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Involvement of the ERK/MAP kinase signalling pathway in milli-calpain activation and myogenic cell migration

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Abstract

Recent research carried out in our laboratory has shown that IGF-1, TGF-β1, and insulin were able to strongly stimulate myoblast migration by increasing milli-calpain expression and activity. However, the signalling pathways involved in these phenomena remain unknown. The aim of this study was to identify the signalling pathway(s) responsible for the effects of IGF-1, TGF-β1, and insulin on myoblast migration and on milli-calpain expression and activity. For this purpose, wound healing assays were carried out in the presence of growth factors with or without specific inhibitors of ERK/MAP kinase and PI3K/Akt pathways. The results clearly showed that the inhibition of the ERK/MAP kinase pathway prevents the effects of growth factors on myoblast migration. Secondly, the expression and the activity of milli-calpain were studied in cells treated with growth factor, alone or with ERK/MAP kinase inhibitor. The results demonstrated that the up-regulation of milli-calpain expression and activity was mediated by the ERK/MAP kinase pathway. Finally, the possible implication of MyoD and myogenin, myogenic regulatory factors able to regulate milli-calpain expression, was studied. Taken together our results clearly showed that the ERK/MAP kinase signalling pathway is responsible for the effects of the three growth factors on myoblast migration and on milli-calpain expression and activity. On the opposite, the PI3K/Akt signalling pathway, MyoD and myogenin seem to be not implicated in these phenomena.

Keywords: Milli-calpain; Myoblast migration; Growth factors; ERK/MAPK pathway

Abbreviations: BCA, bicinchoninic acid; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethyl sulfoxide; DTT, DL-dithiothreitol; EGF, epidermal growth factor; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; ERK, extracellular-signal regulated kinase; FAK, focal adhesion kinase; FBS, foetal bovine serum; IGF-1, insulin-like growth factor-1; MAPK, mitogen-activated protein kinase; MARCKS, myristoylated alanine rich C kinase substrate; MEK, MAPK/ERK kinase; MEF-2, myocyte enhancer factor-2; NBT/BCIP, nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl-1-phosphate; PBS, phosphate buffer saline; PKB, protein kinase B; PI3K, phosphatidylinositol 3 kinase; PVDF, polyvinylidene fluoride; TBS, tris buffer saline; TGF-β1, transforming growth factor-β1

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1. Introduction

The mammalian calpain family includes 14 different calcium-dependent neutral cysteine proteases. The members of this large family of tissue-specific or ubiquitous enzymes are also classified according to the presence or absence of EF-Hand. So-called "typical" calpains contain EF-Hand, whereas "atypical" calpains do not (Goll, Thompson, Li, Wei, & Cong, 2003). μ-Calpain and m-calpain are two ubiquitous members of this family. These typical calpains are heterodimeric enzymes constituted by a catalytic and a regulatory subunit. The μ-calpain catalytic subunit (CAPN1, 80 kDa) is encoded by the *capn1* gene and the m-calpain catalytic subunit (CAPN2, 80 kDa) by *capn2*. CAPN1 and CAPN2 are associated with one of the two small regulatory subunits, Css1 (for Calpain Small Subunit 1) or Css2 (Schád, Farkas, Jékely, Tompa, & Friedrich, 2002). As calpains are proteases, their activities are regulated by numerous factors to prevent uncontrolled proteolysis. Calcium is a necessary factor for calpain activation (Alderton & Steinhardt, 2000; Moldoveanu, Hosfield, Jia, Elce, & Davies, 2001; Pal, Elce, & Jia, 2001). However, the calcium concentrations necessary for calpain activation are higher than the intracellular calcium concentration. Calpain activation thus requires other factors, such as phospholipid binding, autoproteolytic cleavage, and phosphorylation (by ERK) (Fernandez-Montalvan et al., 2006; Glading et al., 2004; Goll et al., 2003; Inomata, Kasai, Nakamura, & Kawashima, 1988; Li, Thompson, & Goll, 2004; Saido, Shibata, Takenawa, Murofushi, & Suzuki, 1992; Suzuki, Saido, & Hirai, 1992). Myogenic regulatory factors (MRF) such as MyoD and myogenin also stimulate calpain activity and expression (Dedieu, Mazères, Dourdin, Cottin, & Brustis, 2003). On the contrary, calpain activity is downregulated by calpastatin, its endogenous inhibitor (Averna et al., 2003; Barnoy, Supino-Rosin, & Kosower, 2000; Temm-Grove, Wert, Thompson, Allen, & Goll, 1999), as well as PKA phosphorylation (Shiraha, Glading, Chou, Jia, & Wells, 2002).

