CKIP-1: A scaffold protein and potential therapeutic target integrating multiple signaling pathways and physiological functions

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The casein kinase 2 interacting protein-1 (CKIP-1) was originally identified as a specific interacting protein of casein kinase 2 (CK2) α subunit (but not α' nor β subunit), and CKIP-1 gene is broadly expressed and encodes a protein with 46 kDa (Bosc et al., 2000). The CKIP-1 protein contains a pleckstrin homology (PH) domain at the N-terminus, a putative leucine zipper (LZ) motif at the C-terminus, and five proline-rich motifs throughout the protein, which could mediate multiple interactions between CKIP-1 and numerous cellular proteins. Based on its protein structure, CKIP-1 is also known as the pleckstrin homology domain-containing family 0, member 1 (PLEKHO1). In this review, we summarize the various CKIP-1-interacting proteins and CKIP-1-involving signaling pathways, analyze the characteristics and mechanisms of CKIP-1 localization, and further provide the potential clinical applications of CKIP-1.

1. The scaffold role of CKIP-1 to mediate interactions with various proteins in multiple signaling pathways

1.1. CKIP-1 and CK2α

Protein kinase CK2 is an important and highly conserved protein serine/threonine kinase, which controls a broad range of biological events by phosphorylating various cellular proteins located in distinct compartments in cells (Glover, 1998; Pinna, 1990). Two catalytic subunits (CK2α and CK2α′) and two regulatory subunits (CK2β) constitute the tetrameric CK2 kinase complex. CK2α and CK2α′ are highly conserved and possess similar catalytic activities in vitro (Bodenbach et al., 1994; Litchfield and Luscher, 1993), but their subcellular localizations and regulation manners are different in cells (Vilk et al., 1999; Yu et al., 1991). Not surprisingly, CKIP-1 interacts with CK2α but not CK2α′ both in vitro and in vivo, and the unique interaction characteristic of CKIP-1 could perhaps explain the functional specialization between the two proteins.
(Olsten et al., 2004). As a CK2-interacting protein, CKIP-1 is neither a substrate nor direct regulator of CK2 kinase activity, while CKIP-1 could redistribute CK2 to the plasma membrane (PM) via its PH domain-mediated interaction with CK2α (Canton et al., 2005). Thus, CKIP-1 is likely to play a role in facilitating the PM recruitment of CK2 to interact with and promote the phosphorylation of PM substrates, such as insulin receptor, caveolin, insulin growth factor-II and dynamin (Olsten et al., 2004). However, whether CKIP-1 is necessary for the phosphorylation of PM substrates by CK2 kinase remains unclear.

1.2. CKIP-1 and CPα

The actin capping protein (CP) plays an important role in actin filament assembly and controls cell shape and movement (Cooper and Sept, 2008). CKIP-1 can directly interact with the α-subunit of CP (CPα) (Fig. 1) and significantly inhibit the capping activity of CP, accelerates F-actin depolymerization and induces an elongated fibroblast cell-like morphology in cells (Fig. 2). Furthermore, peptide “walking arrays” analysis has elucidated two key sites in CKIP-1: Arg-155 and Arg-157; and their substitutions greatly abolish CPα-binding ability and fail to induce the morphological alteration of osteosarcoma cells, indicating that CKIP-1-CPα interaction is required for CKIP-1-modulated cell morphology control (Canton et al., 2006). In addition, CK2 kinase induces the phosphorylation of CPα on Ser9 in vitro, and exhibits an additive effect on the inhibitory activity of CKIP-1 to CP, although the phosphorylation of CPα by CK2 is not necessary in this process (Canton et al., 2005). Regarding the function of CP-CKIP-1-CK2 complex in cell cytoskeletal regulation, we propose that CKIP-1-CK2 interaction may function as a requisite platform for efficient CP binding; or there exits some positive feedback loop for CK2-induced phosphorylation events; or perhaps some other CK2-associated proteins are involved in this process. Together, CKIP-1 could regulate actin cytoskeleton and cell morphology by recruiting CP at the plasma membrane to increase actin polymerization, and CK2 kinase may participate in this regulation.

