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Mini review

Integrin-linked kinase: A new actor in the ageing process?

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ABSTRACT

Integrin-linked kinase (ILK) is a protein located in focal adhesion complexes that is linked to the cytoplasmic domain of integrin receptors. Together with PINCH and parvin, ILK forms the IPP complex, which is associated with conserved intracellular signalling pathways and integrin regulation of the actin cytoskeleton. ILK plays an essential role in a wide variety of cellular functions, including cell migration, differentiation, survival, and division. The present review summarizes recent evidence, suggesting a new role for ILK in organismal ageing and cellular senescence, indicating that ILK is a key regulator of longevity and premature cellular senescence induced by extracellular stressors.

1. Introduction

1.1. Discovery of integrin linked kinase

In 1996, Hannigan and colleagues described a new protein called integrin-linked kinase (ILK), which was involved in cell adhesion and anchorage-dependent cell growth (Hannigan et al., 1996). Since the publication of that study, the structure and function of ILK have been widely studied. ILK is a protein containing ankyrin-repeat and kinase-like domains, which interact with the cytoplasmic domains of the β1 and β3 subunits of integrin receptors (Hannigan et al., 1996; Pasquet et al., 2002). Together with the parvin and PINCH proteins, it forms an intracellular multiprotein complex called the IPP complex and is located at focal adhesion sites (reviewed in Ghatak et al., 2013). The IPP complex connects extracellular matrix (ECM) components with the cytoskeleton, and participates in the transduction of bidirectional signalling between the ECM and intracellular compartments, activating many pathways involved in cell proliferation, migration, and survival (Giancotti and Ruoslahti, 1999). However, the molecular mechanism by which ILK transduces integrin signalling remains controversial.

ILK was initially described as a serine-threonine kinase with the capacity to phosphorylate AKT, glycogen synthase kinase-3β (GSK-3β), and many other substrates (Hannigan et al., 1996; Maydan et al., 2010). The kinetics of ILK kinase activity were also described (Maydan et al., 2010) and more recent studies have further described ILK kinase activity (Hannigan et al., 2011; Peng et al., 2014; Serrano et al., 2013a). However, more detailed studies of the C-terminal kinase domain of ILK subsequently revealed that it is an atypical kinase domain that lacks several residues critical for catalytic activity. The C-terminal kinase domain seems to have evolved to interact with parvin. Nevertheless, studies with purified ILK and crystal structure-function analyses have shown that recombinant ILK expressed in bacterial or mammalian cells shows no kinase activity, but acts as a bona fide pseudokinase when linked to the IPP complex (Fukuda et al., 2011). In contrast, numerous studies have shown that ILK phosphorylates peptide substrates in vitro, indicating that ILK is a bona fide protein kinase, at least in vitro (reviewed in Hannigan et al., 2011). However, the kinase activity of ILK has not been demonstrated in genetic studies in Drosophila (Zervas et al., 2001), *C. elegans* (Mackinnon et al., 2002) or mice (Lange et al., 2009). These studies suggest that some of the biological functions of ILK are independent of kinase or pseudokinase functions, since it has been shown that they are not essential for mouse development and adult life and that ILK can function as an adaptor/scaffold protein. All these studies are extensively discussed and reviewed in Ghatak et al. (2013). Despite this debate, the importance of ILK in relevant functions in cells and in organisms has been extensively reported and reviewed.

1.2. Physiological and pathological functions of ILK

The use of technologies such as Cre-lox driven recombination and RNA interference have yielded important information about tissue-specific roles of ILK (reviewed in McDonald et al., 2008).

ILK is required for physiological processes such as normal bone growth, keratinocyte proliferation and polarization (reviewed in McDonald et al., 2008), and correct wound healing (Serrano et al., 2012). It has also been implicated in myogenic differentiation of

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skeletal muscle cells and smooth muscle cell contraction. In the nervous system, ILK has a role in development, and regulates glial differentiation in the cerebellar region. Indeed, either defects in integrin or ILK deletion from mouse brain resulted in impaired migration of neurons, cortical-lamination defects resembling cobblestone lissencephaly, dysregulation of the glial-cell network, and even premature death. All this work is broadly reviewed in McDonald et al. (2008). ILK also regulates hepatocyte and biliary cell proliferation and extracellular matrix deposition (Gkretsi et al., 2008).

Furthermore, ILK has been implicated in several pathologies. In kidney, increased expression of ILK has been related to tubule interstitial fibrosis and glomerular disease. A role for ILK has been described in the upregulation of extracellular matrix component synthesis in glomerular mesangial cells by stimulating transforming growth factor β1 (TGF-β1) promoter activity through direct activation of AP-1 (Ruiz-Torres et al., 2007).

In addition, pathological involvement of this protein has been described in other tissues. For example, it has been found that ILK activation promotes neuron survival by protection from apoptosis through AKT activation. Furthermore, overexpression of ILK has been described in several human cancers and it is associated with a poor patient prognosis (reviewed in McDonald et al., 2008). In the context of cancer, a role for ILK in lung cancer cell metastasis has been reported, through the AKT-NFκB-αvβ3 signalling axis (Liang et al., 2013).

Moreover, conditional deletion of ILK in cardiomyocytes causes dilated cardiomyopathy and impaired angiogenesis in vascular endothelial cells (reviewed in McDonald et al., 2008). ILK also regulates vascular function, since conditional deletion of ILK in adult animals increased the vascular response to nitric oxide, thereby modulating the soluble guanylate cyclase and protein kinase G system (Serrano et al., 2013b). Lack of ILK promotes aberrant cell proliferation of hair follicle stem cells in their niche, predisposing skin tissue to carcinogenesis (Morgner et al., 2015). More recently, it has been suggested that ILK expression in muscle is a critical component of diet-induced insulin resistance, possibly impairing insulin signalling and insulin perfusion through capillaries (Kang et al., 2016).

