

# Src Family Kinases as Regulators of Angiogenesis: Therapeutic Implications

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**Abstract:** Despite its discovery nearly a century ago, the functions of the Src family of protein tyrosine kinases (SFKs) remain incompletely understood. While much has been learned regarding the functions of Src family kinases in the last few years, new roles for Src, particularly in promoting the progression of cancer towards the metastatic phenotype, continue to emerge. SFKs, through their functions as kinases and adapter proteins in signaling complexes, regulate such diverse cellular events as proliferation, migration, cell cycle control, and apoptosis. In tumor cells, the kinase activity of Src is frequently activated, with greater increases during progressive stages. Likewise, resistance to chemotherapy also corresponds with Src kinase activity. Thus, Src activation is predictive of poor prognosis in several tumors. Recently, selective SFK inhibitors are showing promise in clinical trials in imatinib mesylate (Gleevec, Novartis) resistant chronic myelogenous leukemia. However, *in vitro* studies have suggested that Src inhibitors may hold promise in the treatment of solid tumors such as colon and pancreatic cancer in which new therapeutic inhibitors are desperately needed. This review will summarize briefly the structure and function of Src and the evidence for Src in promoting tumor progression and metastasis. As recent work in this laboratory and others has demonstrated that Src is a regulator of expression of diverse pro-angiogenic factors produced by tumor cells, and a regulator of the endothelial cells that respond to these factors, this review will focus on the role of Src in angiogenesis and potential roles of Src inhibitors as antiangiogenic agents.

**Keywords:** Src kinases, angiogenesis, targeted therapy, chemotherapy, metastasis.

## INTRODUCTION

In 1911, Peyton Rous first described a viral agent capable of inducing tumors in chickens providing evidence for the transmissible nature of cancer [1]. This seminal work went largely unappreciated and uncorroborated until the 1950s, when tumor cells were shown to arise from infection with the Rous sarcoma virus [2]. This finding was confirmed when temperature sensitive activated v-Src mutants failed to transform cells at non-permissive temperatures, demonstrating a requirement for the active virus in cellular transformation [3]. In the 1970s, Brugge and colleagues isolated and identified v-Src as the transforming protein of the oncogenic Rous sarcoma virus utilizing tumor bearing rabbit serum [4]. Additional experiments demonstrated that the viral Src gene (v-Src) has a highly conserved and ubiquitously expressed cellular homologue, c-Src, which is present in normal cells [5]. Src was not only the first proto-oncogene identified, it was also the first demonstrated to possess intrinsic protein kinase activity [6, 7] spawning both a search for similar protein tyrosine kinases and investigations into the role of Src in regulating cellular behavior [4]. The last two decades have witnessed an explosion in Src research, including the recent development of selective small molecule inhibitors that target Src. Early results from clinical trials demonstrate *in vivo* stability and, thus far, limited toxicity of these inhibitors. With increasing knowledge of Src's role in angiogenesis and bone metabolism, the potential clinical

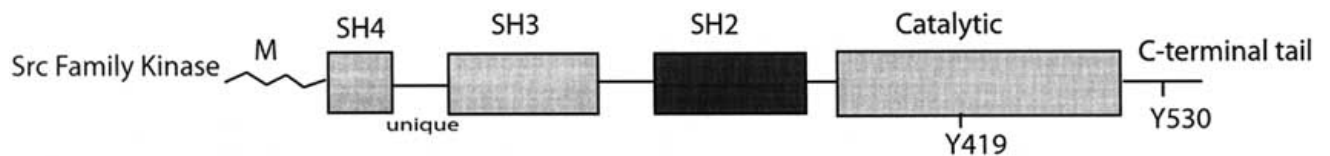
utility of Src inhibition will continue to expand. At this writing, two clinical trials continue to investigate Src inhibition in imatinib mesylate (Gleevec, Novartis) resistant CML patients, and recent studies have shown the effectiveness of combination agents that target both Bcr-Abl and Src in imatinib mesylate resistant CML cell lines [8, 9]. Because Src activation is frequent in a variety of tumors, occurring most often during tumor progression, it is almost certain that Src inhibitors will reach clinical trials for therapy of late stage solid tumors such as colon, prostate and pancreatic, solid tumors in which new therapies are desperately needed. Here, we review the role of Src in tumor progression with special emphasis on angiogenic regulation, and how Src inhibitors might affect this process.