1.3. CKIP-1 and PI3K/AKT

Apart from CK2α and CPα, CKIP-1 directly interacts with phospholipid and serine/threonine kinase Akt (Fig. 1) to regulate phosphoinositide 3-kinase (PI3K) signaling. Upon growth factor activation, PI3K produces a series of 3’ phosphoinositide lipids, which function as second messengers to transmit signals by binding with lipid-associated proteins, such as Akt (Katso et al., 2001). CKIP-1 possesses a wide spectrum of phospholipid binding in vitro, and the 123rd W amino acid plays a central role since that the W123A site mutation dramatically abolishes its binding ability (Olsten et al., 2004). In vivo studies indicate that in mouse myoblast C2C12 cells CKIP-1 specifically interacts with PtdIns-3P, and translocates to the plasma membrane in response to insulin stimulation, through which the N-terminal PH domain and C-terminal autoinhibitory region determine the nucleus-plasma shutting (see Section 2 and Table 2). In PI3K-regulated muscle cell differentiation, CKIP-1 overexpression accelerates the first transient proliferative phase and induces cell differentiation in C2C12 cells in a PI3K-dependent manner (Safi et al., 2004).

The different types of extracellular stimulus and lipids produced contribute to the cellular effects of PI3K pathway, and the PI3K/Akt signaling is the major pathway controlling cell growth and survival. Over-activation of PI3K signaling leads to a competitive growth advantage and metastatic competence, and the dysregulation of PI3K signaling pathway have been broadly observed in human cancers (Hennessy et al., 2005). However, how Akt kinase activity is regulated remains poorly investigated. CKIP-1 binds Akt (Akt1, Akt2, and Akt3) via its PH domain while the LZ region contributed to self dimerization (Fig. 1), and suppresses Akt phosphorylation and decreases Akt kinase activity. Since the inhibitory function on PI3K/Akt signaling, CKIP-1 decreases cell growth both in vitro and in vivo (Fig. 2), providing CKIP-1 protein as a potential tumor suppressor (Tokuda et al., 2007).

1.4. CKIP-1 and AP1/c-Jun

Not only suppressing cell proliferation, CKIP-1 is also implicated in the regulation of cell apoptosis. During tumor necrosis factor (TNF) plus the protein synthesis inhibitor cycloheximide (CHX)-induced apoptosis, CKIP-1 protein is cleaved by caspase-3 from sites of D310 and D345 (the major site D310 in vivo). The C-terminal fragments rather than the full length of CKIP-1 translocate to the cytoplasm and then to the nucleus, where they repress the transcriptional activity of AP-1 (activating protein-1) as a result of direct binding to and inhibiting the phosphorylation of c-Jun (the core subunit of AP-1 transcription factor). Notably, the regulation of AP-1 signaling by CKIP-1 is in a caspase-3-dependent manner, and meanwhile, CKIP-1 could increase apoptosis by activating caspase-3, forming a positive feedback loop with caspase-3 (Zhang et al., 2005) (Fig. 2). In response to DNA damage stresses, such as TNF, CHX and ionizing radiation, CKIP-1 cleavage and fragment translocation may be a common characteristic of the apoptotic responses in a broad range of cells. This function of CKIP-1 may also explain the observations that CKIP-1 expresses at low levels or short-lived in tumor cell lines, strengthening that CKIP-1 may be a crucial master in controlling cell apoptosis and drug sensitivities. This research is interesting, yet provides some more questions. For instance, what induces CKIP-1 cleavage in response to DNA damage stresses? Are there other CKIP-1 C-terminal fragment-binding proteins in vivo? Whether the nucleus translocation of CKIP-1 modulates the activity of other transcription factors or other signaling pathways? How does CKIP-1 cleavage influence the activity of caspase-3?

