increase in the protein levels of receptor family tyrosine kinases that can activate Src and lead to constitutive activation of Src within the tumor. In pancreatic cancer, for instance, insulin-like growth factor-1 is known to be overexpressed, leading to Src activation that is required for cell invasion, proliferation and vascular permeability factor/vascular endothelial growth factor (VEGF) expression that can be experimentally demonstrated [15]. Similar results have been shown in colon cancer in which c-Src activation is activated by epidermal growth factor receptor (EGFR) and by c-Met, the receptor for hepatocyte growth factor in highly metastatic cells lines [16]. Many new therapies have been targeted toward this pathway with some success.

One of the types of tumors in which Src is most frequently activated is colorectal adenocarcinoma [14]. Elevated Src kinase levels correlate with the more metastatic phenotypes of colon cancer. Talamonti *et al.* [17] demonstrated that c-Src activity is highest in hepatic metastases of colon cancer and Termuhlen *et al.* [18] extended these results that increased c-Src activity was present in all extrahepatic colonic metastases. These results indicate that c-Src activation contributes to the malignant, more metastatic phenotype of colon cancer and that the level of c-Src activation can play a role as an independent indicator of prognosis [19]. Irby *et al.* [14] showed that a constitutively active c-Src allele increased the invasiveness of a fibroblast cell line dramatically *in vitro* and *in vivo*. By using a Src kinase inhibitor and a Src dominant-negative mutant they further demonstrated a reversible decrease of cell–cell interactions [14,20]. Moreover, in colon cancer E-cadherin has been shown to be downregulated at the cell membrane when Src Y527F was overexpressed [21] and PP2, a Src kinase inhibitor, reverses the disruption of E-cadherin signaling [22]. In pancreatic cancer, Src has been shown to be upregulated by several mechanisms including tyrosine nitration and an upregulation of insulin-like growth factor-1 receptor, upregulating Akt and Src [11,23]. In pancreatic cancer cell lines, overexpression of activated c-Src has been reported to stimulate proliferation and migration and downregulate E-cadherin [24]. In the same study, E-cadherin downregulation in response to collagen could be suppressed by treatment with inhibitors that have some Src family kinase (SFK) selectivity, including PP1 and herbimycin A, inhibiting the effect of Src activation on pancreatic cancer cells. In addition, inhibition of Src by small interfering RNA (siRNA) or the pharmacologic agent of dasatinib, halts the development of metastases [25]. Src kinases have also been implicated in the control of VEGF critical in promoting angiogenesis. Src kinases may control the VEGF production by tumor cells [26] and control of VEGF receptor-regulated survival of endothelial cells. Gray *et al.* [27] demonstrated a mechanism for c-Src induction of VEGF by which hypoxia-induced VEGF expression requires the activation of c-Src. c-Src activation leads to the downstream activation of signal transducers and activators of transcription 3, which binds to the VEGF promoter hypoxia-inducible factor-1α [27]. Moreover, pharmacological inhibition of SFKs with AP23846 led to decreased production of VEGF and interleukin-8 [28]. *In vivo*, the SFK PP2 also showed a decrease in vessel infiltration in a Gelfoam assay using conditioned media from pancreatic tumor cells [29]. Several studies have demonstrated that inhibition of Src by various tyrosine kinase inhibitors in pancreatic cancer has led to decreased carcinoma cell invasion, proliferation, metastasis and angiogenesis [30,31]. Similar evidence of Src activation playing roles in other solid tumors besides colon and pancreas have also been reported in the literature.

