The Role of Src Family Kinases in Prostate Cancer

Oleg Tatarov and Joanne Edwards

Section of Surgery, Division of Cancer Sciences and Molecular Pathology, University of Glasgow, Royal Infirmary, Glasgow, G31 2ER.

Introduction

In 1911 Peyton Rous described a transmissible agent that could induce sarcoma in chicken, this was later identified as a virus and named Rous Sarcoma Virus (Rous, 1911). Identification of the viral tyrosine kinase v-Src and its cellular counterpart c-Src (later in the text referred as Src), introduced the concept of proto-oncogene which has had a significant impact on the progress of our knowledge of carcinogenesis (Martin, 2001). Since its description, Src has been implicated in a variety of malignancies (Frame, 2002) including prostate cancer (Chang et al. 2007), which is the most commonly diagnosed cancer in men and the second leading cause of cancer-related death in men in the U.K. and U.S. (Jemal et al. 2007).

The Src-family kinases (SFK) comprises of nine members including Src, Fyn, Yes, Blk, Yrk, Fgr, Hck, Lck and Lyn; Src, Fyn and Yes being ubiquitously expressed in all cells while other kinases are tissue specific. Apart from Src, two other family members, Fgr (Edwards et al. 2003) and Lyn (Goldenberg-Furmanov et al. 2004) have been implicated in prostate cancer. All SFK members share similar structure; each protein consists of four Src homology (SH) domains and a unique amino-terminal domain.

High resolution crystallographic analysis of Src revealed the complex nature of structural changes involved in switching between active and inactive state. Src can be locked in an inactive conformation when its negative regulatory tail is phosphorylated at tyrosine Y530 by c-terminal Src kinase (Csk). However, when Src becomes autophosphorylated at tyrosine Y419, which is located in the kinase domain, the protein unfolds assuming its catalytically active conformation. Apart from being a tyrosine kinase, Src may function as a scaffolding molecule being an adaptor for other intracellular proteins that in turn can activate Src by the release of its intramolecular bonds. Another mechanism of Src activation, called peripheral targeting, involves translocation of inactive Src, which is located in the perinuclear region, to the cell periphery where Src becomes attached to the inner surface of cell membrane by its myristoylation fragment (Frame, 2002).

Src interacts with a wide variety of proteins including receptor tyrosine kinases, G-protein coupled receptors, steroid receptors, integrins, other non-receptor protein kinases etc., which is reflected in the multiplicity of resulting cellular biological events (Thomas and Brugge, 1997). Crosstalk between Src and the components of PI3K (phosphatidylinositol 3-kinase) and MAPK (mitogen activated protein kinase) pathways may affect tumor cell proliferation and apoptosis while involvement in focal adhesion complexes, especially FAK (focal adhesion kinase), paxillin and p130CAS (p130 Crk-associate substrate) plays an important part in promoting cell adhesion, migration and invasion (Summy and Gallick, 2006). Considering its unique position at the crossroads of the intracellular signaling networks, Src has become an attractive target in the search of novel prostate cancer therapies (McCarty, 2004).

Receptor Tyrosine Kinases

Epidermal growth factor receptor (EGFR)

Growth factor signaling plays a major role in prostatic oncogenesis (Russell et al. 1998). Receptor protein tyrosine kinases associated with growth factors, control various cell functions including proliferation, apoptosis, differentiation, cell cycle progression etc. EGFR (ErbB-1), Her2/neu (ErbB-2), Her3 (ErbB-3) and Her4 (ErbB-4), closely related EGFR family of transmembrane proteins contribute to the development

Correspondence: Oleg Tatarov, Section of Surgery, Division of Cancer Sciences and Molecular Pathology, University of Glasgow, Royal Infirmary, Glasgow, G31 2ER.

and progression of various malignancies including prostate cancer (Bartlett et al. 2005; Scher et al. 1995). Increased EGFR expression correlates with the tumor progression to hormone independent disease and poor clinical prognosis (Di Lorenzo et al. 2002) while the role of individual family members remains controversial (Edwards et al. 2006).