1.5. CKIP-1 and ATM/p53

With the pro-apoptotic capacity by inhibiting AP-1 signaling in nucleus, CKIP-1 could also recruit nuclear ATM (ataxia-telangiectasia mutated) partially to the plasma membrane. In human breast cancer SK-BR-3 cells, CKIP-1 overexpression delays CHX-mediated p53 degradation accompanied by the upregulation of Ser-15 phosphorylation levels of p53 in an ATM-dependent manner. CKIP-1 interacts with ATM (Fig. 1) and directs a proportion of ATM to the plasma membrane, where ATM is activated by phosphorylation at Ser 1981 (Fig. 2). The authors have established a novel plasma membrane translocation-induced ATM activation and p53 stabilization, indicating that ATM might have certain function at the plasma membrane via scaffold adaptor CKIP-1 (Zhang et al., 2006). Viewed as a nuclear protein involving in DNA damage repair process, ATM may also participate in the regulation of
growth factor/receptor signaling at the plasma membrane. The precise biological role of PM-localized ATM and its relevance to p53 phosphorylation and stabilization are both worthy to be further investigated.

1.6. CKIP-1 and IFP35/Nmi

As a scaffold adaptor mediating numerous protein–protein interactions, CKIP-1 regulates a variety of biological events, such as cell differentiation, apoptosis, cytoskeleton and cell morphology, and cytokine signaling. Interferon (IFN)–induced protein IFP35 and its homologue Nmi (N-Myc interacting protein) are two other CKIP-1 interacting proteins (Fig. 1). IFP35 alone is susceptible to be degraded by the proteasome and thus is a short-lived protein. Nmi forms a heterodimer with IFP35 and stabilizes IFP35 protein. CKIP-1 directly interacts with IFP35 and Nmi, and overexpression of CKIP-1 destabilizes IFP35 by interfering IFP35–Nmi interaction and thus abolishing the protective role of Nmi on IFP35. Thus, the ratio of Nmi to CKIP-1 ultimately controls IFP35 levels. Moreover, similar to IFP35 and Nmi, CKIP-1 expression is also induced by IFN-γ and IL-2 in PBMC (peripheral blood mononuclear cell), suggesting that CKIP-1 may be involved in cytokine signaling and serve as a physiological regulator of IFP35 and Nmi in vivo (Zhang et al., 2007).

1.7. CKIP-1 and Smurf1

CKIP-1 regulates the posttranscriptional modification of proteins via its adaptor role to mediate enzyme–substrate interaction, to facilitate protein phosphorylation, and ubiquitylation as well. The HECT-type ubiquitin–protein ligase (E3) Smurf1 (Smad ubiquitylation regulatory factor 1), controls the stability of numerous important substrate proteins and plays a key role in cell growth, apoptosis, polarization and bone homeostasis (Ozdamar et al., 2005; Yamashita et al., 2005). CKIP-1 has been demonstrated to interact with and promote the E3 ligase activity of Smurf1 partially due to augmenting its affinity to substrates (Fig. 2). Concretely, WW domains of Smurf1 are required for substrate binding, while CKIP-1 targets the short linker region (15 amino acids in length) between the two WW domains of Smurf1. The formation of substrates–Smurf1–CKIP-1 complex may change the conformational structure and facilitates Smurf1–mediated ubiquitylation and degradation of substrates, such as Smad1/5, RhoA and MEKK2 (Lu et al., 2008). Furthermore, to discover the physiological function of CKIP-1 in vivo, the CKIP-1-deficient mice were generated. The CKIP-1−/− mice are born normal, but exhibit an age-dependent increase in bone mass due to the decreased Smurf1 activity towards substrates degradation, suggesting that CKIP-1 functions as a crucial master in controlling osteoblast differentiation and bone formation (Lu et al., 2008). Consistent with the previous studies, the AP-1/c-Jun signaling is increased in CKIP-1−/− cells (Lu et al., 2008). However, whether the dysregulation of CK2, PI3K/Akt, IFP35/Nmi in CKIP-1−/− mice causes some abnormality as well is an important issue to further investigate the physiological function and regulation mechanisms of CKIP-1.

