[Frontiers in Bioscience 8, s1068-1073, September 1, 2003]

SRC IN HUMAN CARCINOGENESIS

Salvatore V. Russello and Scott K. Shore

Fels Institute for Cancer Research & Department of Biochemistry, Temple University School of Medicine, 3307 N. Broad Street, Philadelphia PA 19140

TABLE OF CONTENTS

1. ABSTRACT

The signaling machinery in cells is a complex, multi-factorial network of cross-talking proteins that enables dynamic communication between upstream causal factors and downstream effectors. Non-receptor tyrosine kinases, including Src, are the intermediates of information transfer, controlling pathways as diverse as cell growth, migration, death, and genome maintenance. When expressed as viral genes these proteins are potent carcinogens, yet analogous genetic alterations are rarely observed in human tumors. In seeking to characterize the role of the non-receptor tyrosine kinase Src in neoplasia, arguments can be made that the consequences of mutation, or perturbations in the activity or expression of this protein is a determinative factor in clinical prognosis and pathogenicity. In a variety of tumor types including those derived from the colon and breast, the Src non-receptor tyrosine kinase is either overexpressed or constitutively active in a large percentage of the tumors. Increased expression or activity of Src correlates with the stage and metastatic potential of some neoplasia.

2. INTRODUCTION

Found in 1976 to be the normal homologue of the transforming agent in an avian sarcoma virus, years of study have elucidated the signaling pathways regulated by Src. Moreover, the mechanism by which it is activated has been solved to the level of individual amino acid residues and evidence continues to accumulate for a role in human cancer (reviewed in 1,2). v-Src is the constitutively active tyrosine kinase encoded in the Rous Sarcoma virus (RSV), a chicken tumor virus first identified in the early 1900's (3). Observations that the carboxy-terminal region of v-Src differed from that of the non-transforming Src revealed the importance of this region in regulation of Src kinase activity (4,5). Phosphorylation of Src at the negative regulatory tyrosine 527 causes an intramolecular association of this region with an amino-terminal SH2 domain (6-8). This interaction induces a second intramolecular association between the Src SH3 domain and a linker region lying between the SH2 and kinase domains that is responsible for enzyme inhibition. Displacement of these SH2- and SH3-mediated associations by various ligands has been implicated in Src kinase activation (9,10) whereby the molecule is permissive for both ATP binding and Tyr-416 autophosphorylation , rendering the protein in a fully active conformation and accessible to substrate interactions (11,12)

3. SRC MUTATIONS IN HUMAN CANCER

Because of the oncogenic behavior of mutated oncogenic Src and the role of Src in regulating cell growth and adhesion, a number of studies have attempted to implicate Src mutations in human cancer. The contribution of Src truncation mutants to a subset of advanced human colon cancers has been described by Irby et al. (13). A point mutation introduces a stop codon corresponding to amino acid 531, terminating translation of the mRNA immediately after the carboxy-terminal regulatory tyrosine 530 (corresponding to tyrosine 527 in chicken-derived v-Src) The resultant truncated protein is termed Src 531. DNA samples from normal surrounding tissue and from matched controls were negative for the mutation, demonstrating its tumor-specificity. Protein isolates from tumors harboring the mutation contain enhanced Src kinase activity and in-vitro data supports the finding that truncation of Src at codon 531 activates the kinase. Interestingly, the negative regulatory tyrosine 530 is phosphorylated but this phosphorylation does not inhibit enzymatic activity in in-vitro kinase assays, due likely to the absence of sequences downstream from tyrosine 530. Further, Src 531 can induce biologic transformation, though not as aggressively as v-Src. Stable transfected fibroblasts expressing Src 531 display anchorage independent growth, form foci in transformation assays, and exhibit metastatic behavior in

vivo. Stable expression of Src 531 but not normal Src in fibroblasts increases migration through matrigel and confers metastatic behavior following intravenous injection (13).

Although this evidence has made it tempting to speculate that genetic mutation of Src plays a role in the pathogenesis of colon cancer, additional analysis of colon and other neoplastic specimens have failed to identify changes in Src at codon 531 (14-16) or other activating mutations (17). Indeed, genetic abnormalities in non-receptor tyrosine kinases of tumor samples are rarely observed. A much larger body of evidence implicates changes in protein expression and/or kinase activity as more likely to contribute to human carcinogenesis than mutations (reviewed in 18). A convincing correlation exists between changes in the protein levels/kinase activity of Src and neoplastic transformation of a variety of human cell lines derived from colon, breast, brain, bladder, and pancreatic cancers (see below).