Although the exact mechanism of mitogenic signal transmission from the EGFR is still unknown, cooperation with Src followed by mutual activation appears to be an important event contributing to the aggressive tumor behavior (Maa et al. 1995). Following ligand binding, the receptor precipitates into homo- or heterodimeric complexes with other family members resulting in autophosphorylation at several tyrosine residues (Bromann et al. 2004). These phosphotyrosines regulate downstream signal transmission, stimulate DNA synthesis and cell division as well as serve as the docking sites for various proteins including Src. Physical association between EGFR and SH2 domain of Src induces conformational changes in Src where the catalytic domain becomes available for the interaction with its downstream targets, among them EGFR itself (Biscardi et al. 2000).

Tyrosine Y845 has been identified as the Src specific phosphorylation site, not susceptible to autophosphorylation, within the activation loop of EGFR catalytic domain (Biscardi et al. 1999). Although it does not affect activation of MAP kinase initiated by EGF, Y845 is crucial for the receptor function as its substitution with phenylalanine reduces DNA synthesis induced by EGF. Tyrosine Y845 serves as a relay point integrating EGFR into the complex network of transmembrane and intracellular proteins (Ishizawar and Parsons, 2004). Indeed, transactivation of EGFR can be triggered by multiple extra- and intracellular stimuli including G protein-coupled receptors, cytokines, calcium and zinc ions, integrins, UV light and ionizing radiation (Gross et al. 1999; Knebel et al. 1996; Prenzel et al. 2000; Wu et al. 2002).

Two potential downstream effectors transmitting signals from EGFR phosphorylated at Y845 have been identified: cytochrome *c* oxidase subunit II (Cox II), a mitochondrial enzyme involved in respiratory chain (Boerner et al. 2004) and signal transducer and activator of transcription 5b (STAT5b) implicated in the development of prostate cancer (Ahonen et al. 2003; Amorino et al. 2007; Kloth et al. 2003; Li et al. 2004). EGFR is known to translocate to mitochondria where it associates with Cox II to regulate apoptosis and Src is an integral part of this complex as it co-localizes within mitochondria and phosphorylates a tyrosine residue on Cox II (Miyazaki et al. 2006). Cox Vb, another subunit of cytochrome *c* oxidase is thought to interact with an androgen receptor mutant located in mitochondria although the status of this association in prostate cancer is unknown (Beauchemin et al. 2001).

Insulin-like growth factor receptor (IGFR)

The role of IGF family in prostate carcinogenesis has been extensively studied, while the relationship between IGFR and Src is less well understood (Gennigens et al. 2006). There are two types of IGFR receptors, IGF-1R and IGF-2R binding two ligands, IGF-1 and IGF-2. IGF-1R, a tetramer with higher affinity for IGF-1, is made up of two α subunits that bind the growth factor and two β subunits possessing tyrosine kinase activity. IGF-2R is a monomer without intrinsic kinase activity with higher affinity for IGF-2 that serves mainly as a degradation mechanism for IGF-2. Insulin-like growth factors are located in the extracellular fluid in complexes with various IGF binding proteins (IGFBPs), regulating IGF's physiological activities. IGF-1R is known to promote cell proliferation by activation of MAP kinase cascade and inhibit apoptosis through PI3K-AKT pathway (Gennigens et al. 2006) and studies *in vitro* using various cell lines provide evidence that this process may require Src (Bromann et al. 2004).

IGF-1R, associated with IGF-1 or IGF-2 activates insulin receptor substrate protein (IRS) displaying multiple binding sites for SH2 homology domain containing proteins including Src (Thomas and Brugge, 1997). Using 3T3-L1 murine preadipocytes Boney et al. showed that SFK (Src and Fyn) mediate IGF induced phosphorylation of adaptor protein Shc and MAP kinase stimulation (Boney et al. 2001). Furthermore, introduction of dominant-negative Src abolished phosphorylation of MAP kinase and Shc but not IRS suggesting that SFK are upstream proteins to MAPK.