2. Cell context dependent nuclear/plasma membrane shuttling of CKIP-1 and its physiological relevance

2.1. Cell type-dependent localization of CKIP-1

Large investigations have suggested that the subcellular localization of CKIP-1 highly relies on the cell types. CKIP-1 is localized both at the plasma membrane and in the nucleus in C2C12 myoblasts (Olsten et al., 2004), MC-3T3 and Rat-1 fibroblasts (Safi et al., 2004), human lung cancer Gloc-82 cells and human breast cancer MCF-7 cells (Zhang et al., 2006). In monkey kidney COS-7 cells (Olsten et al., 2004) and human breast cancer SK-BR-3 cells (Zhang et al., 2005), the exogenous CKIP-1 is mainly localized at the plasma membrane, while in human osteosarcoma Saos-2 cells, CKIP-1 shows a prominent nuclear localization (Bosc et al., 2000) (Table 1).
Table 1
CKIP-1 interacting proteins and functions.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Signaling</th>
<th>CKIP-1 functions</th>
<th>Notes for CKIP-1 regulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK2</td>
<td>Ser/Thr kinase</td>
<td>Interact with and redistribute CK2 to the plasma membrane (PM) and nucleus</td>
<td>Facilitate the phosphorylation of substrates in PM</td>
<td>Bosc et al., 2000, Olsten et al. (2004)</td>
</tr>
<tr>
<td>CPu</td>
<td>Cytoskeleton</td>
<td>Inhibit the capping ability of CP by interacting with and recruiting CPu to PM</td>
<td>Cell morphology control</td>
<td>Canton et al. (2005), Canton et al. (2006)</td>
</tr>
<tr>
<td>Akt</td>
<td>PI3K/Akt</td>
<td>Repress Akt phosphorylation and kinase activity</td>
<td>Inhibit cell growth and promote PI3K-mediated muscle cell differentiation</td>
<td>Safo et al. (2004), Tokuda et al. (2007)</td>
</tr>
<tr>
<td>c-Jun</td>
<td>AP-1/c-Jun</td>
<td>Inhibit AP-1 activity via nuclear translocation of C-terminal fragments</td>
<td>Induce cell apoptosis</td>
<td>Zhang et al. (2005)</td>
</tr>
<tr>
<td>ATM</td>
<td>ATM/p53</td>
<td>Recruit nuclear ATM partially to PM, increase ATM activation and p53 stabilization</td>
<td>Mediate ATM function in PM</td>
<td>Zhang et al. (2006)</td>
</tr>
<tr>
<td>IFP35/Nmi</td>
<td>Cytokine</td>
<td>Destabilize IFP35 by interfering IFP35/Nmi interaction</td>
<td>Physiological regulator of IFP35 and Nmi</td>
<td>Zhang et al. (2007)</td>
</tr>
<tr>
<td>Smurf1</td>
<td>BMP</td>
<td>Interact with Smurf1 and augment its affinity to and the degradation of substrates</td>
<td>Control osteoblast differentiation and bone formation</td>
<td>Lu et al. (2008)</td>
</tr>
</tbody>
</table>

Table 2
Cell context-dependent CKIP-1 localization.