4. SURVEY OF SRC EXPRESSION IN SOLID HUMAN TUMORS

4.1. Colon cancer

Colon cancer progresses through a series of genetic and physiologic changes occurring to the normal mucosal cells resulting in polyp growth, and if untreated, eventually metastasis (19). Increases in the transcription of *Src* mRNA has been shown in a number of human colon cancer cell lines, though the vast majority of changes occur post-transcription (20). Moreover, changes in the levels of Src protein and/or kinase activity appear to correlate with the grade of malignancy. Stepwise increases in Src activity have been observed during pre-malignant ulcerative colitis, polyp formation, invasive tumors, and ultimately in metastatic lesions (21-32). In a 2-cm tubulovillous sigmoid adenoma, a 16-fold increase in Src autophosphorylation over adjacent normal mucosa was measured. A 21-fold increase in Src autophosphorylation was noted in a larger polyp containing invasive carcinoma cells (25). A study by Talamonti, et al. (27) corroborates these findings, noting that in small polyps, no enhancement in Src kinase or protein levels was found but in large benign villous adenomas, the specific activity of Src is elevated. Further, in a study of primary lesions, Src specific activity is increased an average of 7-fold over normal mucosa. In samples of metastasis to the liver, these increases are more pronounced (17.9-fold average increase in specific activity). Work with synchronous lesions resected from both primary and metastatic sites support these observations as both contain increases in Src protein levels and specific enzymatic activity (27). These data implicate progressive changes in Src protein levels and kinase activity throughout the pathogenesis of colon cancer. Whether changes in Src aid in the progression of the disease or are a consequence of changes in cellular physiology associated with neoplastic transformation is currently under investigation (32, 33). Answers to this question will have important clinical implications concerning the efficacy of Src kinase inhibitors for treating colon cancer.

4.2. Breast cancer

A connection between Src and breast cancer was first revealed by several animal model systems. Increased Src kinase activity is found in murine breast carcinomas induced by transgenic expression of polyoma T antigen and P185 HER2/ Neu (34,35). However, MMTV-regulated transgenic Src expression results in breast hyperplasia while progression to carcinomas was rare (36). These results support Src expression as a contributor to malignant conversion rather than a causal oncogene. That is, it is unlikely that Src activation is the direct causal event in human mammary cellular transformation. Instead, it is a key contributor to the maintenance and progression of the transformed phenotype. Consistent with the observations from colon cancer, Src protein levels and kinase activity is increased in a large percentage of breast tumors and tumor-derived cell lines. Immunocomplex kinase assays performed with extracts from both breast tumor cells and biopsies obtained from varying stages of malignancy found enhanced Src activity in the tumor-derived samples, relative to surrounding normal tissue (21, 37-44). In breast cancer cell lines, this trend is further corroborated, certain lines contain up to 30-fold increases in Src activity (44). Experiments investigating the mechanism for this activation have found that Src is preferentially dephosphorylated at the negative regulatory tyrosine 530 in breast carcinomas, suggesting that aberrant phosphatase activity may contribute to the observed Src activation (41, 42). Other possible mechanisms for activation of Src kinase include changes in the C-terminal Src kinase, Csk, a negative regulator of Src in vivo or the highly homologous CHK (45). Interestingly, reports of increases in both Csk and Src activity suggest an in vivo insensitivity to the normal regulatory activity (46). Immunohistochemical analysis of both normal and diseased tissue reveals the localization of Src is unique in tumor specimens. Whereas normal breast tissue express low levels of Src in a fine cytoplasmic pattern, tumor tissue contains increased levels of Src protein localized in a granular fashion. In metastatic tumors, expression of Src becomes predominantly perinuclear reflecting a possible change in the function of Src, enhancing mitotic activity in these cells (40, 47, 48).

4.3. Additional solid tumors

Changes in Src expression and/or kinase activity have also been shown to correlate with tumorigenicity in the pancreas, liver, and brain, and bladder. In an immunohistochemical analysis of pancreatic tumor tissue prepared from 13 carcinomas, each sample exhibited increases in Src expression. In 80% of pancreatic tumor lines examined, increases in Src protein expression were noted and kinase activity was limited to cancer lines. Importantly, no changes in Csk protein levels were observed indicating that increases in Src kinase are not due to reductions in Csk (49) although a possibility for oxidative activation of Src by tyrosine nitration has also been proposed (50). Increased levels of interferon-like growth factor receptors have been noted on the surface of transformed pancreatic cells suggesting this may be a downstream consequence of the increased Src activity (51). In hepatocellular carcinoma specimens, Src autophosphorylation is 5 times higher than adjacent normal tissue. Src autophosphorylation levels are inversely