## Src and chemoresistance

An increasing number of reports in the literature are linking Src activation to chemoresistance. Expression of v-Src has been shown to induce cisplatin chemoresistance in gallbladder adenocarcinoma, though this was not a global chemoresistance mechanism [32]. Treatment with the SFK-selective inhibitors herbimycin A and radicicol treatment reversed chemoresistance to cisplatin. In a separate study, gallbladder adenocarcinoma cells transfected with v-Src cell lines showed a 200-fold increase in chemoresistance to gefitinib that was partially reversed with herbimycin A [33]. The downstream effects of activated v-Src on these gallbladder carcinoma cell lines were also examined. The v-Src-transformed cells demonstrated activation of EGFR through Erk and Akt pathways that were inhibited in non-v-Src-transformed cell lines when treated with gefitinib (through EGFR blockade) alone, though not in v-Src-transformed cells. Herbimycin A, however, worked in conjunction with gefitinib to block the activation of the Akt and Erk pathways. Duxbury *et al.* [34] developed a gemcitabine-resistant human pancreatic cancer cell line that when inhibited by PP2 *in vitro*, demonstrated an attenuation of gemcitabine chemoresistance. Furthermore, this group showed that increasing gemcitabine resistance in a panel of cell lines was associated with higher Src phosphorylation levels (implying Src activation) with the highest levels observed in the gemcitabine-resistant pancreatic cancer cell line. That Src activation was associated with this phenotype was suggested by lack of increased Src expression in these cells. This group further validated their results by creating a siRNA against c-Src that increased sensitivity to gemcitabine several different pancreatic cancer cell lines [35]. This mechanism of chemoresistance, however, was not shown to be a global mechanism. Duxbury *et al.* [35] reported that modulating c-Src activity did not change pancreatic adenocarcinoma sensitivity to 5-fluorouracil. In our laboratory, we have also created two pancreatic cancer cell lines resistant to gemcitabine that does not show global chemoresistance to 5-fluorouracil and oxaliplatin (A.N.S. and G.E.G., in preparation). Src also plays a role in the aggressiveness of human tumors. Hiscox *et al.* [36] demonstrated that as breast cancer cells became resistant to tamoxifen they behaved more aggressively with increased motility and invasion. Along with tamoxifen resistance, an increase in Src kinase activity was observed. Pharmacological inhibition of Src with AZD0530, a Src/Abl selective inhibitor, resulted in lower Src activity and inhibition of the motile and invasive nature of the cells [36]. Ovarian tumor cells are known to become quickly resistant to standard chemotherapy [37] and Src is overexpressed in a high proportion of these tumors [38]. Chen *et al.* [39] demonstrated that inhibition of ovarian cancer cells in culture by Src by PP2 or siRNA knockdown enhanced the cytotoxicity of paclitaxel and that perhaps more importantly Src inhibition resensitized chemoresistant ovarian cancer cells to paclitaxel and cisplatin. In colon cancer, similar results were found demonstrating that attenuation of c-Src signaling sensitizes metastatic colon cancer cells to apoptosis induced by oxaliplatin and activation of the Fas death receptor [40]. In some chronic myelogenous leukemia and acute lymphoblastic leukemia patients with tumors resistant to STI571. Donato *et al.* [41] found greatly increased expression and activity of the Src family kinase Lyn and demonstrated that PP2 could induce apoptosis in these cells. This group further demonstrated that Lyn and Hck are upregulated in expression and hyperactive blasts from patients that had relapsed on STI571 therapy [41], demonstrating direct relevance of Src activation and drug resistance in the human disease. Src family kinases have also been shown to play a role in bladder carcinoma invasiveness. Increased Src kinase activity has been shown to be present in low-grade bladder lesions as compared with normal tissues [8], and the overexpression of c-Src has been shown to cause a subpopulation of bladder cancer cells to undergo spontaneous EMT and sensitizes the rest of the population to the scattering activity of other growth factors including hepatocyte growth factor. Overexpression of dominant-negative Src inhibits growth factor-induced cell scattering that occurs with EMT [42] and that SFK inhibition was demonstrated to block EMT [43], thus implicating Src in another mechanism of increasing tumor cell invasiveness.

Taken together, these studies present a strong argument that activation of SFKs plays a role in chemoresistance in multiple tumor types, and further, that inhibition of Src may reverse chemoresistance, thus giving a chemotherapeutic agent a second opportunity for efficacy.