<table>
<thead>
<tr>
<th>CKIP-1</th>
<th>Localization</th>
<th>Cell lines</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full length (1–409)</td>
<td>Plasma membrane (PM) and nucleus</td>
<td>C2C12, MC3T3, Rat-1, Glc-82, MCF-7, COS-7, SW-13</td>
<td>Olsten et al. (2004), Safi et al. (2004), Zhang et al. (2006)</td>
</tr>
<tr>
<td>1–337</td>
<td>Nucleus</td>
<td>C2C12, MCF-7, HepG2</td>
<td>Xi et al. (2010)</td>
</tr>
<tr>
<td>1–382</td>
<td>PM and nucleus</td>
<td>C2C12, MCF-7, HepG2</td>
<td>Xi et al. (2010)</td>
</tr>
<tr>
<td>1–389</td>
<td>Cytoplasm and PM</td>
<td>C2C12, MCF-7, HepG2</td>
<td>Xi et al. (2010)</td>
</tr>
<tr>
<td>C-term1 (338–409)</td>
<td>Nucleus</td>
<td>C2C12, MCF-7, HepG2</td>
<td>Xi et al. (2010)</td>
</tr>
<tr>
<td>W123A</td>
<td>Cytoplasm</td>
<td>Saos-2, C2C12</td>
<td>Olsten et al. (2004), Xi et al. (2010)</td>
</tr>
<tr>
<td>Δ 82–90</td>
<td>Cytoplasm</td>
<td>C2C12</td>
<td>Xi et al. (2010)</td>
</tr>
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</table>

2.2. Key amino acids and particular stimulus in CKIP-1 localization control

The nuclear/plasma membrane distribution of CKIP-1 is determined by some key sites and regulated upon particular stimulus. Although the PH domain is viewed as a plasma membrane-associated domain, the isolated CKIP-1 PH domain alone shows predominantly nuclear distribution in different cell lines, such as myoblast C2C12, hepatic cancer HepG2 and breast cancer MCF-7 cells. A short stretch of basic residues and serine-rich motif “RKSKTSKSL” (amino acid 82–90) seems to function as the nuclear localization signal, and the net charge of basic residues determine the nuclear localization of CKIP-1 (Xi et al., 2010). Additionally, W123 residue is necessary for the integrity of CKIP-1 PH domain and W123A mutant displays the cytoplasm localization and speckles aggregations and fail to bind the phospholipids (Olsten et al., 2004). Notably, the nuclear localization of CKIP-1 after serum starvation relies on the PH domain to target in the nucleus. Interestingly, the C-terminus (or called C-term1) of CKIP-1, especially the region 382–389, could serve as an auto-inhibitory region to inhibit the nuclear distribution of CKIP-1 (Table 2). More importantly, as mediating CKIP-1 self-interaction, C-term1 plays a dominant negative effect and overexpression of C-term1 dramatically enhances the nuclear translocation of CKIP-1. As an important scaffold adapter, the binding of various proteins to different regions in CKIP-1 could regulate its own localization status, and further controls the activity or stability of its interacting proteins, establishing a complicated CKIP-1-centralized regulation network.

2.3. Specific function-dependent localization of CKIP-1

The localization of CKIP-1 changes in response to specific signals, to control the balance of cell growth and apoptosis. In response to serum withdrawal or TNF treatment, CKIP-1 has been reported to translocate from the plasma membrane to the nucleus, through which CKIP-1 is cleaved by and activate the activity of caspase-3, promoting cell apoptosis by repressing AP-1/c-Jun signaling. On the other hand, in response to growth factor stimulation, such as insulin-like growth factor-1 and epidermal growth factor, the endogenous CKIP-1 redistributes to plasma membrane, where it forms a complex with and inhibits the activity of Akt (Tokuda et al., 2007), leading to a “brake” on cell proliferation as a result of PI3K/Akt signaling suppression. When treated with PI3K inhibitors LY294002 or wortmannin, CKIP-1 translocates into the nucleus similar to that after serum starvation, suggesting that CKIP-1 localization is related to PI3K signaling (Hennessy et al., 2005). Therefore, the localization and activity of CKIP-1 is precisely regulated upon distinct stresses, so as to further modulate the activity of various CKIP-1 interacting proteins.

