Nuclear Src

Subjects: Pathology

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Src is the representative member of the Src-family kinases (SFK), a group of tyrosine kinases involved in several cellular processes. Its main function has been for long confined to the plasma membrane/cytoplasm compartment, being a myristoylated protein anchored to the cell membrane and functioning downstream to receptors, most of them lacking intrinsic kinase activity. In the last decades, new roles for some SFKs have been described in the nuclear compartment, suggesting that these proteins can also be involved in directly regulating gene transcription or nucleoskeleton architecture. In this review, we focused on those nuclear functions specifically attributable to Src, by considering its function as both tyrosine kinase and adapting molecule. In particular, we addressed the Src involvement in physiological as well as in pathological conditions, especially in tumors.

Keywords: Tyrosine phosphorylation ; Src-family kinases ; subcellular localization ; nucleus ; oncogenes

1. Introduction

Although tyrosine kinases are well known to function as signaling molecules downstream of extracellular stimuli at the plasma membrane, some SFKs have been described to reside in the nucleus where they regulate tyrosine phosphorylation of nuclear proteins, and/or function as cofactor in multiprotein complexes [1]. Therefore, the roles exerted by Src in the nucleus could be dependent or not on its catalytic activity. Indeed, beside its capability to phosphorylate tyrosine residues on target proteins, the SH2 and SH3 domains in the Src structure are involved in protein-protein interaction that can be independent from Src activation status. In particular, nuclear Src seems to exacebate the activity of oncogenes, and to counteract the protecting function of oncosuppressor, in general by inducing their nuclear export. Here we reviewed the main mechanisms involving Src nuclear functions.

2. Regulation of Gene Transcription and Chromatin Architecture

Changes in the structure of nuclear compartment are frequently observed during transcription, cell differentiation, senescence, cell cycle and tumorigenesis [2], and evidence of active nuclear Src has been reported in different contexts. A study carried out on NT2D1 non-seminoma fibroblasts reveals that Src phosphorylation is constitutively present in the nuclei of these cells, representing a downstream effector of c-MET pathway [3]. c-MET is the membrane receptor of HGF (Hepatocyte Growth Factor). HGF can increase the aggressive and malignant behavior of NT2D1 cells through c-MET activation [4]. The inhibition of Src deletes the HGF-dependent increase of cell proliferation rate, migration and cell invasion. c-MET recruits Src when activated by HGF, and this stimulus seems to be a key point allowing Src to translocate into the nucleus where it interacts with some gene promoters. In this context, a pivotal role is played by the cancer microenvironment, given that in the culture basal conditions (without administration of HGF) the inhibition of Src causes the augment of invasiveness but decreases the cell proliferation rate and migration capability of mouse NT2D1 fibroblasts independently from c-MET pathway, may be due to the Src recruitment by other homeostatic pathways controlling the aggressiveness of these cells [3].

The idea that Src could interact with gene promoters is based on a study that explains a correlation of SFKs with the chromatin structural changes observed following growth factors stimulation [5]. In this study, authors developed a pixel imaging technique of the nucleus to quantitatively detect changes of chromatin structure and condensation levels. They demonstrated that SFK activation by serum-conveyed growth factors localize into the nucleus more frequently in the euchromatin than the heterochromatin areas, and that their kinase activity is required for the chromatin organization, given that growth factor stimulation effects are avoided in mouse embryonic fibroblast SYF cells, which are genetically deficient in expression of Src, Yes, Fyn and Lyn tyrosine kinases. Taken together, this evidence suggested that the SFKs could be useful to create an “open” chromatin more accessible to transcriptional factors. In this context, we recently demonstrated

the Src nuclear localization in osteoblasts and low aggressive osteosarcoma cells [6], and in particular we observed nuclear Src accumulation in hypocondensated chromatin, as demonstrated by the low DRAQ5 staining. This finding, together with the work by Takahashi, strongly suggests a function for nuclear Src in the regulation of transcription.

As regards cancer cells, the protein p300, a large histone acetyltransferase with the function of coactivator, was at first known to be a tumor suppressor but the recent discovery of *p300* gene mutations seems to suggest a role for this enzyme in the oncogenic transformation [7]. In the tumor pancreatic environment, p300 seems to interact with Src, which can in turn activate the pro-migratory genes such as *HMGA2* and *SMYD3* [8]. The binding of Src and p300 to the sequence of DNA depends on chromatin and cell-type background. In those cancers in which Src has been found downregulated, the clinical trials based on Src-inhibitor therapy have proven to be ineffective and data by Paladino et al. provide some explanation about these failing therapies, as Src seems to be more involved in the migratory pathway than in survival signaling. Although these works describe some peculiar roles of Src in specific micro-environment, Src remains a good therapeutic target to prevent tumor metastasis [9].