## Src and epithelial to mesenchymal transition

As discussed above, Src has long been known to promote the aggressive nature of tumor cells and it plays a major role in the motility, invasion and survival of tumor cells. Recently, Src has been implicated as one of the key regulators of EMT. EMT is described as a transformation in which epithelial cells assume a fibroblastic phenotype. Although phenotypic changes associated with the phenomenon have been described since the early 1900s EMT was not recognized as a distinct process until 1982 by Greenburg and Hay [44]. Although the existence of 'true' EMT remains controversial, generically several signaling pathways are common to EMT that normally occur during development and that are also associated with tumor progression [45]. One of the most important changes involved in EMT is the loss of the cadherin-mediated cell–cell contact, the hallmark of epithelial cells. In cells that undergo morphologic and molecular properties of EMT, the loss of E-cadherin decreases the development of stable cell–cell contacts and the formation of adherens junctions, allowing the cells to become more migratory

and less adhesive. Loss of E-cadherin or its translocation is a characteristic of EMT. E-cadherin is also downregulated in colon cancer when Src is overexpressed and PP2 and another SFK inhibitor, now in clinical trial, SKI-606, reverses this loss [21,46]. Similarly in pancreatic cancer, E-cadherin-negative patients have been noted to have larger tumors, distant metastases and increased stage [47]. When E-cadherin is restored to pancreatic cancer cell lines, apoptosis and decreased cell growth result [48]. The downregulation of E-cadherin has been demonstrated in almost every cancer as a negative prognostic indicator and is linked to metastatic disease.

β-Catenin is another factor important in EMT-like changes that occur in tumor progression. As reviewed in Thiery's [49] recent comprehensive article on EMT, when E-cadherin is downregulated from the plasma membrane, it is no longer able to bind to the cytoplasmic β-catenin, allowing β-catenin to be translocated to the nucleus and act as a transcriptional activator, inducing the transcription factors T cell factor-4 and/or lymphoid enhancer factor. In a study by Karni *et al.* [50], it was shown that active Src elevates the expression of β-catenin and enhances the nuclear accumulation of β-catenin contributing the oncogenic phenotype. To support this result, SKI-606 was shown to relocalize β-catenin to E-cadherin and prevent its nuclear translocation, decreasing cell growth and motility. An siRNA knockdown of β-catenin removed the effect of SKI-606 on cell–cell adhesion, showing that c-Src controls β-catenin function as either a cell adhesive promoter or transcriptional activator [46]. Finally, upregulation of vimentin, a mesenchymal cell marker is a hallmark of EMT that is Src regulated [51]. Thus, a strong foundation is emerging that Src activation promotes phenotypes characteristic of both EMT and chemoresistance.

## Chemoresistance and epithelial to mesenchymal transition

Although several mechanisms have been proposed for the activation of Src leading to chemoresistance, no single global theory has been identified, and mechanisms of resistance vary from tumor type to tumor type and cell line to cell line as well as among different chemotherapeutic agents. The data above suggest that, in multiple tumor types and in resistance to multiple agents, Src activation may lead to chemoresistance by promoting EMT. As tumors progress, dedifferentiate and metastasize, an increase in Src activation is frequently observed and the more metastatic (more invasive, more migratory) phenotype is often linked to EMT. Associated with the dedifferentiated, metastatic phenotype is chemoresistance. In addition, in some invasive breast cancers, vimentin, a marker of EMT, becomes expressed and these cell lines are more resistant to chemotherapy [51]. In our laboratory, we have noted that a pancreatic cancer cell line made resistant to gemcitabine has undergone an EMT-like process in which the cells have lost E-cadherin expression, show β-catenin nuclear translocation, express vimentin, and are more invasive and migratory. These results mirror a recent study published by Yang *et al.* [52], in which human colon cancer cells exposed chronically to oxaliplatin show EMT changes though Src activation was not examined. Undoubtedly, not all chemoresistant cells will have activated Src, nor will all chemoresistant cells be shown to be undergoing EMT. Understanding how the EMT process can be reversed, however, may lead to development of novel chemotherapeutic strategies for chemoresistant late-stage tumors. Thus, the emerging link between these phenotypes should spawn considerable work in the future, and has profound therapeutic implications.