Activation of PI3K-AKT pathway is necessary for prostate cancer progression (Majumder and Sellers, 2005) and IGF-1 stimulated activation of AKT by Src may play a role (Cui et al. 2005). Phosphatase and tensin homolog deleted on chromosome ten (PTEN) down regulates PI3K/AKT

pathway inhibiting activation of AKT by IGF-1R signaling. Introduction of a vector carrying PTEN into PC3 prostate cancer cells that normally lack PTEN leads to suppression of cell proliferation and induction of apoptosis by inhibiting translation of IGF-1R precursor (Zhao et al. 2004). Activated Src has been shown to interfere with the binding of PTEN C2 domain to cellular where it can be activated by PI3K (Lu et al. 2003).

Cells transformed by v-Src display constitutive phosphorylation of the β subunit of IGF-1R (Kozma and Weber, 1990). As with EGFR, Src can activate IGF-1R by phosphorylating several tyrosine residues enhancing its catalytic activity (Peterson et al. 1994). Overexpression of constitutively active Src enhances IGF-1-dependent cell proliferation by increasing receptor number and downstream signaling (Flossmann-Kast et al. 1998). Unlike Src-induced phosphorylation of EGFR at the unique site, Src catalyzes phosphorylation of IGF-1R at the same sites that become autophosphorylated following growth factor binding essentially substituting receptor kinase (Peterson et al. 1996).

One of the most remarkable functions of Src is the ability to facilitate cross-talk between various transmembrane and intracellular proteins. Knowlden et al. found that stimulation of IGF-1R by IGF-2 is accompanied by phosphorylation of EGFR at tyrosine Y845 in Src-dependent manner (Knowlden et al. 2005).

Platelet-derived growth factor receptor (PDGFR)

PDGFR signaling is involved in the development and progression of many human malignancies (Yu et al. 2003). Platelet-derived growth factors, members of PDGF/VEGF super family, consist of four distinct proteins: A, B, C and D. A and B subunits form homo- and heterodimers whereas recently discovered C and D form only homodimers. There are two types of PDGF receptors: PDGFRα and PDGFRβ that may form both homo- and heterodimers.

The importance of PDGFR in the development and progression of prostate cancer is well established (Fudge et al. 1994; Fudge et al. 1996; Sitaras et al. 1988), especially its role in metastatic spread and neoplastic angiogenesis (Uehara et al. 2003). Targeted therapies are being investigated as PDGFR inhibitor imatinib is undergoing clinical trials in patients with androgen-resistant prostate cancer (Hofer and Rubin, 2005; Kim et al. 2006; Lin et al. 2006).

PDGFR was the first receptor tyrosine kinase linked to Src (Ralston and Bishop, 1985) and the mechanism of their interaction has been the subject of numerous publications (Bromann et al. 2004). Although the question whether Src is necessary for PDGF-induced DNA synthesis remains controversial, SFK play an important role in PDGFR signalling (Choudhury et al. 2006). Src is recruited to the activated dimerized receptor through an interaction between SH2 homology domain and several autophosphorylation sites located in the juxtamembrane region of PDGFR including tyrosine Y579 (Mori et al. 1993). As a result, the links connecting C-terminal negative regulatory tail with SH2 domain weaken prompting the molecule to assume catalytically active conformation (Alonso et al. 1995).

Oncogenic signals are transmitted through several downstream pathways converging on STAT3 and c-Myc (Bowman et al. 2001). Apart from proliferation and survival, Src is involved in regulation of other cell functions controlled by PDGFR e.g. cell cycle progression and cytoskeletal reorganization (Frame et al. 2002; Roche et al. 1995). Physical association between Src and PDGFR makes it possible for Src to regulate the receptor function by phosphorylating Y934 on PDGFRβ (Hansen et al. 1996). Substitution of Y934 with phenylalanine inhibits PDGF BB-induced DNA synthesis but increases chemotaxis and actin reorganisation implying Src may negatively regulate these cellular responses.