3. Perspectives of therapeutic target potentials of CKIP-1

3.1. CKIP-1 and metabolic skeletal disorders

One common health problem experienced by elderly citizens is osteoporosis, a disease that results in the reduction of bone mass and bone mineral density leading to weak, fragile bones and...
increased risk of fracture in patients. In addition to its societal effect, the condition has a significant financial impact. As all currently approved agents for osteoporosis act on both bone formation and bone resorption processes, new strategies for selectively bone formation without promoting bone resorption are required (Zhang et al., 2012). Based on the observations that CKIP-1 is a negative regulator of bone formation rather than bone resorption both in vitro and in vivo (Lu et al., 2008), Zhang et al. reasoned that silencing this gene in osteoprogenitor cells could promote bone formation and might be useful as a treatment for osteoporosis. They have recently developed the (AspSerSer)₆-liposome system to deliver CKIP-1 siRNAs selectively to bone-formation surfaces, which significantly promotes bone formation in healthy and osteoporotic rats models without activating resorption (Zhang et al., 2012). This study has established a system specifically targeting to the bone formation surface and facilitating the transmission of siRNA drugs to the osteogenic-lineage cells without affecting nonskeletal tissues, thus increasing the efficiency and avoiding the side-effects of RNAi-based bone anabolic therapy. More importantly, this study reveals that CKIP-1 could be a potential promising target for treatment of bone disease such as osteoporosis. In addition to RNA interference, development of small molecular inhibitors of CKIP-1, Smurf1 themselves and their protein–protein interaction should provide another strategy to promote bone formation and treat osteoporosis.

3.2. CKIP-1 and muscle differentiation

While CKIP-1 is a negative regulator for bone formation, Safi et al. indicated that CKIP-1 may be a positive regulator for muscle cell differentiation owing to the observation that CKIP-1 induces muscle cell differentiation in cultured C2C12 myoblasts (Safi et al., 2004). Our studies display that CKIP-1 inhibits myocytes differentiation analyzed from the CKIP-1-/- mice models. CKIP-1 deficient mice showed spontaneous cardiac hypertrophy with age and hypersensitive to pressure overload induced pathological cardiac hypertrophy. Thus, CKIP-1 might be a promising therapeutic target for myocardial hypertrophy (unpublished data). However, the role of CKIP-1 might be cell type-dependent, and the effect of CKIP-1 in skeletal muscle differentiation in vivo is still unknown.

3.3. CKIP-1 and carcinogenesis

As a scaffold protein, CKIP-1 has been reported to regulate numerous important proteins controlling cell survival, such as CK2, Akt, IFP35 and Smurfl. It has been generally demonstrated that the activities of CK2, Akt and Smurfl are elevated in several human cancers and mice models (Daya-Makin et al., 1994; Hennessy et al., 2005; Lu et al., 2008; Seldin and Leder, 1995; Stalter et al., 1994). As discussed above, the cell context-dependent localization of CKIP-1 might take an important part in the activity and subcellular localization of both CK2 and Akt, to regulate their oncogenic activities. In addition, CKIP-1 could induce Akt1 cleavage perhaps partially by activation of caspase-3 (Tsuchiya et al., 2007), destabilize IFP35 by inhibiting the protective role of Nmi (Zhang et al., 2007), and promote the E3 ligase activity and autoubiquitylation of Smurfl (Lu et al., 2008) (Fig. 2). These observations all reveal that CKIP-1 might function as a potential tumor suppressor. Accordingly, CKIP-1 is expressed abundantly in normal tissues, but extremely low in most of the cancer cell lines and some cancer tissues, including small intestine cancer, colorectal cancer, lung cancer and ovary cancer (Tsuchiya et al., 2007; Zhang et al., 2007).

CKIP-1 acts as an important master in controlling the homeostasis and differentiation of osteoblasts and myocytes, whether it also plays a critical role in the development of cancer stem cells or controls the proliferation of cancer cells are interesting topics. CKIP-1 negatively regulates several important pathways, such as TGF-β/BMP signaling, and PI3K/Akt signaling, which participate in cancer stem cell regulation (Banez et al., 2012; Majumdar et al., 2012; Martelli et al., 2011; Massague and Xi, 2012), indicating a potential function of CKIP-1 in stemness maintenance and tumorigenicity control. Thus, the roles and means of CKIP-1 in self-renewal and differentiation of cancer stem cells will be eye-catch.