## SRC: FAMILY MEMBERS, STRUCTURE AND REGULATION

The non-receptor protein tyrosine kinase Src is the prototypical member of a kinase family that includes Yes, Fyn, Lyn, Lck, Hck, Fgr, Yrk, Frk and Blk. This group is collectively known as the Src family kinases (SFKs), and, in contrast to receptor protein tyrosine kinases, is not comprised of transmembrane proteins [10]. Src functions by associating with various intracellular molecules and catalyzing the transfer of phosphate from ATP to tyrosine residues within these proteins.

As a result of mutational studies and structural modeling based on crystallography data, the structure of Src has been well characterized [11]. The c-Src gene product, and all Src family kinases, are comprised of six distinct regions, (Fig. 1).

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**Fig. (1).** Src Family Kinase Domains. Key phosphorylation sites are included. (M) indicates N-terminal myristate.

(1) the Src homology (SH) 4 domain, (2) a poorly conserved sequence termed the unique region, (3) the SH3 domain, (4) the SH2 domain, (5) the protein tyrosine kinase, or SH1, domain and (6) the negative regulatory C-terminal tail [12]. The SH4 domain contains signals for lipid modification of SFKs including a myristoylation site responsible for membrane localization. The unique region follows and, as the name implies, is the domain of greatest sequence diversity among each Src family member. The function of this sequence is unknown but modulation of protein-protein interactions and regulation of catalytic activity have been suggested [13]. The tyrosine kinase/ SH1 domain possesses a bilobed structure with both ATP and substrate binding sites. Within this catalytic domain, Tyr419 (in the human c-Src nomenclature) is noteworthy because its autophosphorylation is required for full Src activation. The SH2 and SH3 domains are globular structures adjacent to the N-terminus and participate in protein-protein interactions. Along with the C-terminal tail, *via* phosphorylation of Tyr530 (human), SH2 and SH3 participate in the negative regulation of Src, as Src is rendered inactive by intramolecular interactions that make the kinase domain less accessible for ATP/ substrate binding. Crystallographic studies have demonstrated interactions between the C-terminus and the SH2 domain and between the kinase and SH3 domain causing Src to assume the less active or “closed” conformational state [14]. These interactions not only limit the catalytic ability of the kinase domain, but hinder the ability of SH2 and SH3 to participate in cell signaling as well. Interestingly, even though Src is expressed widely in normal cells with highest concentrations in platelets, bone and neural tissue [12], *Src*<sup>-/-</sup> mice exhibit normal development of their cardiovascular and neurologic systems and normal platelet function. In fact, the only phenotypic abnormality manifested in these animals is that of osteopetrosis [15], a condition in which bone resorbing osteoclasts fail to function resulting in abnormally dense, brittle bones. This observation has led to escalating efforts for the development of bone-targeted Src inhibitors for a variety of pathologic conditions involving the skeletal system including osteoporosis and treatment of bony metastatic disease.

## SRC AND REGULATION OF BIOLOGIC FUNCTIONS

The cellular functions governed by Src have been extensively reviewed elsewhere [12, 13] and will be summarized here only briefly. As stated above, Src is implicated in a variety of cellular functions including proliferation and migration. Src is implicated in growth and proliferation through interactions with a variety of activated receptor tyrosine kinases. For example, Src is known to form a heterodimer complex with EGFR resulting in

synergistic DNA synthesis upon EGF stimulation [16]. Src is implicated extensively as a regulator of cellular migration *via* interactions with growth factor receptors, integrins and the small GTPases Rac and Rho [17]. Interestingly, recent work from Ding *et al.* demonstrated differential migration of glioblastoma cells upon PDGFR stimulation attributable to differential integrin binding by Src family members [18]. In addition, the study found that activation of individual Src family members was dependent on specific substrate binding and integrin engagement. Fibroblasts from *Src*<sup>-/-</sup> mice are inhibited in migration, further demonstrating a central role for Src in this process. Src activation is also implicated in invasion (reviewed by [19, 20]) and angiogenesis, the latter property of which will be discussed extensively in this review. Thus, the normal functions of Src when tightly regulated are also associated with tumor progression and metastasis, when Src is constitutively activated.