Vascular endothelial growth factor receptor (VEGFR)

Growth and metastatic spread of prostate cancer and other malignancies depend on the progressive increase in blood supply. Angiogenesis is a complex process controlled by various growth factors that include VEGF family proteins (five main isoforms A, B, C, D and E). There are three types of receptors binding VEGFs: VEGFR-1, VEGFR-2 and VEGFR-3; VEGFR-2 being the major tyrosine kinase mediating tumorogenic effects of VEGFs (Delongchamps et al. 2006; Ferrara et al. 2003).

Overexpression of VEGF and its receptors is associated with the development of prostate cancer (Pallares et al. 2006). Targeting VEGF with neutralizing antibodies may reduce primary tumor progression in patients with prostate cancer and prevent formation of metastases (Melnyk et al. 1999). Combined inhibition of VEGFR and other receptor

tyrosine kinases (e.g. EGFR) strategies are being developed and could potentially be used for the treatment of androgen independent prostate cancer resistant to chemotherapy (Busby et al. 2006).

The relationship between Src and VEGF receptors is not fully understood (Rahimi, 2006). Physical association of Src and VEGFR-2 requires the presence of tyrosine Y1212 at the carboxyl terminal of the receptor, although Src activity was not found to be necessary for VEGFR-2 autophosphorylation in response to ligand stimulation (Meyer et al. 2002). Inhibition of Src by adenoviral vector expressing the melanoma differentiation-associated gene-7 (Ad-mda7) has been shown to reduce STAT3 binding to VEGF promoter region, thus suppressing VEGF expression in prostate cancer cell lines (Inoue et al. 2005).

Breakdown of endothelial barrier initiated by VEGF and mediated by Src produces tumor cell intravasation and distant spread by downregulation of Vascular-Endothelial-cadherin (VE-cadherin)-β-catenin system in cell-cell junctions (Weis et al. 2004). Inhibition of Src activity by M475271, the novel Src kinase inhibitor, significantly diminished VE-cadherin-β-catenin phosphorylation as well as improved their association and, as a result, stabilized cells adherens junctions (Ali et al. 2006). Recently, it has been shown that inhibition of VEGF expression by Src-supressed C-kinase substrate (SSeCKS) in MatLyLu (MLL) prostate cancer cells prevented formation of lung metastases in an experimental model, although the inhibitory effect on primary site in this study was minimal, possibly suggesting grater role of VEGF-Src interaction in the establishment of secondary tumor deposits (Su et al. 2006).

Guanosine Phosphate Binding Protein Coupled Receptors (GPCR)

Extracellular signals transmitted through GPCRs regulate many oncogenic processes where Src plays an important part. Conventionally, heptahelical receptor is made up of several membrane spanning protein loops and a heterotrimeric intracellular G protein, consisting of three subunits: α, β and γ. GPCRs bind numerous ligands implicated in the development and progression of prostate cancer, including acetylcholine, bombesin, bradykinin, lysophosphatidic acid, neurotensin etc. (Daaka, 2004). Ligand-activated receptor triggers the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the Gα subunit and its dissociation from the Gβγ subunit. Gα-GTP and Gβγ then participate in regulation of various downstream signaling processes.

Several mechanisms by which GPCRs interact with Src have been described (Luttrell and Luttrell, 2004). Src kinase could be activated by direct association with GPCRs through SH2 or SH3 homology domains, by interactions with heterotrimeric G proteins and indirectly by involvement in GPCR crosstalk with receptor tyrosine kinases and focal adhesion complexes.

Progression of prostatic malignancies to hormone refractory state has been associated with neuroendocrine differentiation of prostate cancer cells that secret various mitogenic factors including bombesin or its human analogue Gastrin-Releasing Peptide (GRP) (Vashchenko and Abrahamsson, 2005). Bombesin, GPCR agonist, was found to induce ERK ½ (MAPK) phosphorylation and DNA synthesis in DU145 and PC3 prostate cancer cell lines that are androgen independent but not in LNCaP cells that are androgen sensitive (Xiao et al. 2003). Furthermore, this process involved GPCR-mediated EGFR transactivation in Src-dependent manner. Treatment of PC3 cells with conditioned culture medium from LNCaP-derived neuroendocrine cells or GPCR agonist neurotensin revealed Src activation and increase in EGFR phosphorylation at Src specific site Y845 (Amorino et al. 2007).