3. Src-Dependent Regulation of Tumor Suppressors

As an example of its catalytic-dependent and independent nuclear functions, Src is able to regulate the localization of INhibitor of Growth 1 (ING1) from nucleus to cytoplasm through phosphorylation-dependent and independent mechanisms, thus contributing to alter the capability of ING1 to induce apoptosis. ING1 plays a role in epigenetic regulation as tumor suppressor, being a stoichiometric member of histone acetlytransferase (HAT) and histone deacetylase (HDAC) complexes. When Src expression and/or activation is altered, as in many types of cancer, the ING1 levels are deregulated accordingly, and decreases following Src activation. Src destabilizes ING1 by phosphorylation, thereby inducing its export from nucleus. The Src phosphorylation-independent mechanism is based on the capacity of Src to bind directly ING1: in this role as cofactor, Src may prompt the degradation of ING1, or, as an alternative, kinase-dead Src may recruit and/or activate other tyrosine kinases to target this tumor suppressor [10].

Another protein that can be altered by Src-dependent kinase activity is the Runt domain transcription factor 3 (RUNX3). RUNX3 is a transcription factor known to be a tumor suppressor involved in proliferation, apoptosis and cellular differentiation. Oxidative stress causes RUNX3 mislocalization in cytoplasm in colon cancer cells. In conditions of oxidative stress, both Src expression and activation is positively regulated in the nucleus by HDAC1, known to involved in the transcription of oncogenes [11] and active Src phosphorylates RUNX3 leading to its cytoplasmic localization [12].

4. Src and Estrogen Receptor

Studies on the subcellular localization of steroid receptors have demonstrated that they can have effects other than the non-genomic action, thereby revealed their ability to interact with target effectors and activate signaling pathways. Src is involved in the regulation of estrogen receptors, which are known to regulate the homeostasis of a variety of tissues, including the bone [13]. Low levels of estrogen deficiency lead to accelerated bone loss and this is the primary cause of postmenopausal osteoporosis [14]. Estrogens are also responsible for an anti-apoptotic effect in osteoblasts [15]. Further studies have demonstrated that Src interacts with the estrogen receptor even in other cells such as the uterine cells and human breast cancer cells. Indeed, in the nuclei of uterine cells, active Src can phosphorylates estrogen receptor α (ERα) and enhances its transcriptional activity due to the activity of SHP2 (Src-Homology Protein2) [16]. SHP2, a protein encoded by the gene *PTPN11*, is generally located in the cytoplasm, but it is also known to translocate in the nucleus when DNA damage occurs [17]. SHP2 enhances Src tyrosine kinase activity by removing its inhibitory phosphorylation and Src, in turn, phosphorylates ERα, thus allowing its binding to the progesterone receptor promoter and driving its transcription [16].

Instead, the study of Castoria and colleagues demonstrates that in the breast cancer tumor environment, Src can promote the tumor progression through its tyrosine kinase activity [18]. The Tyr 537 residue of ERα is a key regulatory site for its activity and localization, and also connects ERα with Src [19]. The stimulation with estradiol promotes Src activity and leads to the phosphorylation of ERα in Tyr537, thus driving the nuclear export of the receptor and regulating hormone responsiveness of DNA synthesis in breast cancer cells [18].

5. Interaction with the Nuclear Envelope Protein Emerin

Emerin is a nuclear inner membrane protein whose gene mutations are related to Emery-Dreifuss Muscular Dystrophy, an X-linked disease [20]. Tifft and coworkers demonstrated that emerin function is regulated by several tyrosine kinases, including Her2, Src and Abl. In particular, Src can mediate the signaling of Her2 by phosphorylation of three specific

tyrosine residues in human emerin: Y59, Y74 and Y95 [21]. These three amino acid residues could not be the only residues phosphorylated by Src, since even the Y4, Y34, Y41, Y105 and Y155 are predicted Src-target sites [22]. Tifft and colleagues demonstrated that the substitutions of the tyrosine with phenylalanine, in the sites recognized by Src, reduced the capability of emerin to bind BAF (barrier-to-autointegration factor, also known as BANF1), a conserved chromatin regulator that also binds lamins. Emerin binds proteins that are crucial for the spatial organization of centrosome and nuclear structure, influences the actin cytoskeletal dynamics and helps to fasten silent chromatin [23]. Emerin is also involved in the mechano-transduction signaling, as it has been described as a downstream detector of mechanical stress. In more detail, emerin binds Lamin A, another nuclear envelop protein, and emerin depletion leads to an increased nuclear rigidity hindering the nuclear adaptation to mechanical forces. Guilluy and colleagues showed that the phosphorylation of Y74 and Y95 of emerin residues by Src mediates the mechanical adaptation of nuclei to mechanical force [24]. Some recent evidence demonstrate that the cells cultured on soft matrices induced emerin phosphorylation and the mislocalization of nuclear envelope proteins in the nucleoplasm [25]. The authors also suggest that emerin is able to reorganize the chromosome territories in cells on softer matrix and they speculate that emerin phosphorylation acts as an upstream regulator of lamin localization resulting in substantial changes of the transcriptional regulation in a substrate stiffness-dependent manner [25].