## References


1 Bjorge JD, Jakymiw A, Fujita DJ. Selected glimpses into the activation and function of Src kinase. *Oncogene* 2000; **19**:5620–5635.
2 Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev* 2003; **22**:337–358.
3 Rous P. A sarcoma of the fowl transmissible by an agent separable from tumor cells. *J Exp Med* 1911; **13**:397–411.
4 Stehelin D, Varmus HE, Bishop JM, Vogt PK. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 1976; **260**:170–173.
5 Okada M, Nada S, Yamanashi Y, Yamamoto T, Nakagawa H. CSK: a protein-tyrosine kinase involved in regulation of src family kinases. *J Biol Chem* 1991; **266**:24249–24252.
6 Jacobs C, Rubsamen H. Expression of pp60c-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. *Cancer Res* 1983; **43**:1696–1702.
7 Jung YD, Nakano K, Liu W, Gallick GE, Ellis LM. Extracellular signal-regulated kinase activation is required for up-regulation of vascular endothelial growth factor by serum starvation in human colon carcinoma cells. *Cancer Res* 1999; **59**:4804–4807.
8 Fanning P, Bulovas K, Saini KS, Libertino JA, Joyce AD, Summerhayes IC. Elevated expression of pp60c-src in low grade human bladder carcinoma. *Cancer Res* 1992; **52**:1457–1462.
9 Takekura N, Yasui W, Yoshida K, Tsujino T, Nakayama H, Kameda T, *et al.* pp60c-src protein kinase activity in human gastric carcinomas. *Int J Cancer* 1990; **45**:847–851.
10 Kiefer PE, Wegmann B, Bacher M, Erbil C, Heidtmann H, Havemann K. Different pattern of expression of cellular oncogenes in human non-small-cell lung cancer cell lines. *J Cancer Res Clin Oncol* 1990; **116**:29–37.
11 Lutz MP, Esser IB, Flossmann-Kast BB, Vogelmann R, Luhrs H, Friess H, *et al.* Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun* 1998; **243**:503–508.
12 Tan YX, Wang HT, Zhang P, Yan ZH, Dai GL, Wu MC, *et al.* c-src activating mutation analysis in Chinese patients with colorectal cancer. *World J Gastroenterol* 2005; **11**:2351–2353.
13 Laghi L, Bianchi P, Orbetegli O, Gennari L, Roncalli M, Malesci A. Lack of mutation at codon 531 of SRC in advanced colorectal cancers from Italian patients. *Br J Cancer* 2001; **84**:196–198.
14 Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, *et al.* Activating SRC mutation in a subset of advanced human colon cancers. *Nat Genet* 1999; **21**:187–190.
15 Zeng H, Datta K, Neid M, Li J, Parangi S, Mukhopadhyay D. Requirement of different signaling pathways mediated by insulin-like growth factor-I receptor for proliferation, invasion, and VPF/VEGF expression in a pancreatic carcinoma cell line. *Biochem Biophys Res Commun* 2003; **302**:46–55.
16 Mao W, Irby R, Coppola D, Fu L, Wloch M, Turner J, *et al.* Activation of c-Src by receptor tyrosine kinases in human colon cancer cells with high metastatic potential. *Oncogene* 1997; **15**:3083–3090.
17 Talamonti MS, Roh MS, Curley SA, Gallick GE. Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J Clin Invest* 1993; **91**:53–60.

18 Termuhlen PM, Curley SA, Talamonti MS, Saboorian MH, Gallick GE. Site-specific differences in pp60c-src activity in human colorectal metastases. *J Surg Res* 1993; **54**:293–298.

