A RENAISSANCE FOR SRC

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The c-SRC non-receptor tyrosine kinase is overexpressed and activated in a large number of human malignancies and has been linked to the development of cancer and progression to distant metastases. These observations have led to the recent targeting of c-SRC for the development of anticancer therapeutics, which show promise as a new avenue for cancer treatment. Despite this, however, the precise functions of c-SRC in cancer remain unclear. In addition to increasing cell proliferation, a key role of c-SRC in cancer seems to be to promote invasion and motility, functions that might contribute to tumour progression.

Despite the fact that c-*SRC* is one of the oldest and most investigated proto-oncogenes[1], we still do not completely understand its role in cancer. Although the c-SRC kinase has been linked with the development and progression of cancer for many years, there has recently been a renewed interest in this oncoprotein as a molecular target for cancer therapy. Whereas several SRC inhibitors have been available for some time for *in vitro* experimentation, compounds that are highly specific for c-SRC and are stable *in vivo* have only recently been developed. These drug-discovery efforts have now borne fruit, and c-SRC inhibitors are entering clinical trials for the first time, creating a resurgence in studies of this molecule in human cancer.

However, despite the progress that has been made in targeting c-SRC for cancer treatment, much remains to be learned about its contribution to cancer progression. In recent years, *in vitro* observations have led to the hypothesis that, in addition to increasing cellular proliferation, a primary role for c-SRC in cancer is to regulate cell adhesion, invasion and motility. What are the molecular mechanisms by which c-SRC regulates these processes and how do these functions of c-SRC contribute to cancer development *in vivo*?

v-Src and c-SRC: an historical perspective

Almost a century ago, Peyton Rous first described a virus that seemed to cause transmissible growth of tumours in chickens, an idea that was controversial because at that time cancers were not thought to be caused by infectious agents[2,3]. Doubts about this were dispelled in the 1950s, when it was clearly demonstrated that a Rous sarcoma virus (RSV)-induced tumour could, in fact, produce infected tumour cells[4]. Definitive confirmation that v-*src* — an RSV gene — was a causative agent of cancer was obtained when Martin identified temperature-sensitive, 'activated' mutants of chicken v-*src* that failed to transform cells at non-permissive temperatures[5]. This model clearly showed that the virus was necessary to maintain the transformed state, as when they were shifted from permissive tumorigenic temperatures to non-permissive temperatures, cells reverted to a normal phenotype.

The v-*src* gene was identified in the viral genome in the 1970s and was subsequently sequenced by Bishop, Hanafusa and Gilbert[6–9]. A great deal of knowledge about the function and mechanism of action of v-*src* comes from studies in which various portions of the gene have been mutated to affect its transformation potential. Interestingly, it was found that various mutations altered the transformation capacity, morphology and host range of this gene. For example, Varmus identified a v-*src* mutant that is defective in transforming rat cells, but is able to transform chicken embryonic fibroblasts[10]. Curiously, it has not been possible to derive a v-*src*-transformed human fibroblast cell line, although we now know that c-*SRC* has a significant role in the development of numerous human cancers. This might be because of the fact that, until recently, the conditions that are required for the transformation of human cells by most oncogenes had not been established. Although not yet reported for c-*SRC*, it has been possible to transform human fibroblasts with oncogenes such as *HRAS* when they are combined with human telomerase reverse transcriptase and the SV40 large T antigen[11].

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Summary

- v-*src* was the first of numerous viral oncogenes to be identified and is among the best studied of these. The cellular counterpart of this oncogene — c-*SRC* — is implicated in a range of human cancers.
- Both overexpression and overactivation of c-SRC can promote the development of cancer. The structural mechanisms by which c-SRC kinase activity is regulated are now well established, and c-SRC is known to have a negative-regulatory domain that is itself regulated through phosphorylation.
- c-SRC kinase activity is regulated by several mechanisms, including activation by receptor tyrosine kinases and cytoplasmic phosphatases. Levels of c-SRC protein can also be regulated, for example, by targeting this protein for degradation in the ubiquitin–proteasome pathway. In addition, c-SRC function can also be modulated by regulation of its cellular localization.
- A wide range of c-SRC substrates have been identified, which has led to a better understanding of c-SRC-mediated signal transduction. These substrates include focal-adhesion proteins, adaptor proteins and transcription factors.
- In addition to cell proliferation, SRC proteins regulate three main cellular functions that ultimately control the behaviour of transformed cells: adhesion, invasion and motility. These functions might also contribute to tumour progression and metastasis.
- Recently, drug-discovery efforts have led to the development of several c-SRC inhibitors for potential use as anticancer therapeutics.

Huebner and Todaro[12] were the first to postulate the existence of viral oncogenes as determinants of cancer and it was later suggested that proto-oncogenes existed as the cellular precursors of retroviral transforming genes[13]. The v-*src* sequence was found to be conserved in the vertebrate genome, which indicated that v-*src* was derived from a normal human gene that had been incorporated into RSV by recombination. It differs in sequence from c-*SRC* in carboxy-terminal deletions and in point mutations throughout the gene[8]. Unlike v-*src*, c-*SRC* is poorly transforming, consistent with a role as a normal cellular proto-oncogene that, when activated, might serve as an oncogene. c-*SRC* was the first of many of such proto-oncogenes to be discovered.

v-*src* was subsequently shown to encode a tyrosine-specific protein kinase[14–16]. Most studies of SRC protein structure and function have been performed with the highly activated avian v-Src, which differs in structure and activity from human c-SRC. Interestingly, v-Src lacks the negative-regulatory C-terminal domain of human c-SRC (FIG. 1; see below), and consequently shows higher levels of activity and transforming ability. In addition, v-*src* contains point mutations throughout its coding region that probably contribute to the high level of intrinsic activity and transforming potential of the v-Src protein[17,18].

c-SRC and human cancer

Of all of the SRC-family kinases — which also include FYN, YES, BLK, YRK, FGR, HCK, LCK and LYN — c-SRC is the one that is most often implicated in cancer. Although the lessons that have been learned from fibroblast models of v-Src transformation are valuable, c-SRC expression in epithelial cells and in cancers might have different effects. For example, although c-SRC is required for fibroblast cell division[19], and might have a role in tumorigenesis by stimulating the proliferation of precancerous cells, it has recently been demonstrated that c-SRC activity does not correlate with increased colon tumour cell proliferation rates *in vitro* or with increased tumour growth rates *in vivo*. Overexpression of c-SRC in human colon cancer cells does not affect cell growth, but it does stimulate the assembly of integrin adhesions, enhancing the ability of cells to spread on a substrate[20]. Similarly, the cooperation of c-SRC and epidermal growth factor receptor (EGFR) regulates the invasiveness of colon cancer cells, but does not seem to influence proliferation[21]. One potential explanation that has been offered for this apparent discrepancy is that c-SRC activation promotes growth during the process of tumorigenesis, but regulates other activities such as adhesion and invasion during the later stages of tumour progression[22]. Another possibility is that SRC proteins have different effects in epithelial cells to those seen in fibroblasts. For these reasons, modelling c-SRC expression and activity in human cancer cells is also important in addition to studies in fibroblasts.