## SRC ACTIVATION AND TUMOR PROGRESSION

The evidence for Src in promoting tumor progression and metastasis has been detailed recently [21] and will only be discussed briefly here. Src is implicated in a variety of human malignancies, both liquid and solid. This is largely based on observations of Src overexpression and increased enzymatic activity in surgical specimens and established tumor cell lines. Despite reports of an activating mutation in the C-terminal region leading to Src activation and increased metastatic potential in colorectal carcinoma [22], mutations do not appear to be a predominant mechanism of Src activation.

The role of Src in human cancer progression and metastasis has been most extensively characterized in colorectal carcinoma, but Src has been implicated in others such as gastric, pancreatic, breast, ovarian, hepatocellular, esophageal, non-small cell lung, and some sarcomas, as well as chronic myelogenous leukemia and multiple myeloma [23-29]. Accepting that no ‘prototypical’ cancer exists, analyzing the large body of work in colorectal cancer provides insight into the role of Src in regulating human malignancies. In colorectal cancer, Src appears to function as a regulator of tumor progression, as experiments have demonstrated increasing Src activity with progression from normal colonic mucosa to pre-malignant dysplastic polyps to primary carcinoma and, finally, to metastatic disease [30]. Src kinase activity also correlates with poor prognosis in patients with colorectal carcinoma [31].

Recent experimental work suggests a role for Src in modulating chemosensitivity to conventional chemotherapeutic agents in solid tumors in addition to CML. Experiments presented by Duxbury and colleagues [32, 33] demonstrate that Src inhibition attenuates chemoresistance of pancreatic carcinoma cell lines treated

with gemcitabine. By utilizing multiple pancreatic cell lines, including a resistant cell line generated by chronic exposure to gemcitabine, they found that Src kinase activity directly correlated with resistance to gemcitabine, resistance to caspase-mediated apoptosis and Akt activity. Additionally, combination therapy with PP2, a pharmacological inhibitor of Src family kinases, and gemcitabine decreased tumor growth and metastatic potential in a murine model of pancreatic cancer as compared to treatment with gemcitabine alone. The exact mechanism by which Src modulates sensitivity to chemotherapy remains unknown, but regulation of the PI3K/ Akt pathway appears likely, as Akt is a known downstream target of Src and constitutively active Akt has been shown to impart chemoresistance to both mitoxantrone and cisplatin in mammary carcinoma cells [34]. From longstanding clinical experience, single agent chemotherapy or individual small molecule inhibitors are unlikely to produce durable results in the majority of cancer patients as resistance and recurrence are commonplace.

## SRC AND ANGIOGENESIS

An emerging and exciting body of work is defining a role for Src in regulating endothelial cell functioning and angiogenesis. Tumors beyond one to two millimeters require the acquisition of a tumor associated blood supply [35-37]. This requires an imbalance between inhibitory and stimulatory factors [38] and occurs *via* two distinct physiological processes: vasculogenesis, the formation of new blood vessels from marrow derived precursor cells, and angiogenesis. Angiogenesis is the development of neocapillaries from existing vasculature and requires proliferation, migration, elaboration of proteases and differentiation, all of which are regulated, in part, by Src. Many experiments have demonstrated a role for Src in vessel growth in normal and pathologic conditions, as *v-Src* is known to induce hemangioma formation in chicks [39]. However, the *Src* *-/-* mice exhibit normal development of their vascular systems, suggesting that Src family kinases have overlapping functions. In agreement with this possibility, mice deficient in Src, Yes and Fyn undergo embryonic lethality at day 9.5, approximately the same time as those mice deficient in VEGFR [40, 41].

Perhaps the best characterized role for Src in regulating angiogenesis is its ability to regulate VEGF expression, particularly VEGF induction by hypoxia. Mukhopadhyay and colleagues demonstrated that hypoxia was sufficient to increase Src kinase activity and VEGF expression in fibroblasts. In addition, they showed that Genistein, a protein kinase inhibitor, and dominant negative mutants of c-Src attenuated the hypoxia-induced VEGF induction seen in untreated control cells [42]. Previous work from this lab has shown that VEGF production varies directly with c-Src expression [43], and experiments by Ellis *et al.* demonstrated that an antisense Src strategy was effective in reducing both constitutive and hypoxic-induced VEGF production in a human colon carcinoma cell line [44]. These experiments also suggested an additional role for Src in augmenting VEGF mRNA expression, as the colon cancer cell line, with high constitutive Src expression and activity, exhibited a much higher fold induction of VEGF in response

to hypoxia than did the fibroblasts (50 vs. 4.5) in the study of Mukhopadhyay. The c-Src antisense clones also generated tumors with decreased vascularity when compared to the parental sense clones in a subcutaneous mouse model suggesting a role for Src in regulating angiogenesis *in vivo*. We have demonstrated recently that hypoxia-induced Src activation leads to binding of both Hif-1 $\alpha$  and STAT3 in a complex on the VEGF promoter, thereby contributing to increased VEGF expression (Gray *et al.*, submitted for publication).