correlated with extent of differentiation, as more robust Src kinase is noted in poorly differentiated hepatocellular carcinomas compared to moderately or well differentiated samples (52,53). Similarly, bladder transitional cell carcinoma contain increases in Src kinase (54) as do cancers of the upper digestive tract (55,56). Human neuroblastoma cell lines contain 20-40 fold increases in Src kinase activity (21, 57-61) which also correlates with their differentiation status (62). Late stage ovarian cancers also contain elevated levels of Src protein and kinase activity, again supporting a role in malignant progression (63, 64). Human melanoma display increased tyrosine phosphorylation that has been linked to Src (65-70) as well as other Src family members (69). Activation of Src has been shown to correlate with the hypophosphorylation of Tyr 530 in transformed melanoma cells (66).

OTHER Src FAMILY MEMBERS OVEREXPRESSED IN SOLID HUMAN TUMORS

While most studies have focused on the role of the Src non-receptor tyrosine kinase in human cancer, changes in other Src family member expression has also been observed in tumor tissues. Using an antibody recognizing Src family kinases, 48% (25/52) of breast tumor specimens analyzed contained elevated expression of Src-related proteins including Fyn and Yes. In 70% of the samples analyzed, increases in the expression or kinase activity relative to normal tissue samples were found (43). In dysplastic colon lesions with high malignant potential and in primary colon cancer, the Yes kinase is activated when compared to normal tissue (70,71). $p56^{lck}$ is a lymphoid specific Src-related tyrosine kinase required for the maturation and proper function of T cells (72-74). Although generally restricted to tissues of the lymphoid lineage, expression of $p56^{lck}$ mRNA has been observed in colon and small lung carcinoma cell lines (75). Higher expression of $p56^{lck}$ mRNA was noted in lines derived from tumor metastasis when compared to levels in lines derived from primary tumors (73). In colon carcinoma tumor tissue, RT-PCR analysis revealed robust expression of $p56^{lck}$ type I transcripts. Corollary immunohistochemical staining detected $p56^{lck}$ expression in primary colon carcinoma but not in adjacent normal tissue**.** Mechanistic studies revel that aberrant expression of $p56^{lck}$ in colon carcinoma may be due to transcriptional activation of the Lck type I promoter as mediated by Ets and HMG-related transcription factors (76,77).

6. SRC ACTIVATION

Src is activated by numerous extracellular stimuli including the binding of ligand to receptor tyrosine kinases (RTKs), through contacts with the extracellular matrix, and by G-protein coupled receptors. Src and numerous other members of the Src family of non-receptor tyrosine kinases including c-Fyn, and c-Yes, have been shown to associate with receptors directly. Following ligand binding and receptor autophosphorylation, the RTK is activated and SH2-mediated docking sites are formed at the autophosphorylation of the cytoplasmic surface of the RTK. This allows transient signaling complexes to form and Src or Src-related proteins to be recruited and activated (reviewed in 78-81). Src can phosphorylate receptors directly, sometimes at residues not normally phosphorylated during RTK autophosphorylation, creating recruitment sites for SH2 containing molecules. Associations have been demonstrated between Src and numerous RTKs including HER2/neu, EGFR, and the estrogen receptor (80, 81). Provocatively, synergistic changes in the expression of Src and RTKs have been correlated with tumorigenesis in cancers derived from the pancreas, breast, lung, and brain and often correlate with poor clinical prognosis (82, reviewed in 18). Further, Src associates with HER2/neu in breast tumors but not in normal breast tissue and in highly metastatic cells of the colon (18). EGFR is upregulated in malignant tissue, the activated receptor associates with Src, and co-expression of both proteins causes synergistic mitogenicity and enhances transformation of murine fibroblast cells. Src exerts these effects presumably through the enhanced phosphorylation of substrates downstream from activated EGFR. Changes in Src signaling have also been noted in the context of angiogenic signaling as inhibition of Src kinase decreases hypoxic induction of VEGF, a ligand upregulated in angiogenic tumor cells (83, 84). Src associates with adhesion proteins, catenins, cadherins, and is activated by integrin-mediated substrate adhesion (81, 85). In signaling downstream from these structures at the cell membrane, Src may aid in circumventing growth controls in tumor types where Src and associated receptors are both overexpressed.