Prostate cancer cells may acquire neuroendocrine phenotype following androgen withdrawal promoting androgen receptor (AR) transactivation by neuropeptides in the transition of prostate cancer from hormone sensitive to hormone refractory state (Burchardt et al. 1999). Src and its substrate FAK participate in bombesin-induced activation of AR, which is inhibited by expressing dominant-negative Src or FAK (Lee et al. 2001). AR activation and its translocation to the nucleus in response to bombesin could be facilitated by Src directly (Desai et al. 2006) or through Src activation of p300, an androgen receptor coactivator with histone acetyltransferase (HAT) activity (Gong et al. 2006).

Nongenotropic Androgen Receptor (AR) Signalling

In prostate cancer AR signaling typically involves binding of androgens to the receptor located in the cytoplasm, dimerisation of the receptor, its translocation to the nucleus, activation of the

transcriptional apparatus resulting in multiple biological events. Prostate cancer cells develop the ability to survive and thrive following androgen withdrawal, which could be explained by several mechanisms (Feldman and Feldman, 2001). Recently, there has been much interest in investigating the role of AR in various transduction processes beyond its conventional role as a ligand-dependent transcription factor (Lange et al. 2007).

As with growth factor receptors, interaction between AR and non-receptor tyrosine kinases including Src, may involve physical association and formation of complexes. SH3 domain of Src is thought to have affinity for AR proline-rich sequences resulting in ability to form complexes where Src can be activated by the release of its intramolecular constrains. Binding of Src to AR in response to stimulation with androgens can result in activation of MAPK pathway and, consequently, induction of S-phase entry in a prostate cancer cell model (Migliaccio et al. 2000). Expression of dominant negative Src as well as treatment with Src inhibitor and androgen antagonist abrogated complex assembly and prevented MAPK activation.

Apart from androgens, interaction involving AR and Src can be initiated by various growth factors and cytokines. Phosphorylation of EGFR following LNCaP cells stimulation with EGF, for example, may be induced by Src which becomes activated after binding to AR and Estrogen Receptor β (ERβ) (Migliaccio et al. 2005). Furthermore, DNA synthesis, cytoskeletal changes and Src activation stimulated by EGF, are inhibited by application of androgen antagonists implying an important role of Src-AR interactions in various oncogenic processes. Activation of Src and its downstream target FAK by IL-8 is necessary for AR transactivation in androgen depleted medium promoting hormone independent growth and migration in prostate cancer cell lines (Lee et al. 2004).

Prostate cancer behavior and underlying molecular basis change in the transition from hormone sensitive to hormone independent disease. Using androgen responsive $LNCaP_{nan}$ cells and androgen independent LNCaP-HP (High Passage) cells Unni et al. observed constitutive activation of Src/MAPK pathway in LNCaP-HP cells whereas $LNCaP_{nan}$ cells required AR stimulation with androgens in order to elicit Src/MAPK activation (Unni et al. 2004).

AR phosphorylation as a mechanism modulating AR signaling has been the subject of several studies (Culig et al. 1995; Gioeli et al. 2002; Heinlein and Chang, 2004). Tyrosine phosphorylation has been shown to regulate AR transcriptional activity, facilitate AR nuclear translocation and stimulate growth of hormone-refractory prostate cancer. Identification of AR phosphorylation sites specific to various protein kinases by the conventional methods has proved difficult due to the rapid and transient nature of this process. Tyrosine Y534 has been proposed as Src-specific AR phosphorylation site, its substitution with phenylalanine significantly reduced AR transcriptional activity. Interestingly, Y534 mutation had dramatic effect on prostate cancer cell growth especially in the low androgen environment suggesting its importance in the transition from hormone sensitive to hormone refractory disease (Guo et al. 2006).