Further, Karni *et al.* have demonstrated the ability of constitutively active Src to induce HIF-1 $\alpha$  under normoxic conditions [45] suggesting that Src activation, regardless of mechanism, can augment VEGF production and angiogenesis. Ongoing work from this laboratory has demonstrated a role for Src in regulating VEGF expression in other tumor types including pancreatic cancer, where Src activation of the PI3kinase/Akt signaling pathway is required (Summy *et al.*, unpublished data). As Src is a regulator of multiple signal transduction pathways, it should not be surprising that Src is important in regulating other pro-angiogenic factors such as bFGF, and IL-8. Recent studies from our laboratory have confirmed that inhibition of Src strongly decreases expression of these pro-angiogenic peptides (Trevino *et al.*, unpublished observations).

In addition to regulating VEGF and bFGF expression, Src regulates responses to these factors in both endothelial and tumor cells. Elicieri and co-workers have shown both bFGF and VEGF are able to induce Src activation in avian endothelial cells [46]. Despite both mitogens being capable of activating Src in this model, only VEGF-induced angiogenesis appears to be Src dependent, as dominant interfering mutants (Src 251) failed to inhibit angiogenesis in bFGF treated cells. Additionally, overexpression of the dominant negative Src induces apoptotic cell death in the VEGF-treated endothelial cells suggesting a survival function for Src during VEGF-induced angiogenesis. Similar results were obtained when utilizing a virus that encodes CSK, a protein tyrosine kinase that inhibits Src activity by phosphorylating Tyr530 in the c-terminal tail.

Although Src-independent angiogenesis can occur with bFGF stimulation, other work implicates Src as a regulator of bFGF induced endothelial function. For example, Kilarski and colleagues showed that PP2, a pharmacological Src family kinase inhibitor, effectively inhibited bFGF induced angiogenesis *in vivo* [47], and *in vitro* experiments demonstrated inhibition of lamellopodia formation and lack of invasive growth and capillary tube formation in PP2 treated cells compared to controls. Overexpression of a kinase-inactive Src resulted in reduced phosphorylation of paxillin and cortactin, suggesting a role for Src in actin cytoskeletal rearrangement and migration. Finally in this regard, recent evidence has demonstrated that tumor cells themselves express VEGF receptors [48], and that stimulation of these receptors augments Src kinase activity and tumor cell migration (Lesslie *et al.*, unpublished).

Ongoing work continues to define the role of Src in regulating endothelial cell functioning and angiogenesis. In a microvascular hepatic murine endothelial cell line, increased Src activity, as measured by phosphorylation at Tyr416, has been observed following bFGF and EGF stimulation.

Furthermore, treatment with a potent Src kinase inhibitor not only abrogates Src phosphorylation following mitogen stimulation, but markedly diminishes proliferation and migration as well. While the effects of Src inhibition on kinase activity and migration are expected, the finding of an anti-proliferatory effect is somewhat novel, as previous work has failed to demonstrate a requirement for Src in endothelial proliferation [49]. The mechanism for the effect on proliferation is unclear. Growth arrest is one possibility, or the hepatic endothelial cells, like the avian endothelial cells discussed previously, require Src (or one or more Src family kinases) for survival. Indeed, preliminary work has demonstrated morphological changes by light microscopy and nuclear features consistent with early apoptosis on TUNEL staining in the hepatic murine endothelial cell line treated with a Src kinase inhibitor. Additional work lies ahead to fully define the role of SFKs in regulating angiogenesis and endothelial cell behavior.