All these studies indicate an important role for ILK in the physiology and pathophysiology of organisms. However, only a few studies have suggested a role for ILK in cellular senescence and organism ageing. The aim of this review is to examine current information and raise new questions regarding the role of ILK in ageing.

2. Role of ILK in cellular senescence

ILK may play a role in cellular senescence, a basic process that most likely contributes to organismal ageing, which was first described by Hayflick as a permanent arrest of the cell cycle (Hayflick and Moorhead, 1961). Cellular senescence can be induced by successive telomere shortening during the replicative life of the cell, or by intra- or extracellular stress. Senescent cells have a specific phenotype characterized by increased size, increased activity of senescence-associated-β-galactasidase (SA-β-Gal), increased expression of cell cycle inhibitors such as p16, p53, and p21, accumulation of senescence-associated heterochromatic foci (SAHF), and induction of a senescence-associated secretory phenotype (SASP), among other properties. Studies have shown that cellular senescence is involved in ageing-related organ dysfunction (Baker et al., 2016), in physiological processes such as embryonic development, in maintaining the balance between cell death and excessive proliferation in normal tissues, and in many pathological events (reviewed in Muñoz-Espín and Serrano, 2014).

The first study describing a role for ILK in the ageing process appeared in 2004 (Li et al., 2004). This article reported higher expression of ILK in primary cultures of mesangial and tubular epithelial cells isolated from a 28-month-old rat compared with a 3-month-old rat. ILK expression was positively correlated with SA-β-Gal staining. The same authors subsequently reported that overexpression of ILK in young cultured fibroblasts induced cellular senescence, whereas knockdown of ILK by RNA silencing prevented the phenotypic changes associated with senescence (Chen et al., 2006).

Overexpression of ILK has also been described in cells that enter senescence after prolonged exposure to extracellular stressors such as increased concentrations of extracellular phosphate or oxidative stress. However, the role for ILK in cellular senescence is not very clear, since several researchers have demonstrated that repression or inactivation of ILK in cancer cells promotes cellular senescence. It seems that, depending on the physiopathological context, ILK plays different roles with respect to cellular senescence. Zhu and colleagues (Zhu et al., 2014) have reported that peroxisome proliferator-activated receptor-β/δ induces cellular senescence and tumour suppression in a model of skin cancer by repressing ILK expression, which in turn decreases AKT activation. In another study, ILK inactivation by a chemotherapy drug induced senescence in cells expressing the retinoblastoma tumour protein suppressor, a well-known cell cycle regulator at the G1 checkpoint (Duminuco et al., 2015).

These differing roles for ILK in senescence of transformed cells versus normal cells could be due to their function in the mitotic process. ILK localizes in centromeres, while cancer cells have an increased number of centromeres that contributes to multipolar division. When ILK is inactivated by anti-tumour drugs in cancer cells, they undergo catastrophic anaphase due to centrosome declustering (Sikkema et al., 2014). This can result in tumour cell senescence. However, why ILK overexpression has different consequences in cancer cells than in normal somatic cells remains to be elucidated.

2.1. ILK and senescence signalling pathways

A role for ILK in the ageing process is suggested by the connection between ILK and some conserved signalling pathways involved in the regulation of senescence.

Oxidative stress is perhaps the best-studied mechanism involved in cellular senescence. A recent study by our group showed that oxidative stress induces cellular senescence in renal cells by increasing ILK protein expression and activity. Interestingly, when ILK expression was downregulated by specific RNA interference, oxidative stress did not provoke cellular senescence in these cells (Troyano-Suárez et al., 2015). Oxidative stress is also involved in hyperphosphatemia-induced ILK-dependent senescence (Troyano et al., 2015).

Additionally, the effect of hyperphosphatemia on ILK expression described above is mediated by other well-known ageing-related pathways, such as the insulin growth factor-1 (IGF-1) receptor/FOXO pathways. Hyperphosphatemia induces overexpression of ILK by activating the IGF-1 receptor and then inhibiting the activity of FOXO transcription factor, leading to an increase in reactive oxygen species production (Troyano et al., 2015).

Twenty years ago, a new protein named Klotho was discovered as an ageing-related protein. Klotho mutation in mice results in a short lifespan, arteriosclerosis, and infertility, all of which are ageing-like phenotypes (Kuro-o et al., 1997). Klotho has glucoronidase activity and acts as a co-receptor of the fibroblast growth factor 23 to regulate phosphate homeostasis. Klotho is mainly expressed in the kidney, where its expression has been reported to decrease with age (Doi et al., 2011). In this context, we recently reported a new and interesting relationship between ILK and Klotho. We found that ILK also exerts a direct effect on mRNA transcription of the Klotho protein in renal cells, and demonstrated that when ILK is overexpressed, Klotho mRNA transcription is repressed in these cells (Troyano-Suárez et al., 2015). The downregulation of Klotho protein is a mechanism by which ILK promotes senescence in renal cells.

Some studies have established a direct relationship between glycogen synthase kinase-3 (GSK-3) and ILK activity. In particular, ILK phosphorylation of GSK-3β inhibits its activity (Maydan et al., 2010). The GSK-3 protein family has been implicated in ageing. GSK-3 has two