Adhesion, Migration and Invasion

Transformation of normal epithelium into cancer is frequently associated with acquisition of highly motile invasive phenotype, which is a feature of epithelial to mesenchymal transition (EMT). The main characteristics of EMT are the disruption of cell-cell adherens junctions and altered cell-matrix focal adhesions assembly leading to the loosening of cell-cell contacts, thus increasing migratory capacity of cancer cells. Src appears to control both processes by destabilizing dynamic regulation of adherens junctions as well as participating in focal adhesions complexes (Frame, 2002).

Adherens junctions are made up of cadherins (E-cadherin and N-cadherin), transmembrane proteins connecting the cells by their extracellular portions, and catenins (α-catenin, β-catenin, γ-catenin and p120catenin) linking cadherins with actin cytoskeleton inside the cell. In prostate cancer, expression of E-cadherin α-catenin, β-catenin and p120catenin have been found lower in higher grade tumors compare to lower grade specimens and benign tissue, whereas expression of N-cadherin, which is thought to produce more dynamic adherens junctions, is increased in more aggressive prostatic tumors (Jaggi et al. 2005; Jaggi et al. 2006). Although the role of Src in deregulation of E-cadherin *in vitro* has been well documented (Avizienyte et al. 2004), the status of their relationship in prostate cancer is currently unknown.

Complex structures involved in cell-extracellular matrix (ECM) contacts, termed focal adhesions, consist of more than 50 various proteins. Facilitating

cell movement requires a well orchestrated mechanism of focal adhesions assembly at the leading edge of the moving cell accompanied by disassembly at the back and reorganization of actin cytoskeleton (Brunton et al. 2004). Integrins, transmembrane proteins, form clusters at focal adhesions; they provide physical links between ECM and the cytoskeleton and serve as receptors transmitting extracellular stimuli. Following integrin engagement, FAK is recruited to the focal adhesions and rapidly becomes autophosphorylated on tyrosine Y397, which is a high affinity Src docking site. Src induces tyrosine phosphorylation of large number of proteins including FAK (Y566, Y577, Y861, Y925), Paxillin (Y118), p130CAS (Y410), Shc and various other substrates (Carragher and Frame, 2004).

In prostate cancer cell lines expression of FAK was found to be higher in invasive highly tumorigenic PC3 and DU145 compare to non-invasive LNCaP cells (Slack et al. 2001). Inhibition of FAK by overexpression of FRNK (Focal adhesion kinase—Related Non-Kinase) and Src by PP2 significantly reduced migration of prostate cancer cells underlying the importance of FAK/Src signaling in cell motility. As mentioned, LNCaP cells have the potential to acquire hormone independent features following IL-8 stimulation due to transactivation of AR by FAK and Src. Activation of both FAK and Src was necessary for IL-8 induced cell migration whereas Src activity was also required for androgen independent growth (Lee et al. 2004).

Recently, there has been much interest in studying the role of Proline-rich tyrosine kinase 2 (PYK2) in prostate cancer. PYK2 is a member of FAK family kinases, protein that is structurally and functionally related to FAK. Although the expression of PYK2 is decreased in high grade prostate cancer specimens compare to low grade (Stanzione et al. 2001), it is thought to play an important part in regulation of prostate cancer cells motility. Leupaxin, a member of the paxillin family of adaptor proteins, has been found to associate with PYK2 in complexes containing Src and protein tyrosine phosphatase-proline-, glutamate-, serine-, and threonine-rich sequence (PTP-PEST) (Sahu et al. 2007). Inhibition of leupaxin in PC3 cells using siRNA approach led to reduced cell migration, whereas overexpression of leupaxin induced complex formation with PYK2 and Src and, as a result, increased prostate cancer cell migration.

Metastasis suppressor KAI1/CD82 has recently been linked with regulation of adhesive and invasive properties of prostate cancer cells utilising Src-dependent pathways. Loss of KAI1/CD82 expression has been shown to correlate with progression of prostate cancer and other malignancies to metastatic disease (Dong et al. 1996; Friess et al. 1998; Guo et al. 1998; Huang et al. 1998; Liu et al. 2000; Liu et al. 2003). Stable transfection of KAI1/CD82 into invasive DU145 cells induced homotypic aggregation of the cells which was reversed by the treatment with anti-KAI1/CD82 antibody (Jee et al. 2003). Interestingly, transfection of KAI1/CD82-positive DU145 cells with Src lacking catalytic domain produced similar effect as antibody suppression of KAI1/CD82, suggesting direct involvement of Src in KAI1/CD82 signaling.