19 Allgayer H, Boyd DD, Heiss MM, Abdalla EK, Curley SA, Gallick GE. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer* 2002; **94**:344–351.

20 Irby RB, Yeatman TJ. Increased Src activity disrupts cadherin/catenin-mediated homotypic adhesion in human colon cancer and transformed rodent cells. *Cancer Res* 2002; **62**:2669–2674.

21 Avizienyte E, Wyke AW, Jones RJ, McLean GW, Westhoff MA, Brunton VG, *et al*. Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. *Nat Cell Biol* 2002; **4**:632–638.

22 Nam JS, Ino Y, Sakamoto M, Hirohashi S. Src family kinase inhibitor PP2 restores the E-cadherin/catenin cell adhesion system in human cancer cells and reduces cancer metastasis. *Clin Cancer Res* 2002; **8**:2430–2436.

23 MacMillan-Crow LA, Greendorfer JS, Vickers SM, Thompson JA. Tyrosine nitration of c-SRC tyrosine kinase in human pancreatic ductal adenocarcinoma. *Arch Biochem Biophys* 2000; **377**:350–356.

24 Menke A, Philippi C, Vogelmann R, Seidel B, Lutz MP, Adler G, *et al*. Down-regulation of E-cadherin gene expression by collagen type I and type III in pancreatic cancer cell lines. *Cancer Res* 2001; **61**:3508–3517.

25 Trevino JG, Summy JM, Lesslie DP, Parikh NU, Hong DS, Lee FY, *et al*. Inhibition of SRC expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am J Pathol* 2006; **168**:962–972.

26 Ellis LM, Staley CA, Liu W, Fleming RY, Parikh NU, Bucana CD, *et al*. Down-regulation of vascular endothelial growth factor in a human colon carcinoma cell line transfected with an antisense expression vector specific for c-src. *J Biol Chem* 1998; **273**:1052–1057.

27 Gray MJ, Zhang J, Ellis LM, Semenza GL, Evans DB, Watowich SS, *et al*. HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. *Oncogene* 2005; **24**:3110–3120.

28 Summy JM, Trevino JG, Lesslie DP, Baker CH, Shakespeare WC, Wang Y, *et al*. AP23846, a novel and highly potent Src family kinase inhibitor, reduces vascular endothelial growth factor and interleukin-8 expression in human solid tumor cell lines and abrogates downstream angiogenic processes. *Mol Cancer Ther* 2005; **4**:1900–1911.

29 Trevino JG, Summy JM, Gray MJ, Nilsson MB, Lesslie DP, Baker CH, *et al*. Expression and activity of SRC regulate interleukin-8 expression in pancreatic adenocarcinoma cells: implications for angiogenesis. *Cancer Res* 2005; **65**:7214–7222.

30 Ito H, Gardner-Thorpe J, Zinner MJ, Ashley SW, Whang EE. Inhibition of tyrosine kinase Src suppresses pancreatic cancer invasiveness. *Surgery* 2003; **134**:221–226.

31 Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N. SRC family kinases: potential targets for the treatment of human cancer and leukemia. *Curr Pharm Des* 2003; **9**:2043–2059.

32 Masumoto N, Nakano S, Fujishima H, Kohno K, Niho Y. v-src induces cisplatin resistance by increasing the repair of cisplatin-DNA interstrand cross-links in human gallbladder adenocarcinoma cells. *Int J Cancer* 1999; **80**:731–737.

33 Qin B, Ariyama H, Baba E, Tanaka R, Kusaba H, Harada M, *et al*. Activated Src and Ras induce gefitinib resistance by activation of signaling pathways downstream of epidermal growth factor receptor in human gallbladder adenocarcinoma cells. *Cancer Chemother Pharmacol* 2006; **58**:577–584.

34 Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. Inhibition of SRC tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells. *Clin Cancer Res* 2004; **10**:2307–2318.

35 Duxbury MS, Ito H, Zinner MJ, Ashley SW, Whang EE. siRNA directed against c-Src enhances pancreatic adenocarcinoma cell gemcitabine chemosensitivity. *J Am Coll Surg* 2004; **198**:953–959.