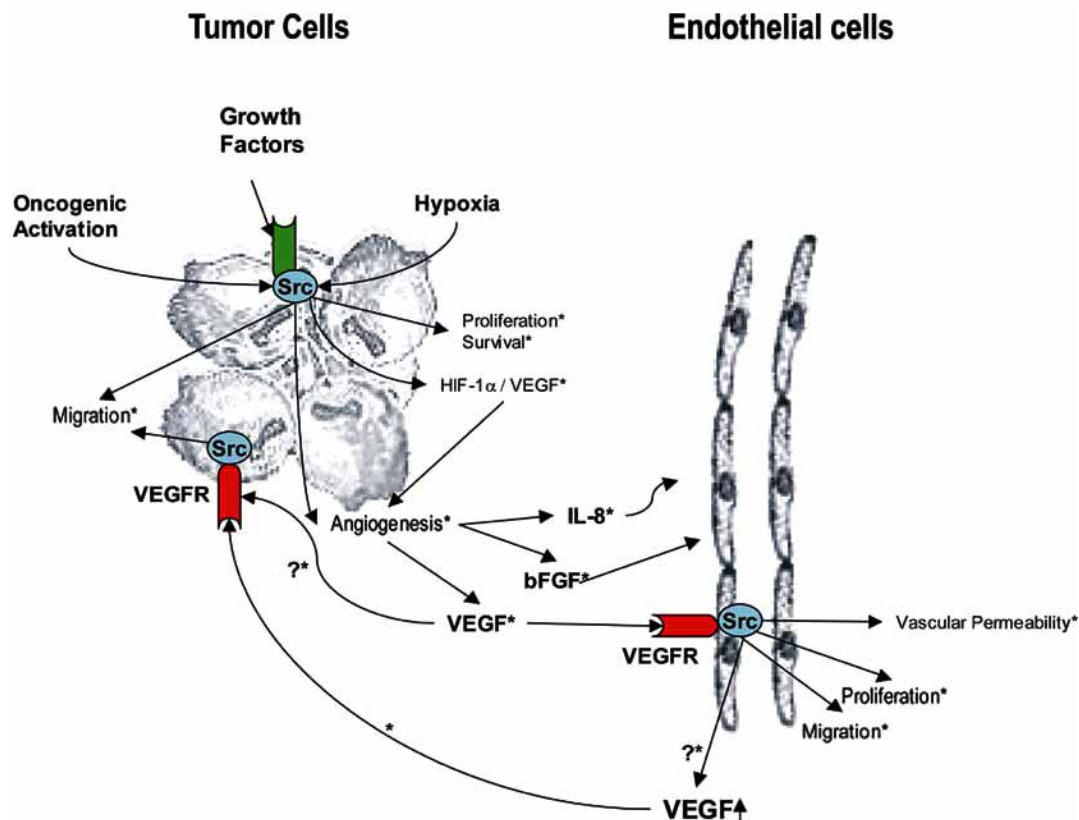
## FUTURE DIRECTIONS

The potential clinical utility of Src kinase inhibitors appears to be multi-faceted. First Src inhibitors could be utilized to enhance chemosensitivity to existing chemotherapeutic agents, thereby maximizing anti-tumor activity while minimizing untoward side effects and toxicity. Work cited previously [32, 33] demonstrates the efficacy of Src inhibition on sensitizing pancreatic adenocarcinoma cells to gemcitabine. The same study, however, demonstrates no such effect when using

combination therapy with PP2 and 5-FU and, in fact, shows a trend towards increasing resistance to 5-FU with Src inhibition in some pancreatic cell lines. Also, constitutively active Akt, a well known downstream effector molecule of Src, failed to impart chemoresistance to the cell cycle specific agent paclitaxel in mammary carcinoma cells [34]. Therefore, and not surprisingly, the effectiveness of Src kinase inhibitors on augmenting chemosensitivity is likely to be tumor and/ or drug specific. Current experimental work continues to investigate combination therapy with Src kinase inhibitors and chemotherapeutic agents in a variety of solid tumors including colorectal, pancreatic and ovarian carcinomas.

Another foreseeable application for Src kinase inhibitors are as direct targeted anti-cancer agents, most likely in combination with other small molecule inhibitors. The ongoing work with imatinib mesylate resistance and CML, while exciting, does not fully encompass the disease spectrum likely to benefit from this therapy. As Src is frequently overexpressed or aberrantly activated in solid tumors, clinical trials to explore the efficacy of these agents in advanced stage disease are likely forthcoming. If one examines the regulatory functions governed by Src in tumor progression, angiogenesis and the cross-talk between tumor cells and endothelial cells, (Fig. 2), the potential clinical utility begins to emerge.