Overexpression of the c-SRC protein and an increase in its specific activity have been observed in numerous cancer types[23]. Most notably, gastrointestinal-tract cancers such as colorectal cancer show a progressive increase in c-SRC activity as the tumour stage advances — metastatic lesions, both intrahepatic and extrahepatic, often have the highest levels of c-SRC activity[24]. This indicates a potential role for c-SRC in mediating tumour progression. Paradoxically, the most aggressive tumours, which show poor differentiation, often display reduced levels of c-SRC protein and lower levels of c-SRC activity when compared with well- to moderately differentiated tumours that are less aggressive[25]. This interesting observation, however, might be explained by the presence of overexpressed receptor tyrosine kinases such as EGFR in poorly differentiated tumours, which can synergistically activate c-SRC (see below)[26,27]. Therefore, low levels of c-SRC might be compensated for by high levels of a receptor tyrosine kinase.

Beyond colorectal cancer, increased c-SRC activity has been demonstrated in several other gastrointestinal malignancies, including hepatocellular, pancreatic, gastric and oesophageal malignancies[23], as well as in breast[28], ovarian[29] and lung cancers[29]. Hepatocellular cancers[30,31] and colon carcinomas[32] are of interest because they can both overexpress c-SRC and underexpress the negative-regulatory c-SRC tyrosine kinase (CSK) protein concurrently, leading to higher levels of c-SRC activation. Increased specific activity of c-SRC can also occur in the presence of relatively normal levels of c-SRC protein expression (see below).

Mechanisms of c-SRC regulation and activation

c-SRC structure. Both the avian and human forms of the c-SRC protein are composed of a C-terminal tail containing a negative-regulatory tyrosine residue (Tyr527, chicken; Tyr530, human), four SRC homology (SH) DOMAINS[33] and a unique amino-terminal domain (FIG. 1a). The SH domains consist of the SH1 kinase domain, which contains the autophosphorylation site

SH DOMAINS
SRC homology domains are distinct regions of amino-acid homology that possess well-defined biochemical functions.

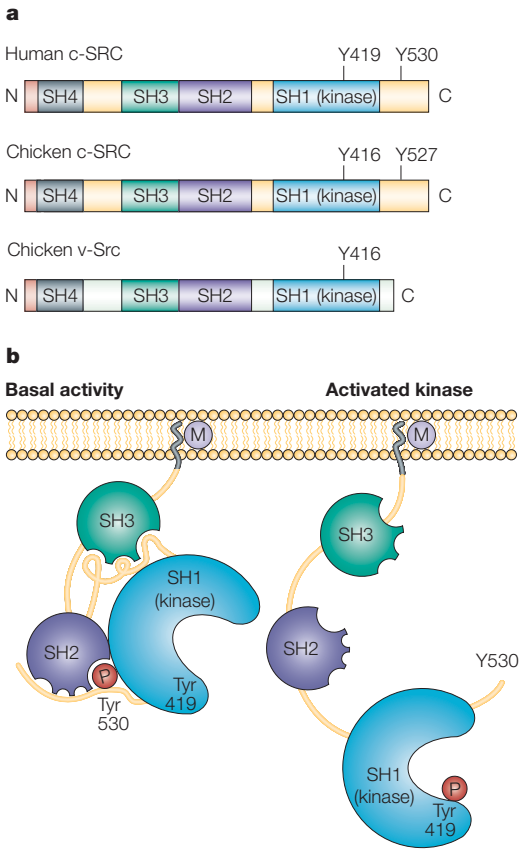

Figure 1 | **Structure and activation of SRC proteins.** **a** | Comparison of the molecular structures of human c-SRC, chicken c-SRC and chicken v-Src. All three proteins contain four SRC homology (SH) domains and a unique amino-terminal domain of unknown function. The SH1 domain contains the kinase domain and a conserved tyrosine residue involved in autophosphorylation (Tyr419 in human c-SRC; Tyr416 in chicken SRC proteins). Chicken v-Src lacks the carboxy-terminal negative-regulatory domain and contains 12 substituted carboxy-terminal amino acids, as well as numerous point mutations throughout the molecule, explaining the high level of activity of this protein. **b** | Inactivation of human c-SRC occurs when its C-terminal Tyr530 is phosphorylated and it binds back to the SH2 domain. This interaction and an interaction between the SH3 domain and the kinase domain result in a closed molecular structure with diminished access of substrates to the kinase domain. Conversely, c-SRC activation occurs with removal of the C-terminal phosphotyrosine, displacement of inhibitory intramolecular interactions, and opening of the c-SRC molecular structure. Full activation involves phosphorylation at Tyr419. M, myristoylation; P, phosphorylation. Part **b** modified with permission from REF. 1 © (2001) Nature Publishing Group.

(Tyr416, chicken; Tyr419, human); the SH2 domain, which interacts with the negative-regulatory Tyr527 and binds to the platelet-derived growth factor receptor (PDGFR)[34]; the SH3 domain, which promotes intramolecular contact with the kinase domain in the inactive form of the protein; and the SH4 domain, which contains the MYRISTOYLATION site that is important for membrane localization. The autophosphorylation site is important because it has been shown to be required for full SRC activation. The functions of the N-terminal domain are not well defined, but mutation of this region seems to reduce the transforming potential of v-Src[17].

The C-terminal tail and the SH2 and SH3 domains are involved in the negative regulation of c-SRC. The C-terminus contains the Tyr527 (human Tyr530) residue, which can bind to the SH2 domain when phosphorylated. Crystallographic studies have shown that interactions between the C-terminus and the SH2 domain, and between the kinase domain and the SH3 domain, cause the c-SRC molecule to assume a closed configuration that covers the kinase domain and reduces its potential for substrate interaction (FIG. 1b)[35]. Mutational studies, primarily involving avian forms of SRC, have clearly elucidated a closed, inactive conformation and an open, active state. When the C-terminal tyrosine is phosphorylated, SRC is inactive; when dephosphorylated, SRC is active, with the potential for autophosphorylation and for downstream interactions with and phosphorylation of SRC substrates. So, phosphatases that dephosphorylate human c-SRC at Tyr530 can bring about activation, even when protein levels are normal. v-Src, unlike c-SRC, is constitutively active because it lacks this crucial C-terminal negative-regulatory region.

c-SRC is able to activate downstream targets either because it is activated or because of high levels of the protein. v-Src is a highly active protein that can transform cells when expressed at low levels. c-SRC, on the other hand, is only mildly transforming, even when very high levels of the protein are expressed[36]. As cancer advances, both high levels of c-SRC protein and c-SRC kinase activity have been observed[29]. Collectively, these observations indicate the potential importance of both protein levels and specific activity in various phases of tumour development and progression. The various means by which c-SRC levels and activity are regulated are discussed below and are shown in FIG. 2.