36 Hiscox S, Morgan L, Green TP, Barrow D, Gee J, Nicholson RI. Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells. *Breast Cancer Res Treat* 2006; **97**:263–274 [Epub 7 December 2005].

37 NIH consensus conference. Ovarian cancer. Screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer. *JAMA* 1995; **273**:491–497.

38 Wiener JR, Windham TC, Estrella VC, Parikh NU, Thall PF, Deavers MT, *et al*. Activated SRC protein tyrosine kinase is overexpressed in late-stage human ovarian cancers. *Gynecol Oncol* 2003; **88**:73–79.

39 Chen T, Pengetnze Y, Taylor CC. Src inhibition enhances paclitaxel cytotoxicity in ovarian cancer cells by caspase-9-independent activation of caspase-3. *Mol Cancer Ther* 2005; **4**:217–224.

40 Griffiths GJ, Koh MY, Brunton VG, Cawthorne C, Reeves NA, Greaves M, *et al*. Expression of kinase-defective mutants of c-Src in human metastatic colon cancer cells decreases Bcl-xL and increases oxaliplatin- and Fas-induced apoptosis. *J Biol Chem* 2004; **279**:46113–46121.

41 Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, *et al*. BCR-ABL independence and LYN kinase overexpression in chronic myelogenous leukemia cells selected for resistance to STI571. *Blood* 2003; **101**:690–698.

42 Rodier JM, Valles AM, Denoyelle M, Thiery JP, Boyer B. pp60c-src is a positive regulator of growth factor-induced cell scattering in a rat bladder carcinoma cell line. *J Cell Biol* 1995; **131**:761–773.

43 Cattan N, Rochet N, Mazeau C, Zanghellini E, Mari B, Chauzy C, *et al*. Establishment of two new human bladder carcinoma cell lines, CAL 29 and CAL 185. Comparative study of cell scattering and epithelial to mesenchyme transition induced by growth factors. *Br J Cancer* 2001; **85**:1412–1417.

44 Greenburg G, Hay ED. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J Cell Biol* 1982; **95**:333–339.

45 Tarin D, Thompson EW, Newgreen DF. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* 2005; **65**:5996–6000; discussion 6000–6001.

46 Coluccia AM, Benati D, Dekhil H, De Filippo A, Lan C, Gambacorti-Passerini C. SKI-606 decreases growth and motility of colorectal cancer cells by preventing pp60(c-Src)-dependent tyrosine phosphorylation of beta-catenin and its nuclear signaling. *Cancer Res* 2006; **66**:2279–2286.

47 Shin SJ, Kim KO, Kim MK, Lee KH, Hyun MS, Kim KJ, *et al*. Expression of E-cadherin and uPA and their association with the prognosis of pancreatic cancer. *Jpn J Clin Oncol* 2005; **35**:342–348.

48 Lowy AM, Knight J, Groden J. Restoration of E-cadherin/beta-catenin expression in pancreatic cancer cells inhibits growth by induction of apoptosis. *Surgery* 2002; **132**:141–148.

49 Thiery JP. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2002; **2**:442–454.

50 Karni R, Gus Y, Dor Y, Meyuhas O, Levitzki A. Active Src elevates the expression of beta-catenin by enhancement of cap-dependent translation. *Mol Cell Biol* 2005; **25**:5031–5039.

51 Korsching E, Packeisen J, Liedtke C, Hungermann D, Wulfing P, van Diest PJ, *et al*. The origin of vimentin expression in invasive breast cancer: epithelial-mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential? *J Pathol* 2005; **206**:451–457.

52 Yang AD, Fan F, Camp R, van Buren G, Liu W, Somcio R, *et al*. Chronic oxaliplatin resistance induces epithelial to mesenchymal transition in colorectal cancer cell lines. *Clin Cancer Res* 2006; **12**:4147–4153.