In this model Src kinase inhibition, would simultaneously target tumor cells (inhibiting their propensity to progress and metastasize), endothelial cells (limiting their ability to grow and migrate/ extravasate) and



**Fig. (2).** Model by which Src activity mediates biologic functioning of tumor cells and endothelial cells, and promotes intercellular signaling to promote tumor progression and metastasis. \*Indicates the signaling molecules and/ or cellular functions potentially affected by Src kinase inhibition.

limit signaling between the two (inhibiting 'cross-talk' that further promotes tumor progression and metastasis).

## CONCLUSIONS

In summary, while Src has been implicated as a key regulator or tumor progression and metastasis for decades, new roles and functions for this proto-oncogene continue to emerge. Recent exciting work implicates Src as a regulator of the angiogenic response and sensitivity to standard chemotherapeutic regimens. As most experts agree that single agent treatment of human malignancy is destined for resistance and failure, the development of Src kinase inhibitors, with their effects on tumor cells (primary and metastatic) and activated endothelial cells as well as their ability to potentiate existing chemotherapeutic agents and augment other small molecule inhibitors, will continue to generate interest and enthusiasm in clinical oncology.

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## REFERENCES

- [1] Rous P.A. Transmission of a malignant new growth by means of a cell-free filtrate. *Journal of the American Medical Association* 1911; 56: 198.
- [2] Rubin H. Quantitative relations between causative virus and cell in the Rous no. 1 chicken sarcoma. *Virology* 1955; 1(5): 445-73.
- [3] Martin GS. Rous sarcoma virus: a function required for the maintenance of the transformed state. *Nature* 1970; 227(262): 1021-3.
- [4] Brugge JS, Erikson RL. Identification of a transformation-specific antigen induced by an avian sarcoma virus. *Nature* 1977; 269(5626): 346-8.
- [5] 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(5547): 170-3.
- [6] Collett MS, Erikson RL. Protein kinase activity associated with the avian sarcoma virus src gene product. *Proc Natl Acad Sci U S A* 1978; 75(4): 2021-4.
- [7] Levinson AD, Oppermann H, Levintow L, Varmus HE, Bishop JM. Evidence that the transforming gene of avian sarcoma virus encodes a protein kinase associated with a phosphoprotein. *Cell* 1978; 15(2): 561-72.
- [8] Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 2004; 305(5682): 399-401.
- [9] Tipping AJ, Baluch S, Barnes DJ, *et al.* Efficacy of dual-specific Bcr-Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate. *Leukemia* 2004; 18(8): 1352-6.
- [10] Neet K, Hunter T. Vertebrate non-receptor protein-tyrosine kinase families. *Genes Cells* 1996; 1(2): 147-69.
- [11] Xu W, Harrison SC, Eck MJ. Three-dimensional structure of the tyrosine kinase c-Src. *Nature* 1997; 385(6617): 595-602.
- [12] Brown MT, Cooper JA. Regulation, substrates and functions of src. *Biochim Biophys Acta* 1996; 1287(2-3): 121-49.
- [13] Thomas SM, Brugge JS. Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol* 1997; 13: 513-609.
- [14] Yamaguchi H, Hendrickson WA. Structural basis for activation of human lymphocyte kinase Lck upon tyrosine phosphorylation. *Nature* 1996; 384(6608): 484-9.
- [15] Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 1991; 64(4): 693-702.
- [16] Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ. c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. *J Biol Chem* 1999 19; 274(12): 8335-43.
- [17] Timpson P, Jones GE, Frame MC, Brunton VG. Coordination of cell polarization and migration by the Rho family GTPases requires Src tyrosine kinase activity. *Curr Biol* 2001; 11(23): 1836-46.
- [18] Ding Q, Stewart J Jr., Olman MA, Klobe MR, Gladson CL. The pattern of enhancement of Src kinase activity on platelet-derived growth factor stimulation of glioblastoma cells is affected by the integrin engaged. *J Biol Chem* 2003; 278(41): 39882-91.
- [19] Frame MC. Src in cancer: deregulation and consequences for cell behaviour. *Biochim Biophys Acta* 2002; 1602(2): 114-30.
- [20] Mareel M, Leroy A. Clinical, cellular, and molecular aspects of cancer invasion. *Physiol Rev* 2003; 83(2): 337-76.
- [21] Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev* 2003; 22(4): 337-58.
- [22] Irby RB, Yeatman TJ. Role of Src expression and activation in human cancer. *Oncogene* 2000; 19(49): 5636-42.
- [23] Masaki T, Shiratori Y, Okada H, *et al.* pp60c-src activation in gastric carcinoma: a preliminary study. *Am J Gastroenterol* 2000; 95(3): 837-8.
- [24] Lutz MP, Esser IB, Flossmann-Kast BB, *et al.* Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun* 1998; 243(2): 503-8.
- [25] 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(4): 1696-702.
- [26] Budde RJ, Ke S, Levin VA. Activity of pp60c-src in 60 different cell lines derived from human tumors. *Cancer Biochem Biophys* 1994; 14(3): 171-5.
- [27] Masaki T, Okada M, Tokuda M, *et al.* Reduced C-terminal Src kinase (Csk) activities in hepatocellular carcinoma. *Hepatology* 1999; 29(2): 379-84.
- [28] Wang X, Wang C, Huang G, Xiao H. [A study of c-src gene express product pp60c-src in esophageal carcinoma]. *Hua Xi Yi Ke Da Xue Xue Bao* 1995; 26(2): 197-201.
- [29] Hallek M, Neumann C, Schaffer M, *et al.* Signal transduction of interleukin-6 involves tyrosine phosphorylation of multiple cytosolic proteins and activation of Src-family kinases Fyn, Hck, and Lyn in multiple myeloma cell lines. *Exp Hematol* 1997; 25(13): 1367-77.
- [30] 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(1): 53-60.
- [31] Aligayer 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 15; 94(2): 344-51.
- [32] 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(7): 2307-18.
- [33] 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(6): 953-9.
- [34] Schmidt M, Hovelmann S, Beckers TL. A novel form of constitutively active farnesylated Akt1 prevents mammary epithelial cells from anoikis and suppresses chemotherapy-induced apoptosis. *Br J Cancer* 2002; 87(8): 924-32.
- [35] Folkman J. Tumor angiogenesis: a possible control point in tumor growth. *Ann Intern Med* 1975; 82(1): 96-100.
- [36] Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990; 82(1): 4-6.
- [37] Fidler IJ. Critical determinants of metastasis. *Semin Cancer Biol* 2002; 12(2): 89-96.
- [38] Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; 86(3): 353-64.
- [39] Stoker AW, Hatier C, Bissell MJ. The embryonic environment strongly attenuates v-src oncogenesis in mesenchymal and epithelial tissues, but not in endothelia. *J Cell Biol* 1990; 111(1): 217-28.
- [40] Fong GH, Rossant J, Gertsenstein M, Breitman ML. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 1995; 376(6535): 66-70.