Intramolecular regulation. Inactivation of c-SRC by phosphorylation of the terminal tyrosine residue is now known to be performed by CSK[37] and its homologue CHK, resulting in the closed, inactive c-SRC conformation described above. CSK is structurally related to c-SRC, but lacks the negative-regulatory domain of the c-SRC C-terminus, and there is now evidence that reduced expression of CSK might have a role in c-SRC activation in human cancer[31]. Conversely, the C-terminal phosphate of c-SRC can be removed by several protein phosphatases that function as activators of c-SRC. Protein tyrosine phosphatase-α (PTPα) has been shown to dephosphorylate the terminal tyrosine residue[38] *in vitro* and *in vivo*, and PTP1, SH2-containing phosphatase 1 (SHP1) and SHP2 might also regulate c-SRC[39]. The most direct evidence for a role in c-SRC activation in cancer among these phosphatases is for PTP1B, which is present at higher levels in breast cancer cell lines and can dephosphorylate c-SRC[40].

MYRISTOYLATION
Refers to the accession of fatty moieties that allow association with the inner layer of the plasma membrane.

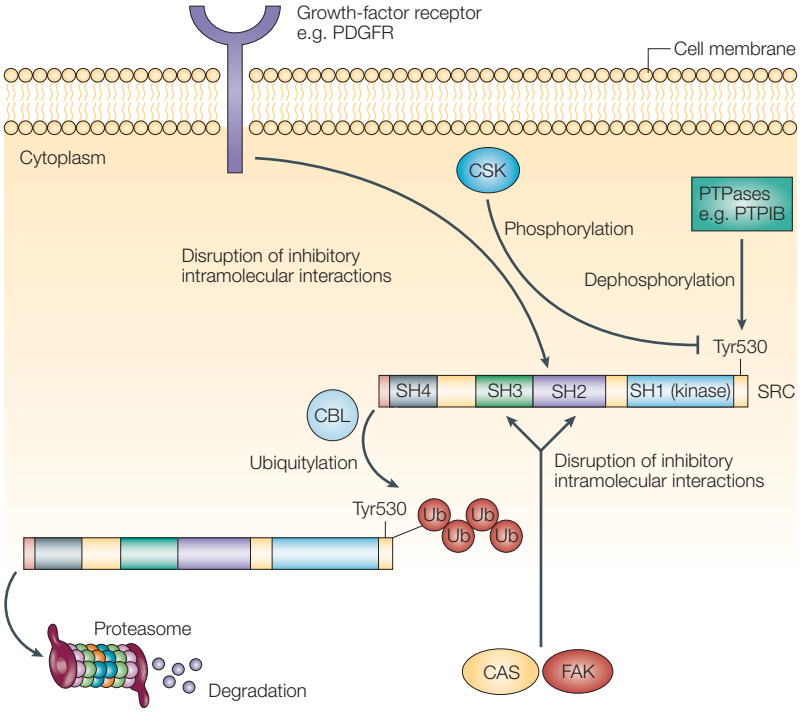

Figure 2 | **Regulation of c-SRC.** c-SRC is regulated in terms of both protein levels and levels of activity by a range of mechanisms. Inactivation of c-SRC is carried out by c-SRC tyrosine kinase (CSK), which phosphorylates a conserved tyrosine residue in the c-SRC carboxy-terminal domain (Tyr530). This is reversed by phosphatases such as protein tyrosine phosphatase 1B (PTP1B), which leads to c-SRC activation. Activation of growth-factor receptors leads to their association with the c-SRC SRC homology 2 (SH2) domain, which disrupts inhibitory intramolecular interactions to promote c-SRC activation. Other proteins, such as CRK-associated substrate (CAS) and FAK, bind to the c-SRC SH2 and SH3 domains to promote c-SRC activation by a similar mechanism. Levels of c-SRC protein are negatively regulated by the E3 ubiquitin ligase CBL, which leads to c-SRC ubiquitylation and subsequent degradation by the proteasome.

In addition, the direct binding of focal-adhesion kinase (FAK)[41] or its molecular partner CRK-associated substrate (CAS, also known as P130^{CAS}; see below) to the SH2 and SH3 (REF. 43) domains of c-SRC also results in the open, active configuration of c-SRC, as the intramolecular interactions that maintain the closed configuration are displaced.

Receptor-mediated activation. c-SRC can also be activated as a result of extracellular signalling. c-SRC is overexpressed and/or activated in a wide range of tumours that also overexpress several receptor tyrosine kinases, indicating the potential for cooperative or even synergistic interactions to promote tumorigenesis. In support of this, when EGFR and c-SRC are co-transfected into fibroblasts, their combined action results in increased proliferation, invasiveness and tumorigenesis[44]. Murine models also support the notion that overexpression of ERBB family members leads to c-SRC activation[45]. It is now clear that interactions with ligand-activated receptor tyrosine kinases, such as EGFR[44], PDGFR[46,47], ERBB2 (also known as HER2/NEU)[45], fibroblast growth factor receptor[48], colony-stimulating factor 1 (REF. 49) and hepatocyte growth factor[27] can result in augmented and even synergistic c-SRC activation, probably by disrupting the intramolecular interactions that hold c-SRC in a closed configuration.

Other mechanisms of c-SRC regulation. Other modes of c-SRC regulation include ubiquitylation, with subsequent degradation by the proteasome. The CBL ubiquitin ligase has been shown to be important in suppressing v-Src transformation through ubquitin-dependent protein degradation[50]. Recent evidence indicates that the ubiquitin–proteasome pathway is deregulated in cancer cells, which might allow c-SRC activation[51]. There is also evidence that c-SRC is activated through other, less clearly elucidated means, such as through nitric-oxide signalling[52].

One of the most intriguing potential means for c-SRC activation is through naturally occurring mutational events. There are two reports of rare, but potentially significant, point mutations that truncate c-SRC just C-terminal to the regulatory Tyr530, resulting in c-SRC activation in colon cancer[36] and endometrial cancer[53]. Other studies of different cancer populations, however, have failed to identify this mutation, although these studies used potentially less sensitive approaches[54–57].

Regulation of c-SRC localization. Like RAS, the localization of c-SRC within the cellular infrastructure is important. The association of c-SRC with the plasma membrane is considered essential for cellular transformation[58], and the autophosphorylation of Tyr419 that occurs with membrane targeting, which is enabled by interactions with activated receptor tyrosine kinases, is associated with the highest level of c-SRC transforming activity[59]. Inactive c-SRC is localized at perinuclear sites, but c-SRC activation causes its SH3 domain to become indirectly associated with actin. Activated c-SRC is ultimately translocated to the cell periphery to sites of cell adhesion, where it attaches to the plasma membrane inner surface through its myristoylated SH4 domain[60]. This tethered location allows for interactions with membrane-bound receptor tyrosine kinases and integrins associated with adhesion functions. The localization of c-SRC at the membrane–cytoskeletal interface in focal adhesions, LAMELLIPODIA and FILOPODIA (see below) seems to be regulated by the G PROTEINS RHOA, RAC1 and CDC42 (REF. 61).