7. CONCLUDING REMARKS

The biological significance of altered Src signaling in human neoplasia that represents an intersection between gross analysis and the molecular dissection of signal transduction circuits. Analysis of Src expression in diverse solid human tumors has consistently revealed an enhancement in Src kinase activity compared to normal surrounding tissue. The difficulty in identifying genetic alterations responsible for this activation, analogous to the activated v-Src genotype, has instead supported a role for Src in the maintenance and progression of the transformed phenotype. Src signaling in numerous solid tumors activating diverse signal transduction pathways identifies it a credible target in cancer treatment. Because of the knowledge of its activation and the presence of domains responsible for protein interactions and assembling signal complexes, multiple targets are available for rational drug design, including inhibition of ATP binding and blocking peptides. The precise downstream effectors of Src signaling are still being defined, as are effects extending well beyond the cell membrane now to the levels of specific gene expression and regulation.

8. REFERENCES

1. Bjorge, J.D., A. Jakymiw, & D.J. Fujita: Selected glimpses into the activation and function of Src kinase. *Oncogene* 19, 5620-5635 (2000)
2. Irby R.B. & T.J. Yeatman: Role of Src expression and activation in human cancer. *Oncogene* 19, 5636-42 (2000)

3. Rous, P.: A sarcoma of the fowl transmissible by an agent separate from the tumour cells. *J Exp Med* 13, 397-411 (1911)
4. Takeya T. & H. Hanafusa: Structure and sequence of the cellular gene homologous to the RSV src gene and the mechanism for generating the transforming virus. *Cell* 32, 881-90 (1983)
5. Cooper, J.A., K.L. Gould, C.A. Cartwright & T. Hunter: Tyr 527 is phosphorylated in pp60c-Src: implications for regulation. *Science* 231, 1431-1434 (1986)
6. Roussel, R.R., S.R. Brodeur, D. Shalloway, & A.P. Laudano: Selective binding of activated pp60c-src by an immobilized synthetic phosphopeptide modeled on the carboxyl terminus of pp60c-src. *Proc Nat Acad Sci USA* 88, 10696-10700 (1991)
7. Liu, X., S.R. Brodeur, G. Gish, Z. Songyang, L.C. Cantley, A.P. Laudano, & T. Pawson: Regulation of c-src tyrosine kinase activity by the Src SH2 domain. *Oncogene* 8, 1119-1126 (1993)
8. Weijland, A., J.C. Williams, G. Neubauer, S.A. Courtneidge, R.K. Wierenga, & G. Superti-Furga: Src regulated by C-terminal phosphorylation is monomeric. *Proc Nat Acad Sci USA* 94, 3590-3595 (1997)
9. Alexandropoulos, K. & D. Baltimore: Coordinate activation of c-Src by SH3- and SH2-binding sites on novel p130 Cas-related protein, Sin. *Genes Dev* 10, 1341-1355 (1996)
10. Burnham, M.R., P.J. Bruce-Staskal, M.T. Harte, C.L. Weidow, A. Ma, S.A. Weed, & A.H. Bouton: Regulation of c-Src activity and function by the adapter protein CAS. Mol Cell Biol 20, 5865-5878 (2000)
11. Xu, W., S.C. Harrison, & M.C. Eck: Three dimensional structure of the tyrosine kinase c-Src. Nature 385, 595-602 (1997)
12. Williams, J.C. A. Weijland, S. Gonfloni, A. Thompson, S.A. Courtneidge, G. Superti-Furga, & R.K. Wierenga: The 2.35A crystal structure of the inactivated form of chicken src; a dynamic model with multiple regulatory interactions: *J Mol Biol* 274, 757-775 (1997)
13. Irby R.B., W. Mao, D. Coppola, J. Kang, J.M. Loubeau, W. Trudeau, R., D.J. Fujita, R. Jove & T.J. Yeatman: Activating SRC mutation in a subset of advanced human colon cancers. *Nat Genet* 21, 187-90 (1999)
14. Wang, P., F. Fromowitz, M. Koslow, N. Hagag, B. Johnson, & M. Viola: c-Src structure in human cancers with elevated pp60c-src activity. *Brit J Cancer* 64, 531-533 (1991)