Previous studies have shown that the calpain family may be implicated in several pathological phenomena, such as cataract formation, psoriasis, tumoral invasion, diabetes, rheumatoid arthritis, ischemia, neurodegenerative diseases, and muscular dystrophies (Biswas, Harris, Singh, & Phoenix, 2004; Cox, Hayes, Roe, Tsuchiya, & Bell, 2004; David, Shearer, & Shih, 1993; Mamoune, Luo, Lauffenburger, & Wells, 2003; Matsushita, Shimada, Kawara, Takehara, & Sato, 2005; Menard & El-Amine, 1996; Richard et al., 2000; Saito, Elce, Hamos, & Nixon, 1993; Tidball & Spencer, 2000). Moreover, calpains are also thought to be involved in numerous physiological and biological processes, such as signal transduction, cell-cycle regulation, apoptosis, myogenesis, cell adhesion, spreading, and motility (Atencio, Ramachandra, Shabram, & Demers, 2000; Carragher & Frame, 2002; Cottin et al., 1994; Dedieu et al., 2004; Huttenlocher et al., 1997; Mazères, Leloup, Daury, Cottin, & Brustis, 2006; Potter et al., 1998; Sato & Kawashima, 2001; Tan, Wu, De Veyra, & Greer, 2006).

Myoblast migration is an early, critical stage in myogenesis. Calpains may play a pivotal role in this process, since calpain inhibition by chemical inhibitors or calpastatin overexpression dramatically reduces myoblast motility (Dedieu et al., 2004). Inhibiting migration prevents myoblast alignment and fusion, and, thus, formation of multinucleated myotubes. Calpains are involved in cell migration, possibly by cleaving numerous proteins present in focal adhesions, including FAK (focal adhesion kinase), desmin, vinculin, paxillin, MARCKS (myristoylated alanin-rich C kinase substrate), and talin (Bhatt, Kaverina, Otey, & Huttenlocher, 2002; Dourdin et al., 1999; Goll, Dayton, Singh, & Robson, 1991). These proteins form complexes and link the actin cytoskeleton to integrins. The assembly and disassembly of these complexes, in particular by calpains, is responsible for cell migration (Lambrechts, Van Troys, & Ampe, 2004; Petit & Thiery, 2000).

Cell migration may be enhanced by numerous factors, particularly growth factors (Lafrenière, Mills, Tremblay, & Fahime, 2004). Epidermal growth factor (EGF), for example, is able to strongly increase fibroblast motility by activating calpains via the ERK/MAP kinase pathway (Glading, Chang, Lauffenburger, & Wells, 2000; Satish, Babu, Tran, Hebda, & Wells, 2004). Our recent work on myoblast migration showed that insulin-like growth factor (IGF-1), transforming growth factor (TGF-β1), and insulin were capable of increasing myoblast motility significantly by enhancing m-calpain expression and activity (Leloup, Mazères, Daury, Cottin, & Brustis, 2006). Calpain activity was also shown to be required for growth factor-mediated migration. Indeed, inhibiting calpain activity by adding calpeptin or overexpressing calpastatin blocked the effects of growth factors on myoblast migration (Leloup et al., 2006).

However, the signalling pathways involved in this phenomenon were not elucidated. Several signalling pathways were potentially responsible for myoblast migration and m-calpain activation by growth factors such as IGF-1, TGF-β1, and insulin. The ERK/MAPK signalling pathway may phosphorylate m-calpain on serine 50, thus enhancing its activity (Glading et al., 2000). This pathway may also increase the expres-