Downstream effects of c-SRC activation. Experimentally constructed chicken v-*src* mutants were observed to have a wide range of activity, with different effects in different cellular backgrounds. This led to the proposal that there were probably several interacting substrates that would have variable effects when activated by c-SRC. The identification of a large number of c-SRC substrates was facilitated by the development of phosphotyrosine antibodies that can identify substrates directly phosphorylated by c-SRC. This supports the notion that c-SRC is a crucial molecule in a complex network of interacting proteins. c-SRC substrates include focal-adhesion proteins, adaptor proteins and transcription factors[33,62], which interact with c-SRC to either directly or indirectly

LAMELLIPODIA
Thin, sheet-like cell extensions of cytoplasm found at the leading edge of crawling cells. They form transient adhesions with the cell substrate, enabling the cell to move along a surface.

FILOPODIA
Small membrane projections, rich in actin, which emanate from the leading edge of the cell in the direction of movement.

G PROTEINS
GTP-binding intracellular-membrane-associated proteins that are activated by receptor stimulation.

Box 1 | **The SRC-induced transcriptome**

Recent advances in gene-expression profiling have permitted the global analysis of changes in gene expression induced by SRC activation, permitting a new window into the effects of SRC proteins on the cell. We have evaluated the effects of overexpressing wild-type c-SRC and various forms of activated SRC (c-SRC531, v-Src) on downstream gene expression[113]. Our data corroborated previous reports of upregulation of hypoxia-inducible factor 1, cathepsin L and cyclin D1, whereas MARCKS, fibronectin and DRS transcripts were downregulated. These experiments have indicated that a large number of genes, including those regulating the cell cycle, the cytoskeleton, cellular transcription and lysosomal proteins, are affected by SRC protein in the process of cellular transformation. Moreover, several of these genes are also co-regulated in human colon cancer tissues arrayed by stage, indicating that there might be an underlying SRC fingerprint in human colon cancer that can be modelled from SRC-overexpressing cell lines. See the Publication list of the Program for Genomic Application in the online links box for complete gene-expression profiles of cells overexpressing various forms of SRC.

alter the phenotype of the affected cell. Many of these molecules are now the focus of intense scrutiny to determine their relationship to the phenotype produced by c-SRC expression in normal and cancerous cells.

c-SRC overexpression and/or increased activity also cause secondary changes in the expression of numerous genes, the overall effect of which presumably contributes to the observed phenotypic changes linked to c-SRC overexpression or overactivation. Using gene-expression profiling techniques, it has recently become possible to assess the global effect of c-SRC expression on other genes. This global change in gene expression induced by c-SRC is referred to as the SRC fingerprint (BOX 1)[23].

DENSITY INHIBITION
The process by which non-transformed cells limit their proliferation when a certain density is reached due to physical contact with other cells or colonies.

The SRC phenotype

Our understanding of the function of SRC proteins has primarily come from infection or transfection of fibroblasts with the highly activated v-*src* oncogene. One need only look at v-Src-transformed fibroblasts under the microscope to gain an insight into the biological effects of activated SRC proteins in normal cells — v-Src transformation of normal fibroblasts is a visible event, leading to morphological changes in transfected cells. The cells round up, disaggregate and begin to float in the culture medium, as they lose the intercellular, integrin-based cytoskeletal attachments that normally ensure that they bind to the substratum in an ordered monolayer (FIG. 3). v-Src-transformed cells are also more motile and more able to invade the basement-membrane matrix. Over the course of several weeks, v-Src transformation can result in overgrown clumps of cells, known as foci, that form where cells lose their DENSITY INHIBITION — a hallmark of a cancer cell. These changes are consistent with the processes that are needed for a cancer cell to disaggregate from the primary tumour, invade the surrounding tissue and metastasize to distant organ sites. In addition to these effects, v-Src increases the proliferation rates of normal cells — doubling times are reduced and nutrient requirements are increased *in vitro*. *In vivo*, transfected cells grow rapidly to form visible tumours within days of injection, and these tumours are capable of local invasion and metastasis to distant sites.

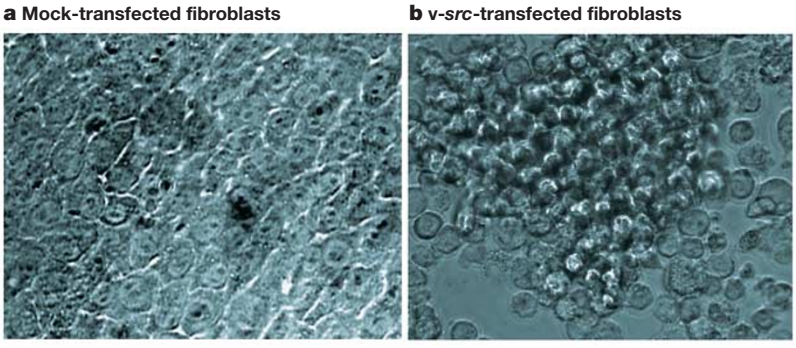

Figure 3 | **Transformation of fibroblasts by v-*src*.** v-*src* transfection produces a transformed phenotype, which is easily visualized in these photomicrographs. **a** | Shows a normal rat fibroblast culture stably transfected with a control vector. The cells have a fusiform morphology and show density inhibition. **b** | Shows the effects of v-*src* transfection on cellular morphology, with evidence of cells rounding up, detaching from the culture plate and forming visible foci, showing a loss of density inhibition. These cells demonstrate the characteristics of diminished intercelluar adhesion, decreased substrate adhesion and increased motility that are common to transformed cells with metastatic potential.

In addition to the well-established role of SRC in regulating cellular proliferation, there is accumulating evidence that c-SRC also acts to affect adhesion, invasion and motility events, particularly in epithelial cells and in cancer cells during the later stages of cancer progression[22]. Although antisense treatment[63] and c-SRC-specific inhibitors[64] seem to suppress tumour-cell proliferation, overexpression of c-SRC or activated forms of c-SRC do not seem to primarily affect cellular proliferation rates of human cancer cells. Interestingly, however, both c-*SRC*-transfected fibroblasts and cancer cells show decreased intercellular adhesion, higher levels of motility and an increased ability to invade substrates such as matrigel. Indeed, c-SRC is expressed at high levels in colonic polyps and adenomas early in the course of colon cancer development[65], but there are also large increases in c-SRC-specific activity in later stages of cancer progression (FIG. 4)[24,66]. SRC-family kinases seem to have a role in mitotic progression from G2 to M phase in the cell cycle, which might explain their effects on proliferation[19]. However, the development of high levels of specific c-SRC kinase activity, seen in the later stages of cancer[29,36,67], seems to contribute to metastatic potential through effects on adhesion, invasion and motility[20].