15. Daigo Y, Y. Furukawa, T. Kawasoe, H.Ishiguro, M. Fujita, S. Sugai, S. Nakamori, G.J. Liefers, R.A. Tollenaar, C.J. van de Velde, & Y. Nakamura: Absence of genetic alteration at codon 531 of the human c-src gene in 479 advanced colorectal cancers from Japanese and Caucasian patients. *Cancer Res* 59, 4222-4224 (1999)
16. Laghi, L., P. Bianchi, O. Orbetegli, L. Gennari, M. Roncelli & A. Malesci: Lack of mutation at codon 531 of SRC in advanced colorectal cancers from Italian patients. *Brit J Cancer* 84, 196-198 (2001)
17. Wang, N.M., K.T. Yeh, C.H. Tsai, S.J. Chen, & J.G. Chang: No evidence of correlation between mutation at codon 531 of src and the risk of colon cancer in Chinese. *Cancer Lett* 150, 201-04 (2000)
18. Biscardi, J.S., D.A. Tice, & S.J. Parsons: c-Src, receptor tyrosine kinases, and human cancer: *Adv Cancer Res*, 61-119 (1999)
19. Fearon, E.R. & B. Vogelstein: A genetic model for colorectal tumorigenesis. *Cell* 61, 759-769 (1990)
20. Dehm S., M.A. Senger, & K. Bonham K: SRC transcriptional activation in a subset of human colon cancer cell lines. *FEBS Lett* 487, 367-71 (2001)
21. Rosen, N., J.B. Bolen, A.M. Schwartz, P. Cohen, V. DeSeau, & M.A. Israel: Analysis of pp60c-src protein kinase activity in human tumor cell lines and tissues. *J Biol Chem* 261, 13754-13759 (1986)
22. Bolen, J.B, A. Veillette, A.M. Schwartz, V. Deseu & N. Rosen: Activation of pp60c-src protein kinase activity in human colon carcinoma. Proceedings of the National Academy of Sciences of the United States of America 84, 2251-2255 (1987)
23. Lundy J, J. Chen, P. Wang, F. Fromowitz, A. Schuss, S. Lynch, J. Brugge & M.V. Viola: Phenotypic and genetic alterations in pre-cancerous cells in the colon. *Anticancer Res* 8, 1005-1013 (1988)
24. Cartwright, C.A., M.P. Kamps, A.I. Meisler, J.M. Pipas & W. Eckhart: pp60c-src activation in human colon carcinoma. *J Clin Invest* 83, 2025-2033 (1989)
25. Cartwright C.A., A.I. Meisler, & W. Eckhart: Activation of the pp60c-src protein kinase is an early event in colonic carcinogenesis. *Proc Nat Acad Sci USA* 87, 558-62 (1990)
26. Weber T.K., G. Steele, & I.C. Summerhayes: pp60c-src activity in well and poorly differentiated human colon carcinomas and cell lines. *J Clin Invest* 90, 815-21 (1992)
27. Talamonti, M.S., M.S. Roh MS, S.A. Curley, & G.E. Gallick: Increase in activity and level of pp60c-src in progressive stages of human colorectal cancer. *J Clin Invest* 91, 53-60 (1993)
28. Termuhlen P.M., S.A.Curley, M.S. Talamonti, M.H. Saboorian, & G.E. Gallick: Site-specific differences in pp60c-src activity in human colorectal metastases. *J Surg Res* 54, 293-298 (1993)
29. Cartwright C.A., C.A. Coad, & B.M. Egbert: Elevated c-Src tyrosine kinase activity in premalignant epithelia of ulcerative colitis. .*J Clin Invest* 93, 509-515 (1994)
30. Mao, W., R. Irby, D. Coppola, L. Fu, M. Wloch, J. Turner, H. Yu, R. Garcia, R. Jove & T.J. Yeatman: Activation of c-Src by receptor tyrosine kinases in human colon cancer cells of high metastatic potential. *Oncogene* 15, 3083-3090 (1997)
31. Iravani S., W. Mao, L. Fu, R. Karl, T. Yeatman, R. Jove, & D. Coppola: Elevated c-Src protein expression is an early event in colonic neoplasia. *Lab Invest* 78, 365-71 (1998)
32. Rustgi A.K: c-src tyrosine kinase activity: a marker of dysplasia in ulcerative colitis. *Gastroenterology* 107, 1565-1566 (1994)
33. Malek R.L., R.B. Irby, Q.M. Guo, K. Lee, S. Wong, M. He, J. Tsai, B. Frank, E.T. Liu, J. Quackenbush, R. Jove, T.J. Yeatman, & N.H. Lee: Identification of Src transformation fingerprint in human colon cancer. *Oncogene* 21, 7256-65 (2002)
34. Guy, C., S. Muthuswamy, R. Cardiff, P. Soriano & W. Muller: Activation of the c-Src tyrosine kinase is required