6. Src and the Mechanotransduction

The involvement of cytoplasmic Src in the cell response to mechanical stimulation has been well characterized, especially in its crucial role of triggering the tyrosine phosphorylation cascade thought to be pivotal for mechanosensing [26]. Indeed, extracellular matrix proteins interaction with integrins induces their activation and the assembly of the focal adhesion complex proteins. This process, known as cell mechanotransduction, identifies involved proteins as mechanosensors, able to perceive and transduce mechanical stimuli into biochemical signals. Following integrin activation, the membrane-bound Src is responsible of an increase in focal adhesion kinase (FAK) and paxillin tyrosine phosphorylation, described as a first response to several mechanical stimuli, to such an extent that Src and FAK inhibitors are able to block the response to mechanical stimulation as the cyclic stretch [26].

In the context of mechanobiology, the Hippo pathway has been described to be relevant in regulating tissue growth and organ size [27]. The main function of the Hippo pathway is to inhibit Yes-associated proiein (YAP) and Tafazzin (TAZ) transcription co-activators, thereby regulating cell proliferation, apoptosis, and stemness in response to extracellular and intracellular signals, among which cell-cell contact, cell polarity, mechanical cues, ligands of G-protein coupled receptors and cellular energy status [28]. When YAP and TAZ are slightly phosphorylated they are more concentrated in the nucleus, thus leading to cell proliferation, wound healing or tissue regeneration [29]. Contrariwise, high levels of phosphorylation lead to cell quiescence [30]. It is also known that mechanical signals and phosphorylation can modulate YAP1 functions [31]. This may be related to Src-mediated phosphorylation of YAP1 in Tyr357 [32]. As a transcriptional factor, YAP1 is very important and two types of pathway are involved in its regulation: the “canonical” way (through the negative LATS1/2 regulation) and, as recently discovered, the SFK dependent way [33].

Ege and colleagues described for the first time the dominance of YAP1 nuclear export as the key point regulating its subcellular localization. Although serine phosphorylation is the first trigger required for YAP1 nuclear export, the inhibition of SFK activity by dasatinib in cancer related fibroblasts (CAFs) reduces the YAP1 nuclear localization leading to a higher citoplasmic content resembling normal fibroblasts. Indeed, CAF treatment with Src-family kinase inhibitors, such as dasatinib, affects the subcellular distribution of YAP1 by increasing the dissociation rate of YAP1 from chromatin thus inducing YAP1 export from nucleus. Among Src-mediated control of YAP1, its phosphorylation in Y357 functions as an independent mechanism for YAP1 activity regulation. Y357 phosphorylation seems to be not involved in controlling YAP1 subcellular localization, but in reducing its transcriptional competence. The evidence that YAP1 transcriptional activity is altered even when nuclear export is blocked suggests that this crucial phosphorylation may occur in the nucleus and that depends on nuclear Src activity [32].

Given the crucial roles of Src in the bone cells [34][35] and the great relevance of mechanical loads in the bone homeostasis [36], it is worth to mention the nuclear Src functions in osteoblast cells in response to mechanical stimulation. Indeed, external mechanical loads as the interstitial fluid shear stress are sensed at the membrane by integrins that transmit the message through ERK, Src and RhoA to actin stress fibers in the cytoskeleton [37]. Osteocytes, the most abundant cells of the bone tissue, reside into the mineralized matrix and are capable of sensing mechanical cues applied to the bone, to which they react triggering mechanisms involved in controlling osteoblast and osteoclast activities [38]. In particular, osteocytes respond to mechanical loading inducing the formation of a Src/Pyk2/MBD2 complex that suppresses anabolic gene expression [39]. Once activated by oscillatory fluid shear stress, Pyk2 and Src translocate into the nucleus, where they associate with methyl-CpG-binding domain protein 2 (MBD2), a protein involved in DNA methylation.

Therefore, the formation of a nuclear Pyk2/Src complex in osteocytes is related to altered transcription and epigenome regulation, leading to the suppression of anabolic gene expression, likely a mechanism to prevent an over-reaction to physical stimuli [39].

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