Molecular mechanisms of c-SRC function

Adhesion, invasion and motility, although representing independent cellular functions, are related events that require several well-orchestrated molecular interactions. For example, motility, by definition, requires alterations in cell–cell and cell–matrix adhesion, and invasion requires alterations in both adhesion and motility. The assembly and disassembly of intercellular junctions that mediate adhesion is associated with both significant cytoskeletal alterations and integrin signalling[68], which allow morphological changes and alterations in motility and invasiveness.

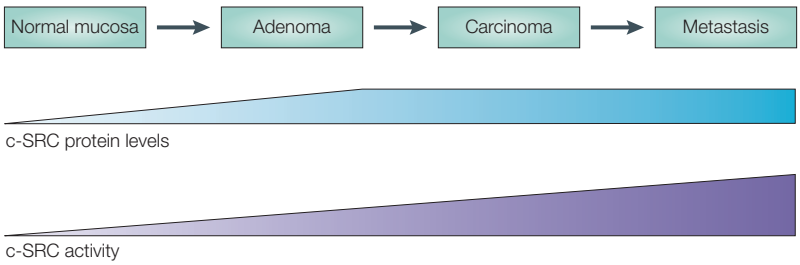

Figure 4 | **Levels of c-SRC expression and activity in colorectal cancer progression.** Sporadic colon cancer is frequently the result of the sequential accumulation of numerous genetic alterations, with neoplastic progression from normal mucosa, to formation of polyps (adenomas), to invasive cancer (carcinoma), which then metastasizes. Both c-SRC overexpression and c-SRC activation, with increased specific activity, are prominent features of the adenoma–carcinoma sequence. Both c-SRC overexpression and activation seem to be early features of adenoma formation, whereas continuing increases in c-SRC activity are seen with progression to cancer and later metastatic stages.

Adhesion. Two principal subcellular structures regulate adhesion, invasion and motility — focal adhesions and adherens junctions — both of which are regulated by c-SRC (FIG. 5,6)[69,70]. Focal adhesions form at the sites where integrins link the actin cytoskeleton to extracellular-matrix (ECM) proteins[71]. In addition to their role as cell–matrix attachment structures, providing the structural and mechanical properties that are necessary for adhesion, they also participate in cell-signalling processes that regulate proliferation and gene transcription[72]. They are composed of over 50 different proteins — such as talin, vinculin, α-actinin, c-SRC, FAK, CAS and paxillin — that assemble into supramolecular structures[73]. These cytoskeletal proteins are recruited to focal adhesions to mediate cellular migration. They are associated with cytoskeletal stress fibres, composed of actin and myosin, which control the shape and, ultimately, the motility of the cell.

Focal adhesions are dynamic structures that assemble to allow cells to adhere to the ECM and disperse to promote cell suspension. This process is regulated by crosstalk between the integrins and other cell-surface molecules, such as CADHERINS, SELECTINS, other cell-adhesion molecules, syndecans, G-protein-coupled receptors, receptor tyrosine kinases and the actin cytoskeleton, all of which converge to regulate RHOA. RHOA is a small GTP-binding protein that regulates actin cytoskeletal organization. Its activation is the cornerstone of focal-adhesion assembly and is absolutely required for this process[74].

Focal adhesions are disassembled when the cell needs to move along or away from the ECM. c-SRC contributes to this process — its expression leads to disruption of focal adhesions and actin stress fibres. This occurs during normal cellular migration and during mitosis, when cells round up and lose their matrix attachments. Focal adhesions are also disassembled during transformation, during which the integrity of these structures is disrupted, permitting increased motility[75].

In order for cells to adhere to each other, they must form stable adherens junctions mediated by homotypic interactions between E-cadherin molecules on neighbouring cells (FIG. 5)[72,76]. This process requires E-cadherin to first recruit β-catenin to its C terminus, which promotes its transport through the secretory pathway to the plasma membrane[77]. Next, α-catenin is recruited from the cytoplasm to bind to the complex, providing a link to the actin cytoskeleton[78]. α-catenin can bind to actin filaments either directly or indirectly, through intermediate molecules such as vinculin and VASP[79]. These interactions with the actin cytoskeleton stabilize adherens junctions. An additional protein that regulates adhesion is p120 catenin, a cytoplasmic protein that interacts with cadherins, including E-cadherin[80], and is thought to positively or negatively regulate adhesion, depending on the circumstances.

The mechanisms by which SRC proteins affect adhesion have recently been clarified. SRC-family kinases have a key role in focal-adhesion disassembly and turnover (FIG. 6). c-SRC seems to be necessary for integrins to block downstream signalling by RHOA through activation of p190 RHOGAP, leading to focal-adhesion disruption[81]. Conversely, expression of kinase-inactive v-Src results in the formation of enlarged focal adhesions[82]. Transfection of cancer cells with activated c-SRC leads to the formation of disorganized, essentially less effective focal-adhesion structures, or even to disassembly of these structures, with a reduction of cell clustering[83]. c-SRC is also thought to affect the integrity of focal adhesions through its effects on the RRAS protein. Recent results indicate that v-Src can induce tyrosine phosphorylation of RRAS, which is responsible for maintaining integrin activity in cells. RRAS and v-Src form a complex that suppresses integrin activity and reduces cell-matrix adhesion[84]. c-SRC also regulates the stability of focal adhesion through its interaction with FAK (see below).

c-SRC disrupts adherens junctions by suppressing E-cadherin localization and function at these crucial contact points. In addition, c-SRC and other tyrosine kinases induce the tyrosine phosphorylation and ubiquitylation of the E-cadherin complex, which induces endocytosis of E-cadherin[85] (FIG. 6). Therefore, activated c-SRC not only promotes the release of cells from the matrix, but also the release of cells from each other. Phosphorylation of FAK by c-SRC is also required for disruption of E-cadherin cell–cell contacts (see below).

Motility. Motility is a highly regulated, multi-step event that includes formation of cellular protrusions (lamellipodia, filopodia), their attachment to the ECM, traction and release of adhesions from other cells (adherens junctions) and from the basement-membrane matrix (focal adhesions). Cells are thought to move directionally by forming and extending protrusions, forming stable attachments near the leading edge of these protrusions, and propulsion forward.

CADHERINS
A group of functionally related glycoproteins that are involved in calcium-dependent cell-to-cell adhesion.

SELECTINS
A family of cell-adhesion molecules consisting of a lectin-like domain, an epidermal growth-factor-like domain, and a variable number of domains that are homologous to complement-binding proteins. Selectins mediate the binding of leukocytes to the vascular endothelium.