for the induction of mammary tumors in transgenic mice. *Genes Dev* 8, 23-32 (1994)
35. Muthuswamy, S., P. Siegel, D. Dankort, M. Webster & W. Muller: Mammary tumors expressing the neu proto-oncogene possess elevated c-Src tyrosine kinase activity. *Mol Cell Biol* 14, 735-743 (1994)
36. Webster, M.A., R.D. Cardiff, & W.J. Muller: Induction of mammary epithelial hyperplasias and mammary tumors in transgenic mice expressing a murine mammary tumor virus/activated c-src fusion gene. *Proc Nat Acad Sci USA* 92, 7849-7853 (1995)
37. Hennipman, A., B.A. van Oirshot, J. Smits, G., Rijksen, & G.E.J. Staal: Tyrosine kinase activity in breast cancer, benign breast disease and normal breast tissue. *Cancer Res* 49, 516-521 (1989)
38. Jacobs, C. & H. Rubsman: Expression of ppc-src protein kinase in adult and fetal human tissue: high activities in some sarcomas and mammary carcinomas. *Cancer Res* 43, 1696-1702 (1983)
39. Ottenhoff-Kalff, A.E., G. Rijksen, E.A.C.M. van Beurden, A. Hennipman, A.A. Michels, & G.E.J. Staal: Characterization of protein tyrosine kinases from human breast cancer; involvement of the c-src oncogene product. *Cancer Res* 52, 4773-4778 (1992)
40. Verbeek, B.S., T.M. Vroom, S. S. Adriaasen-Slot, A.E. Ottenhoff-Kalff, J.N.G. Geertzema, A. Hennipman & G. Rijksen: c-Src protein expression is increased in human breast cancer. An immunohistochemical and biochemical analysis. *J Pathol* 180, 383-388 (1996)
41. Egan C., A. Pang, D. Durda, H.C. Cheng, J.H. Wang & D.J. Fujita: Activation of Src in human breast tumor cell lines: elevated levels of phosphotyrosine phosphatase activity that preferentially recognizes the Src carboxy terminal negative regulatory tyrosine 530. *Oncogene* 18, 1227-37 (1999)
42. Bjorge, J.D., A. Pang, & D.J.Fujita: Identification of protein-tyrosine Phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. *J Biol Chem* 275, 41439– 41446 (2000)
43. Reissig D, J. Clement, J. Sanger, A. Berndt, H. Kosmehl, & F. D. Bohmer: Elevated activity and expression of Src-family kinases in human breast carcinoma tissue versus matched non-tumor tissue. *J Cancer Res Clin Oncol* 127, 226-30 (2001)
44. Belsches-Jablonski, A.P., J.S. Biscardi, D.R. Peavy, D.A. Tice, D.A. Romney, & S.J. Parsons: Src family kinases and HER2 interactions in human breast cancer cell growth and survival. *Oncogene* 20, 1465-1475 (2001)
45. Bougeret, C, S. Jiang, I. Keydar, & H. Avraham: Functional analysis of Csk and CHK kinases in breast cancer cells. J Biol Chem, 33711-33720 (2001)
46. Benistant, C., J.-F. Bourgaux, H. Chapuis, N. Mottet, S. Roche & J.-P. Bali: The COOH-terminal Src kinase Csk is a tumor antigen in human carcinoma. *Cancer Res* 61, 1415-1420 (2001)
47. Chackalaparampil, I. & D. Shalloway: Altered phosphorylation and activation of pp60c-src during fibroblast mitosis. Cell 52, 801-810 (1988)
48. Kaplan, K.B., J.R. Swedlow, H.E. Varmus, & D.O. Morgan: Association of p60c-src with endosomal membranes in mammalian fibroblasts. *J Biol Chem* 118, 321-333 (1992)