Recently, a sequence homology and bioinformatics analysis revealed the potential tumor suppression ability of three proteins (DEAH box helicase protein, CKIP-1 and caspase 10), as a result of the peptide sequences homologous to an active region of pigment epithelium-derived factor (PEDF), an important angiogenesis inhibitor (Koskimaki et al., 2012). Strikingly, the peptide derived from DEAH box helicase protein displayed significant tumor suppression in breast orthotopic xenograft model in severe combined immunodeficient mice (Koskimaki et al., 2012). The corresponding sequence in CKIP-1 is located at the central region (aa 186–196, TLDLIEEDPS). However, the experimental verification of this sequence of CKIP-1 was not performed. So, whether CKIP-1 possesses similar activities in regulating cell adhesion, migration and proliferation is an exciting story and needs further research.

3.4. Structure, limitation, and potential therapeutic targets

WW domains are 40-amino acid residue units characterized by two highly conserved tryptophans (W), which are composed of a single three stranded β-sheet. Generally, WW domains bind a PXX(S/T) sequence known as the PY motif (Macias et al., 1996, 2002). Aragon et al. had shown the detailed view of Smurf1 WW domain bound to the PY motif of Smad1 by crystal structure analysis. In addition, they illustrated the role of glycogen synthase kinase-3 (GSK3) phosphorylation in Smad1 to enhance the binding capacity of Smurf1 with Smad1 (Aragon et al., 2011). Although lack of structure evidence for CKIP-1/Smurf1/Smad complex till now, Lu et al. had pointed out the key amino acids in Smurf1 linker region which controlled Smurf1–CKIP-1 association, providing the possibility that CKIP-1 could increase Smurf1–Smad1/5 interaction probably by altering the structure of the complex (Lu et al., 2008). Therefore, the small molecular inhibitors of protein–protein interaction for CKIP-1/Smurf1 would be promising.

Although viewed as a promising target in osteoporosis, the role of CKIP-1 in other organs or tissues in vivo are still unclear at this stage. Our previous analysis of CKIP-1 expression in human tissues revealed that CKIP-1 is expressed abundantly in skeletal muscle and heart, moderately in kidney, liver, brain, and placenta, and sparingly in the pancreas and lung (Zhang et al., 2007). Additionally, a dot blot containing a total of 68 normal tissues and 8 human cancer lines indicated that CKIP-1 was widely expressed in the examined adult and fetal tissues (Zhang et al., 2007). Consistent with this wide expression profiling, our unpublished data showed that CKIP-1 deficient mice displayed spontaneous cardiac hypertrophy with age, hypersensitive to pressure overload-induced pathological cardiac hypertrophy and also resistance to endotoxin infection. So there exist limitation in targeting CKIP-1 as drug target, and researches on the conditional modulation of CKIP-1 in animal models and the functions of CKIP-1 in distinct physiological and pathological situations will be much important. Based on the reported knockout mice phenotype, CKIP-1 is an inhibitor of bone formation but not a regulator of bone resorption (Lu et al., 2008), suggesting that the siRNA targeting CKIP-1 might be promising in osteoporosis therapy. To prevent the possible side-effects, Zhang et al. developed a bone formation surface-specific siRNA delivery system, i.e. (AspSerSer)₆-liposome, and therefore targeted CKIP-1 in restricted bone formation surface (Zhang et al., 2012). Such strategy efficiently escaped the potential limitation of wide CKIP-1 expression and should be encouraged in further CKIP-1 studies.
In summary, given the importance of CKIP-1 in cell growth and apoptosis control, it might be a novel therapeutic biomarker involving multiple important signaling pathways through regulating protein expression, activity or localization.

Conflicts of interest
None.

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