- [41] Shalaby F, Rossant J, Yamaguchi TP, *et al.* Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 1995; 376(6535): 62-6.
- [42] Mukhopadhyay D, Tsiokas L, Zhou XM, Foster D, Brugge JS, Sukhatme VP. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. *Nature* 1995; 375(6532): 577-81.
- [43] Fleming RY, Ellis LM, Parikh NU, Liu W, Staley CA, Gallick GE. Regulation of vascular endothelial growth factor expression in human colon carcinoma cells by activity of src kinase. *Surgery* 1997; 122(2): 501-7.
- [44] Ellis LM, Staley CA, Liu W, *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(2): 1052-7.
- [45] Karni R, Dor Y, Keshet E, Meyuhas O, Levitzki A. Activated pp60c-Src leads to elevated hypoxia-inducible factor (HIF)-1alpha expression under normoxia. *J Biol Chem* 2002 8; 277(45): 42919-25.
- [46] Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. *Mol Cell* 1999; 4(6): 915-24.
- [47] Kilarski WW, Jura N, Gerwins P. Inactivation of Src family kinases inhibits angiogenesis *in vivo*: implications for a mechanism involving organization of the actin cytoskeleton. *Exp Cell Res* 2003; 291(1): 70-82.
- [48] Boocock CA, Charnock-Jones DS, Sharkey AM, *et al.* Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. *J Natl Cancer Inst* 1995; 87(7): 506-16.
- [49] Steinle JJ, Meininger CJ, Chowdhury U, Wu G, Granger HJ. Role of ephrin B2 in human retinal endothelial cell proliferation and migration. *Cell Signal* 2003; 15(11): 1011-7.

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