49. Lutz, M.P., I.B.S. Eber, B.B.M. Flossman-kast, R. Vogelmann, H. Luhrs, H. Friess, M.W. Buchler & G. Alder: Overexpression and activation of the tyrosine kinase src in human pancreatic carcinoma. *Biochem Biophys Rres Commun* 243, 503-508 (1998)
50. Macmillian-Crow, L.A., J.S.S Greendorfer, S.M. Vickers & J.M. Thompson: Tyrosine nitration of c-SRC tyrosine kinase in human pancreatic ductal adenocarcinoma. *Arch Biochem Biophys* 377, 350-356 (2000)
51. Flossmann-Kast, B.B., P.M. Jehle, A. Hoeflich, G. Adler & M.P. Lutz: Src stimulates insulin-like growth factor I (IGF-I)-dependent cell proliferation by increasing IGF-I receptor number in human pancreatic carcinoma cells. *Cancer Res* 58, 3551-3554 (1998)
52. Masaki,T., M. Okada, Y. Shiratori, W. Rengifo, K. Matsumoto, S. Maeda, N. Kato, F. Kanai, Y. Komatsu, M. Nishioka & M. Omata: pp60c-src activation in hepatocellular carcinoma of humans and LEC rats. *Hepatology* 27, 1257-1264 (1998)
53. Masaki,T., M. Okada, M. Tokuda, Y. Shiratori, O. Hatase, M. Nishioka & M. Omata: Reduced C-terminal src kinase (Csk) activities in human hepatocellular carcinoma. *Hepatology* 29, 379-382 (1999)
54. Benistant C., H. Chapuis, N. Mottet, J. Noletti, E. Crapez, J.P. Bali, & S. Roche: Deregulation of the cytoplasmic tyrosine kinase cSrc in the absence of a truncating mutation at codon 531 in human bladder carcinoma. *Biochem Biophys Res Commun* 273, 425-430 (2000)
55. Kumble, , S., M.B. Omary, C.A. Cartwright, & G.Triadafilopoulos: Src activation in malignant and premalignant epithelia of Barrett's esophagus. *Gasteroenterology* 112, 348-356 (1997)
56. Takekura, , N., W. Yasui, K. Yoshida, T. Tsujino, H. Nakayama, T. Kameda, H. Yokozaki, Y. Nishimura, H. Ito, & E. Tahara: pp60c-src protein kinase activity in human gastric carcinomas. *Int J Cancer* 45, 847-851 (1990)
57. Bolen, J.B., N. Rosen, & M.A. Israel: Increased pp60c-src tyrosyl kinase activity in human neuroblastomas is associated with amino-terminal tyrosine phosphorylation of the src gene product. *Proc Nat Acad Sci USA* 82, 7275-9 (1985)
58. Veillette A., J. O'Shaughnessy, I.D. Horak, M.A. Israel, D. Yee, N. Rosen, D.J. Fujita, H.J.Kung, J.L. Biedler, & J.B Bolen: Coordinate alteration of pp60c-src abundance and c-*src* RNA expression in human neuroblastoma variants. *Oncogene* 4, 421-427 (1989)
59. Bjelfman C.F., I. Hedborg, M. Johansson, M. Nordenskjold, & S. Pahlman: Expression of the neuronal form of pp60c-src in neuroblastoma in relation to clinical stage and prognosis. *Cancer Res* 50, 6908-6914 (1990)
60. Matsunaga T., H. Takahashi, N. Ohnuma, M. Tanabe, H. Yoshida, J. Iwai, H. Shirasawa, & B. Simizu: Expression of N-myc and c-src protooncogenes correlating to the undifferentiated phenotype and prognosis of primary neuroblastomas. *Cancer Res* 51, 3148-3152 (1991)
61. Matsunaga T., H. Shirasawa, H. Enomoto, H. Yoshida, J. Iwai, M. Tanabe, K. Kawamura, T. Etoh, & N. Ohnuma: Neuronal src and trk a protooncogene expression in neuroblastomas and patient prognosis. *Int J Cancer* 79, 226-31 (1998)