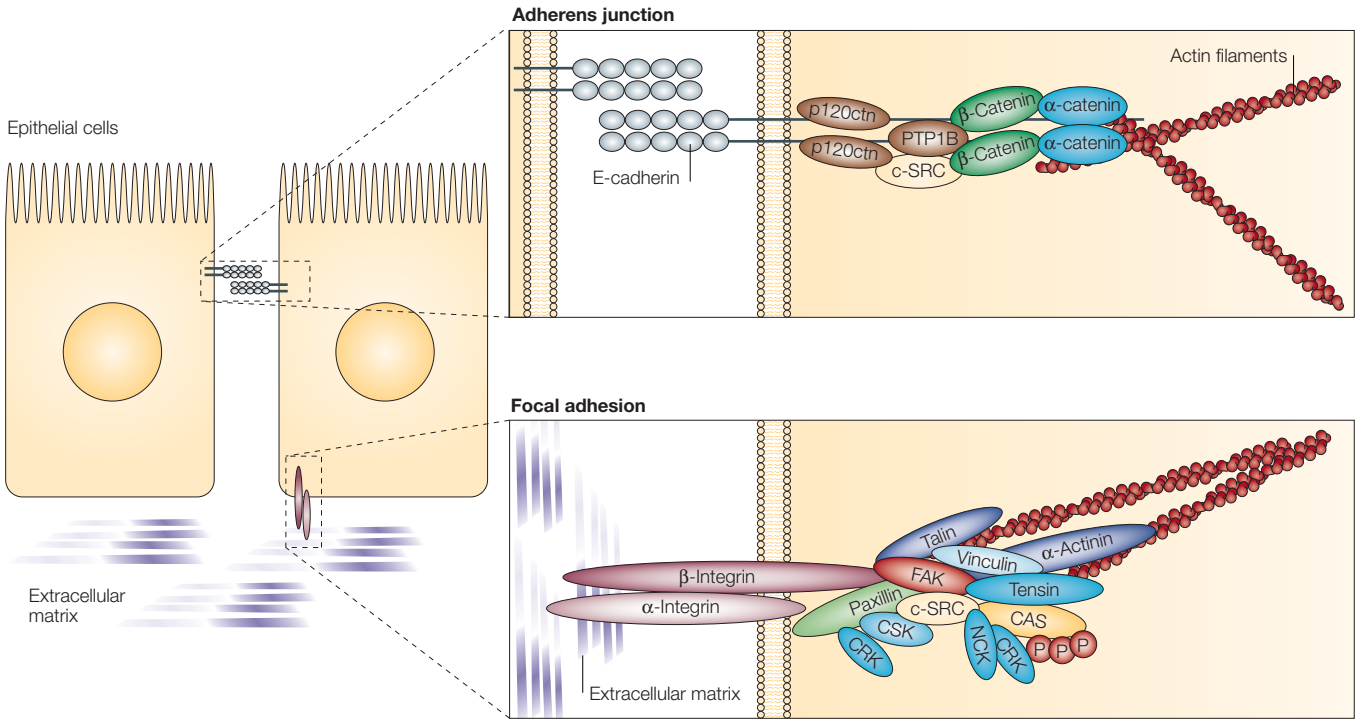

Figure 5 | **Cell adhesion at adherens junctions and focal adhesions.** Two main types of junction mediate adhesion in epithelial cells — adherens junctions and focal adhesions. Adherens junctions facilitate cell–cell adhesion through homotypic binding between E-cadherin molecules on adjacent cells. A cytoplasmic complex consisting of α-catenin, β-catenin and p120 catenin (p120ctn) links E-cadherin homodimers to the actin cytoskeleton. c-SRC associates with this complex and, when activated, is able to promote the disruption of the adherens junction. Protein tyrosine phosphatase 1B (PTP1B), which also localizes to adherens junctions, can dephosphorylate and activate c-SRC. At focal adhesions, heterodimers of α- and β-integrin subunits bind the extracellular matrix through their extracellular domains. Their cytoplasmic domains bind to a complex consisting of a range of proteins, including paxilin, talin, vinculin, tensin and α-actinin, which connect integrins to the actin cytoskeleton. Several signalling molecules also associate with this complex, including focal-adhesion kinase (FAK) and c-SRC, which can promote the turnover of the focal adhesion when activated, to promote cellular motility. c-SRC tyrosine kinase (CSK) is a kinase that associates with focal adhesions, and is responsible for phosphorylating and inactivating c-SRC to maintain focal-adhesion integrity when motility is not required. CRK and NCK are adaptor proteins and c-SRC substrates that contain SH2 and SH3 domains and have a key role in regulating tyrosine-kinase signalling, including that of c-SRC. They serve to recruit proline-rich effector molecules to tyrosine-phosphorylated kinases or their substrates, and have been implicated in the reorganization of the actin cytoskeleton that is required for cell movement. CRK-associated substrate (CAS) is a tyrosine-kinase substrate implicated in integrin-mediated control of cell behaviour. Phosphorylation of CAS by SRC-family kinases promotes cell motility because of its effects on the actin cytoskeleton. Figure modified with permission from REF. 71 © (2000) Academic Press.

METALLOPROTEINASES
A group of enzymes that can break down extracellular matrix proteins and require zinc or calcium atoms for catalytic activity. Matrix metalloproteinases are involved in wound healing, angiogenesis, and tumour-cell metastasis.

TIMPs
Tissue inhibitors of metalloproteinases are a family of secreted proteins that have a crucial role in regulating the activity of metalloproteinases. They influence the activation of the pro-metalloproteinase and act to modulate proteolysis of the extracellular matrix, notably during tissue remodelling and inflammatory processes.

This is followed by release of adhesions and retraction of the rear end[86]. The process whereby adhesions at the cell front disassemble as new adhesions form has been referred to as 'adhesion turnover'[87]. Integrin–matrix contacts stabilize adhesions by recruiting cytoskeletal and signalling proteins. Cytoskeletal actin polymerization then propels the cell forward. Small adhesions can transmit stong forces for rapid movement and ultimately mature into larger focal adhesions[88].

However, focal adhesions tend to inhibit cellular migration unless they are countered by other cellular defects such as E-cadherin loss. So, both disruption of adherens junctions and focal-adhesion turnover are required for cellular motility, both of which are regulated by c-SRC. Identifying the mechanisms for the formation and disassembly of these structures is now the focus of much investigation.

Invasion. Cellular invasion, like intercellular homotypic adhesion, is regulated by the expression of E-cadherin, as loss of E-cadherin facilitates invasion. Definitive studies in transgenic mice indicate that E-cadherin is an invasion suppressor[89] and overexpression of E-cadherin can, in fact, counteract the acquisition of the invasive phenotype[90]. As mentioned previously, c-SRC can affect E-cadherin levels and interactions, and this probably affects invasion, as well as homotypic adhesion. In addition, c-SRC might affect invasion by regulating matrix METALLOPROTEINASES (MMPs) and tissue inhibitors of metalloproteinases (TIMPs; FIG. 6)[91]. Evidence indicates that FAK signalling to c-JUN N-terminal kinase can promote the expression of MMP2 and MMP9 (REF. 92). These principles probably operate in both developmental and neoplastic processes.