KAI1/CD82 has been implicated in the crosstalk between integrins and growth factor receptors as an important factor regulating cell migration and invasion (Sridhar and Miranti, 2006). Re-expression of KAI1/CD82 in PC3 cells resulted in dramatic reduction in their ability to invade matrigel. Furthermore, activation of hepatocyte growth factor receptor c-Met in response to integrin- or ligand-induced stimulation was attenuated in KAI1/CD82-positive PC3 cells. Inhibition of invasive properties was linked with down regulation of Src signaling; activation of Src as well as its substrates FAK and p130CAS was significantly reduced upon PC3 cells transfection with KAI1/CD82 cDNA.

Modulation of Src Activity in Prostate Cancer and Therapeutic Implication

Recent advances in research investigating involvement of Src in multiple signaling networks have led to the increased interest in the development of Src inhibitors. As Src can be activated through several mechanisms, various strategies targeting specific activation processes have been considered.

Normal and cancer cells maintain Src activity in balance using a variety of endogenous activators and inhibitors. According to their mechanism of action, Src inhibitors could be divided into two main categories: catalytic and non-catalytic (Chong et al. 2005). Catalytic inhibitors mainly suppress Src tyrosine kinase activity whereas non-catalytic interfere with protein-protein interactions and affect intramolecular displacement. Csk represents a typical example of endogenous catalytic Src

inhibitor, although its role in prostate cancer has not been completely understood. Recently described protein DOC-2/DAB2 (differentially expressed in ovarian cancer-2/disabled 2), an endogenous non-catalytic Src inhibitor, acts by displacing AR from AR/Src complex *in vitro* thus suppressing AR induced activation of Src and its downstream effectors MAPK and AKT (Zhoul et al. 2005).

Despite almost one hundred years history of research and the amount of evidence implicating Src kinase in cancer, the development of orally bio-available stable *in vivo* clinically effective synthetic Src inhibitors has only been achieved within the last several years. As with endogenous Src inhibitors, similar principles apply to the development of synthetic compounds: catalytic inhibitors target the kinase domain by interacting with its ATP binding pocket whereas non-catalytic inhibitors block Src association with the substrates or modify intramolecular bonds. Dasatinib (BMS-354825, Bristol-Myers Squibb), SKI-606 (Wyeth) and AZD0530 (AstraZeneca) are the leading synthetic catalytic small molecule inhibitors of Src and structurally similar kinase Abl that have progressed beyond the laboratory experiments and are currently undergoing clinical testing (Boschelli et al. 2001; Hennequin et al. 2006; Lombardo et al. 2004). Dasatinib (BMS-354825) has emerged as a frontrunner; it has recently been given the U.S. Food and Drug Administration Agency approval for the use in adults with chronic myeloid leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph + ALL) with resistance to prior therapy.

Evaluation of Src inhibitors in prostate cancer has been the subject of several publications. Recchia et al. reported the effect of CGP77675 and CGP76030, pyrrolopyrimidine class Src inhibitors in prostate cancer *in vitro*. Using PC3 prostate cancer cell line the authors found significant reduction in proliferation, adhesion, migration and invasion following treatment with the inhibitory compounds (Recchia et al. 2003). However, IC_{50} for the proliferation assay as assessed by thymidine incorporation test was above 2 μM, indicating that inhibition of other tyrosine kinases might also be responsible.