62. Mellstrom K, C. Bjelfman, U. Hammerling, & S. Pahlman: Expression of c-src in cultured human neuroblastoma and small-cell lung carcinoma cell lines correlates with neurocrine differentiation. *Mol Cell Biol* 7, 4178-84 (1987)
63. Budde R.J., S. Ke S, & V.A. Levin: Activity of pp60c-src in 60 different cell lines derived from human tumors. *Cancer Biochem Biophys* 14, 171-175 (1994)
64. Weiner, J.R., T. C. Windham, V.C. Estrella, N.U. Parikh, P.F. Thall, M.T. Deavers, R.C. Bast Jr, & G.E. Gallick: Activated Src protein tyrosine kinase is overexpressed in late-sage human ovarian cancers. *Gynecol Oncol* 88, 73-79 (2003)
65. Barnekow A, E. Paul, & M. Schartl: Expression of the c-src protooncogene in human skin tumors. *Cancer Res* 47, 235-240 (1987)
66. O'Connor T.J., E. Neufeld, J. Bechberger, & D.J. Fujita.: pp60c-src in human melanocytes and melanoma cells exhibits elevated specific activity and reduced tyrosine 530 phosphorylation compared to human fibroblast pp60c-src. *Cell Growth Diff* 3, 435-442 (1992)
67. O'Connor T.J., J.D. Bjorge, H.C. Cheng, J.H. Wang, & D.J. Fujita.: Mechanism of c-SRC activation in human melanocytes: elevated level of protein tyrosine phosphatase activity directed against the carboxy-terminal regulatory tyrosine. *Cell Growth Diff* 6, 123-130 (1995)
68. O'Connor, T.J. & D.J. Fujita: Differentiation of B16 murine melanoma cells is associated with an increased level of c-SRC. *Melanoma Res* 5, 5-13 (1995)
69. Loganzo, F. Jr, J.S. Dosik, Y. Zhao, M.J. Vidal, D.M. Nanus, M. Sudol, & A.P. Albino: Elevated expression of protein tyrosine kinase c-Yes, but not c-Src, in human malignant melanoma. *Oncogene* 8, 2637-2644 (1993)
70. Niu G, T. Bowman, M. Huang, S. Shivers, D. Reintgen, A. Daud, A. Chang, A. Kraker, R. Jove, & H. Yu: Roles of activated Src and Stat3 signaling in melanoma tumor cell growth. *Oncogene* 21, 7001-7010 (2002)
71. Pena S.V., M.F. Melhem, A.I.Meisler, & C.A. Cartwright: Elevated c-yes tyrosine kinase activity in premalignant lesions of the colon. *Gastroenterology* 108, 117-24 (1995)
72. Park J., A.I. Meisler, & C.A. Cartwright: c-Yes tyrosine kinase activity in human colon carcinoma. *Oncogene* 8, 2627-2635 (1993)
73. Veillette A., F.M. Foss, E.A. Sausville, J.B. Bolen, & N. Rosen: Expression of the Lck tyrosine kinase gene in human colon carcinoma and other non-lymphoid human tumor cell lines. *Oncogene Res* 14, 357-374 (1987)
74. Veillette, A., M.A. Bookman, E.M. Horak, & J.B. Bolen: The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. *Cell* 55, 301-308 (1988)
75. Veillette, A., I.D. Horak, E.M. Horak, M.A. Bookman, & J.B. Bolen: Alterations in the lymphocyte-specific protein tyrosine kinase (p56lck) during T-cell activation. *Mol Cell Biol* 8, 4353-4361 (1988)
76. McCracken S., C.S. Kim, Y. Xu, M. Minden, & N.G.Miyamoto: An alternative pathway for expression of p56lck from type I promoter transcripts in colon carcinoma. *Oncogene* 15, 2929-2937 (1997)
77. Nakamura K., Y. Chijiiwa Y, & H. Nawata H: Augmented expression of *LCK* message directed from the downstream promoter in human colorectal cancer specimens *Eur J Cancer* 32A, 1401-1407 (1996)
78. Martin G.S.: The hunting of the Src. *Nat Rev Mol Cell Biol* 2, 467-475 (2001)
79. Ma Y.C. & X.Y. Huang: Novel regulation and function of Src tyrosine kinase. *Cell Mol Life Sci* 59, 456-62 (2002)
80. Pellicena P. & W.T. Miller: Coupling kinase activation to substrate recognition in SRC-family tyrosine kinases. *Front Biosci* 7, d256-67 (2002)
81. Thomas S.M. & J.S. Brugge: Cellular functions regulated by Src family kinases. *Ann Rev Cell Dev Biol* 13, 513-609 (1997)
82. Slamon D.J., W. Godolphin, L.A. Jones, J.A. Holt, S.G. Wong, D.E. Keith, W.J. Levin, S.G. Stuart, J. Udove, A. Ullrich, & M.F. Press: Studies of the HER-2/neu protooncogene in human breast and ovarian cancer. Science 244, 707-712 (1989)
83. Mukhopadhyay D., L. Tsiokas, X.M. Zhou, D. Foster, J.S. Brugge, & V.P. Sukhatme: Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. *Nature* 375, 577-581 (1995)
84. Schlessinger J: New roles for Src kinases in control of cell survival and angiogenesis. *Cell* 100, 293-296 (2000)
85. Frame M.C., V.J. Fincham, N.O. Carragher & J.A. Wyke: v-Src's hold over actin and cell adhesions. *Nat Rev Mol Cell Biol* 3, 233-245 (2002)

Key Words: Non-Receptor Tyrosine Kinase, Receptor Tyrosine Kinase, Phosphorylation, Colon Cancer, Breast Cancer, Review

Send correspondence to: Dr. Scott Shore, Fels Institute for Cancer Research & Department of Biochemistry, Temple University School of Medicine, 3307 N. Broad Street, Philadelphia PA 19140, Tel: 215 707-3359, Fax:215-707-2102, E-mail: sks@temple.edu