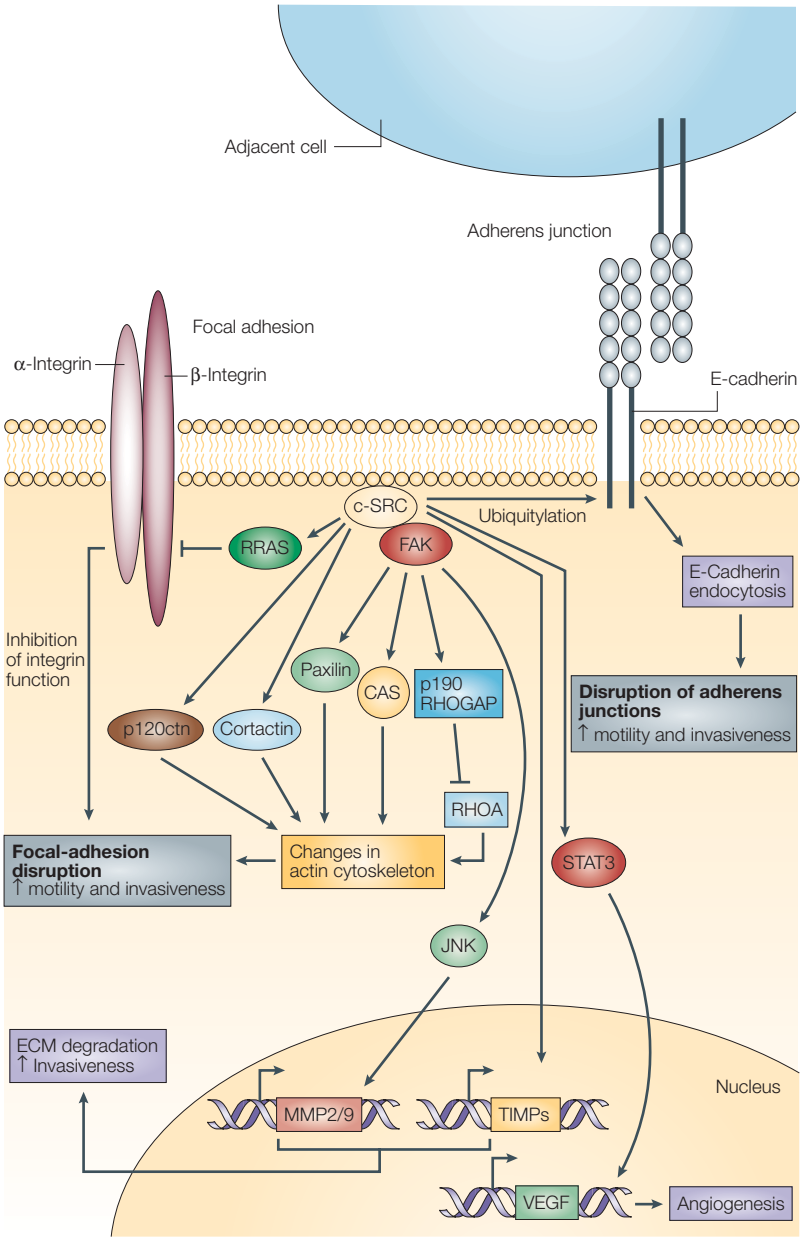

Figure 6 | **Effects of c-SRC on tumour-cell behaviour.** c-SRC exerts its effects on tumour-cell behaviour through a range of mechanisms mediated by interactions with various substrates and binding partners. A selection of these mechanisms is illustrated here. Motility and invasiveness depend on the loss of cell–cell adhesion mediated by E-cadherin. c-SRC promotes this by stimulating the ubiquitylation of E-cadherin, leading to its endocytosis. Turnover of focal adhesions is also required for motility and invasiveness, and c-SRC promotes this in various ways. Several of these mechanisms are mediated by the binding and activation of FAK, which phosphorylates substrates such as paxillin, CAS and p190 RHOGAP to bring about changes in the cytoskeleton that lead to focal-adhesion disruption. c-SRC also brings about similar changes independently of FAK through its interactions with cytoskeleton-associated proteins such as p120 catenin (p120ctn) and cortactin. Phosphorylation of RRAS by c-SRC inhibits integrin function, which also leads to focal-adhesion turnover. c-SRC activity also leads to changes in the expression of several genes that contribute to tumour progression. Activation of FAK stimulates the c-JUN amino-terminal kinase (JNK) signalling pathway, ultimately leading to increased expression of the matrix metalloproteinases MMP2 and MMP9; c-SRC also induces the expression of various tissue inhibitors of metalloproteinases (TIMPs). MMPs promote the breakdown of the extracellular matrix (ECM) that is required for tumour invasion of surrounding tissues. Signal transducer and activator of transcription 3 (STAT3) activation by c-SRC leads to increased expression of vascular endothelial growth factor (VEGF), a signalling molecule that promotes tumour angiogenesis.

Effects mediated by FAK. The relationship between c-SRC and FAK deserves special attention. FAK was one of the first identified c-SRC substrates and is thought to regulate growth-factor- and integrin-mediated cellular motility, adhesion and invasion, as well as cell proliferation and survival[93,94]. Numerous malignant neoplasms demonstrate concordant c-SRC and FAK overexpression and activation, which can lead to increased invasion and metastasis[95].

FAK is a non-receptor tyrosine kinase involved in the regulation of cell-cycle progression, cell survival and cell migration[93]. It localizes to the focal adhesions that form between cells growing in the presence of ECM constituents, such as fibronectin, and regulates both cell–cell and cell–matrix adhesion. It is phosphorylated after cellular contact with the ECM and can be activated by both growth factors and integrins, supporting the notion that integrins are signalling molecules as well as mediators of cell adhesion[68]. Integrin clustering, actin polymerization and actomyosin contractility facilitate FAK autophosphorylation and activation[92]. FAK associates with integrins through its COOH domain (FIG. 5) and forms transient signalling complexes with c-SRC. c-SRC induces tyrosine phosphorylation and activation of FAK, which results in the loss of focal adhesions[82] (FIG. 6).

Similar to c-SRC-transfected cells, FAK-transfected cells show increased motility and invasiveness[96]. The role of FAK in motility seems to be distinct from that in invasion[92]: it localizes to lamellipodia and INVADOPODIA — cytoplasmic protrusions that are involved in invasive behaviour — to promote invasion, and to focal contacts to promote integrin-stimulated cell motility. v-*src* transfected into *Fak*$^{-/-}$ mouse embryo fibroblasts readily causes transformation[97] and converts a rounded cell to a fusiform cell that is more mobile[92], indicating that Fak is not required for the increased motility that is induced in v-*src*-transfected cells. However, Fak is required for the ability of v-*src* to induce cellular invasiveness, as transfection with v-*src* does not rescue invasion properties of cells that do not express Fak.