Controversy surrounding the role of Src in proliferation of solid tumors is partly due to the lack of specificity many small molecule inhibitors often display. PP1 and PP2 for example, pyrazolo-pyrimidine based competitive inhibitors of ATP binding are extensively used in tissue culture including prostate cancer cell lines, to study the cellular features of Src inhibition (Hanke et al. 1996). Pyrazolo-pyrimidine compounds have been applied in proliferation and migration studies in order to elicit the effect of Src inhibition in prostate cancer cells stimulated with various growth factors (Lee et al. 2004; Slack et al. 2001; Zhoul et al. 2005). These chemicals are proven, however, to inhibit other tyrosine kinases including PDGFβ, stem cells factor (SCF) receptor c-Kit, tyrosine kinase Ret etc. with an *in vitro* IC_{50} below 10 μM, concentration used in these experiments (Bondzi et al. 2000; Tatton et al. 2003; Waltenberger et al. 1999). Recently developed SI35 and SI40 pyrazolo(3, 4-d)pyrimidines were effective in reducing EGF stimulated PC3 cells proliferation at concentrations 5 μM and higher, whereas much lower doses of the inhibitors were used to elicit the reduction in cell adhesion and migration (Angelucci et al. 2006).

BMS-354825 (Dastinib), a potent biochemical Src kinase inhibitor (K_i = 96 pM) (Lombardo et al. 2004) has been applied in experiments *in vitro* and *in vivo* using various cancer models, including lung cancer (Song et al. 2006), pancreatic adenocarcinoma cells (Trevino et al. 2006), head and neck tumors (Johnson et al. 2005) and human sarcoma cells (Shor et al. 2007). In human prostate cancer cell line DU-145, dasatinib suppressed Src/FAK/p130CAS signaling, inhibited MMP-9 activity and, consequently, reduced cell adhesion, migration and invasion (Nam et al. 2005).

Physical associations between Src and its substrates provide an attractive targeting mechanism in search of inhibitory substances. Detailed structural analysis of substrate binding sites may allow synthesis of short peptides preventing substrate docking. Sequence based peptide inhibitor KRX-123 has demonstrated effectiveness against Lyn, a member of SFK, in hormone-refractory prostate cancer cell lines and explants in nude mice (Goldenberg-Furmanov et al. 2004). Similar approach has been used to construct a short peptide mimicking AR proline-rich sequences that interact with SH3 domain of Src (Migliaccio et al. 2007). This 10 amino-acid long synthetic substance prevented AR/Src complex assembly, which resulted in diminished androgen or EGF induced DNA synthesis in AR positive LNCaP cells and inhibited LNCaP xenografts growth in nude mice.

Experiments with Src knockout mice revealed two important aspects of Src inhibition: first, the mice were reasonably healthy promising low

toxicity for specific Src inhibitors; second, the animals developed osteopetrosis due to deficient osteoclast function, the unexpected finding that may lead to the discovery of new class of drugs for the treatment of osreoporosis (Soriano et al. 1991; Susva et al. 2000). Furthermore, when investigating the action of a purine based Src inhibitor AP23451 on osteolytic bone metastases in mice, the size of tumor infiltrating the bone marrow cavity was significantly reduced (Boyce et al. 2003). This was not observed in cases treated with zoledronic acid alone to prevent osteolysis caused by metastases, the effect that has important implications for prostate cancer where secondary deposits in bones represent a serious problem (Pinski and Dorff, 2005). Thus, Src inhibitors are thought to have the ability to break the vicious cycle of metastasis-induced bone re-absorption followed by the release of growth factors that in turn promote tumor cell growth (Boyce et al. 2006).

Conclusion

The discovery of proto-oncogene Src that has given us a fascinated insight into the normal and tumor cell biology, is finally bearing fruits as the research is gradually moving from the laboratory bench to the patient's bedside (Summy and Gallick, 2006). In prostate cancer, however, there are relatively few publications compare to other malignancies and, as a result, many questions remain unanswered. For example: what is the relationship between Src expression or activation and prostate cancer progression in clinical settings; how the behavior of Src kinase changes in the transition from hormone-sensitive to hormone-independent disease etc. To date, there are no publications suggesting the correlation between Src expression or activation and clinical parameters studying human prostate tumor specimens. At the moment, the best timing to administer the inhibitory compounds is unknown as there are no definitive end points. Further research, therefore, is necessary so that Src in the treatment of advanced tumors truly stays at the center stage.

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