Although it is not strictly required for increased motility, the recruitment of c-SRC to FAK at focal adhesions promotes turnover of these junctions, contributing to cellular motility. FAK also becomes phosphorylated and activated in response to integrin-mediated binding of cells to the ECM, indicating an important role in cell adhesion and/or migration[98]. *Fak*$^{-/-}$ cells transfected with dominant-negative c-*SRC* have larger than normal, less organized and presumably less effective focal adhesions, and impaired motility[98]. It has been suggested that the increase in the number and size of the focal adhesions in *Fak*$^{-/-}$ and *Src*-null mouse cells is because of an inhibition of adhesion turnover, the process responsible for cellular motility[98].

Recently, using quantitative assays to determine levels of kinase and adaptor molecules in focal adhesions, it has been demonstrated that the FAK–c-SRC complex, CAS, paxillin, extracellular signal-regulated kinase (ERK) and myosin light-chain kinase (MLCK)

are crucial for adhesion turnover at the cell front[99]. The FAK–c-SRC complex phosphorylates paxillin and CAS, which can then recruit other molecules to adhesions and so regulate actin cytoskeletal organization[100]. c-SRC, FAK and CAS form a trimeric complex that is stabilized by c-SRC SH2 binding to FAK and c-SRC SH3 binding to CAS. ERK is a target of FAK–c-SRC signalling and phosphorylates and activates MLCK, regulating adhesion disassembly.

Effects mediated through other targets. In addition to FAK, c-SRC also phosphorylates other targets — including p190 RHOGAP, p120 RASGAP and cortactin — that probably have an effect on motility (FIG. 6). As described above, c-SRC activates p190 RHOGAP, which in turn inhibits RHOA, and this normally promotes assembly of focal adhesions. In combination, these molecular interactions that are regulated by c-SRC lead to the loss of actin organization and to the disruption of cellular adhesions, resulting in cell detachment from the ECM[22].

c-SRC and tumour metastasis

As discussed previously, in many cancers increased c-SRC kinase activity is associated with advanced-stage tumours that readily metastasize and is thought to have an important role in the increased metastatic potential of these tumours. c-SRC is proposed to affect the processes of tumour growth and cancer-cell adhesion, motility and invasion, which directly influence metastatic potential. Different effects for c-SRC have been observed when expressed *in vitro* and *in vivo*. For example, c-SRC expression in cancer cells might have more significant effects on growth rates *in vivo*[101] than on tumour-cell proliferation rates *in vitro*[20]. Despite these effects on tumour growth rates, overexpression of wild-type c-SRC does not seem to be sufficient to enhance metastatic potential[101], whereas more active forms of SRC such as c-SRC531 — a truncated form that lacks C-terminal inhibitory amino acids — and v-Src[23,36] might be capable of permitting cells to achieve distant organ colonization[102]. Consistent with this, overexpression of Csk, a negative regulator of Src, suppresses metastasis[103] in mouse models. These experimental data are consistent with the model requiring high levels of Src-specific activity for the enhancement of metastatic potential. Moreover, SRC expression and activity seem to be significantly increased in metastatic lesions that are derived from more than one organ site[66].

SRC proteins promote angiogenesis

SRC proteins also seem to regulate molecules associated with angiogenesis. For example, v-Src has been shown to induce vascular endothelial growth factor (VEGF) expression through signal transducer and activator of transcription 3 (STAT3) activation[104,105] (FIG. 6). STAT3 activation by v-Src has also been shown to contribute to other biological properties of cancer cells, including cell growth and survival, and tumour-cell immune evasion[106]. c-SRC is required for hypoxia-induced VEGF production and *VEGF* expression is inhibited by antisense c-*SRC*. In recent studies, endothelial cells expressing kinase-inactive c-SRC failed to demonstrate the telltale signs of angiogenesis, including spreading and formation of cord-like structures[107]. In addition, substrates such as cortactin and paxillin show reduced phosphorylation in these cells, which is likely to affect the organization of the actin cytoskeleton, presumably inhibiting the motility of these cells that is required for angiogenesis. c-SRC inhibitors, such as PP2, SU11333 and CGP77675 inhibit invasive growth, sprouting of endothelial and vascular smooth muscle cells, and VEGF-induced vascular permeability[108].

c-SRC as a target of drug therapy

After many years of fruitful investigation, c-SRC has recently become a target for drug therapy[109]. Because it is commonly activated in a large number of human cancers and because its mechanisms of activation are now better understood, drug-development programmes have been successfully able to target c-SRC activation. Numerous c-SRC inhibitors are entering early Phase I trials or are in preclinical trials with efforts underway to develop c-SRC-inhibitory compounds that target the kinase domain, including compounds developed by Wyeth (SKI-606)[110] and Sugen (SU6656)[109], and Ariad Pharmaceuticals — AP23464 (REF. 111) and AP23451 (a bone-tissue-targeted c-SRC kinase inhibitor developed for osteoporosis therapy). The ubiquitous overexpression of c-SRC in cancer and its minimal deletion phenotype has indicated that c-SRC inhibition might not have significant toxicity, and its effect on human colon cancer cells seems to be growth-inhibitory. However, the fact that the non-catalytic domains of c-SRC might cause integrin assembly — with potential biological consequences — indicates that c-SRC inhibition, by targeting the kinase domain, might only be partially effective[83]. Nonetheless, the emerging strategy for targeting c-SRC holds significant promise in the therapy of solid tumours that show high levels of c-SRC activity. Interestingly, recent data indicate that targeting c-SRC and FAK simultaneously might be very effective in promoting apoptosis of colon cancer cells[112] and raises the potential for combined therapy strategies.

Conclusions

c-*SRC* is the oldest and best-studied proto-oncogene, and there is clear evidence for its participation in normal cells in proliferation, maintenance of normal intercellular contacts and cell motility. The role of c-SRC in human cancer development and progression, however, is still not fully understood. When activated through various mechanisms — from stimulation by growth factors to activating mutations — c-SRC might produce a defined neoplastic programme that ultimately results in a transformed phenotype, with increased cellular proliferation, invasion and motility, as well as decreased intercellular and cell–matrix adhesion. We are just beginning to unravel the many pathways that intersect with c-SRC,

INVADOPODIA
Invadopodia are specialized plasma-membrane structures associated with invading cells and extracellular-matrix degradation. These cellular protrusions are enriched in integrins and their associated tyrosine-kinase signalling molecules, metalloproteinases, and actin and actin-associated proteins.

its many substrates and the gene-expression products that are upregulated as a result of its activity. This should lead to a clearer understanding of how the numerous proteins that interact with c-SRC relate to each other in molecular pathways that lead to malignancy and tumour progression.

Because c-SRC activation is found in a large percentage of common solid tumour types, and because the mechanistic links of c-SRC to processes promoting tumour progression are now being realized, there has been a clear rationale for targeting c-SRC in cancer therapy through drug-discovery efforts. c-SRC, a molecule that has been investigated over the course of nearly a century, is now being rediscovered, with new potential to subvert its detrimental actions through the drug-targeted therapy of human cancer.

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Competing interests statement

The author declares that he has no competing financial interests.

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