The International Journal of Biochemistry & Cell Biology 43 (2011) 1104–1113

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

c-Jun is required for TGF-β-mediated cellular migration via nuclear Ca^{2+} signaling

Einsley Janowski[a,1], Xuanmao Jiao[b,c,1], Sanjay Katiyar[b,c], Michael P. Lisanti[b,c], Manran Liu[b,e], Richard G. Pestell[b,c,*,2], Martin Morad[a,d,**,2]

[a] Department of Pharmacology, Georgetown University, 3900 Reservoir Road, NW, Washington, DC 20057, United States
[b] Kimmel Cancer Center, Department of Cancer Biology, Thomas Jefferson University, Philadelphia, PA 19107, United States
[c] Jefferson Stem Cell and Regenerative Medicine Center, Thomas Jefferson University, Philadelphia, PA 19107, United States
[d] Blue Cross Blue Shield Endowed Professor for Cardiovascular Health, Director of SC Cardiac Signaling Center and Department of Cell Biology and Regenerative Medicine of MUSC, Charleston, SC 29401, United States
[e] Chongqing Medical University, Medical College Road, Yuzhong District 1, 400016, China

ARTICLE INFO

Article history:
Received 5 November 2010
Received in revised form 18 March 2011
Accepted 21 March 2011
Available online 5 April 2011

Keywords:
Calcium
Calcium imaging
Cell migration
Inositol phosphates
Tranforming growth factor beta

ABSTRACT

Tumor progression involves the acquisition of invasiveness through a basement membrane. The *c-jun* proto-oncogene is overexpressed in human tumors and has been identified at the leading edge of human breast tumors. TGF-β plays a bifunctional role in tumorigenesis and cellular migration. Although c-Jun and the activator protein 1 (AP-1) complex have been implicated in human cancer, the molecular mechanisms governing cellular migration via c-Jun and the role of c-Jun in TGF-β signaling remains poorly understood. Here, we analyze TGF-β mediated cellular migration in mouse embryo fibroblasts using floxed *c-jun* transgenic mice. We compared the *c-jun* wild type with the *c-jun* knockout cells through the use of Cre recombinase. Herein, TGF-β stimulated cellular migration and intracellular calcium release requiring endogenous c-Jun. TGF-β mediated Ca^{2+} release was independent of extracellular calcium and was suppressed by both U73122 and neomycin, pharmacological inhibitors of the breakdown of PIP_2 into IP_3. Unlike TGF-β-mediated Ca^{2+} release, which was c-Jun dependent, ATP mediated Ca^{2+} release was c-Jun independent. These studies identify a novel pathway by which TGF-β regulates cellular migration and Ca^{2+} release via endogenous c-Jun.

1. Introduction

Tumor onset and progression requires the acquisition of distinct cellular properties including increased cellular motility and invasiveness. The *c-jun* proto-oncogene was one of the first transcription factors to be identified and is frequently overexpressed in human tumors as determined by histopathology (Shaulian and Karin, 2002). c-Jun N-terminal kinase (JNK), a subgroup of mitogen-activated protein (MAP) kinases whose phosphorylation plays a key role in stress signals (Kyriakis, 1999), has been shown to be constitutively phosphorylated and activated in sporadic human cancers (Yamagata et al., 2005). c-jun promotes cellular proliferation and DNA synthesis via induction of cell-cycle proteins including Cyclin D_1 (Albanese et al., 1995; Shaulian and Karin, 2002), and recent studies demonstrated a c-Jun abundance governs progenitor or stem cells expansion in vivo (Jiao et al., 2010).

The role of c-jun in cellular migration and invasiveness is less well understood (Xia and Karin, 2004). Genetic deletion of c-Jun in transgenic mice is embryonic lethal (Eferl et al., 1999), with c-Jun and JunB having non-redundant roles in placentation, hepatogenesis, and heart development (Eferl and Wagner, 2003). c-Jun together with JNK, regulates cellular migration (Jiao et al., 2008b; Katiyar et al., 2007a; Xia and Karin, 2004). *c-jun*$^{-/-}$ fibroblasts derived from transgenic mice encoding a c-Jun gene flanked by LoxP sites (*c-jun*$^{f/f}$) exhibited flattened morphology, increased cellular diameter, and reduced cellular migratory activity in culture compared with the *c-jun*$^{f/f}$ 3T3 cells. This phenotype could be rescued in the *c-jun*$^{-/-}$ cells by transduction with a retrovirus expressing c-Jun (Jiao et al., 2008b). c-Jun promotes fibroblast migration (Eferl and Wagner, 2003) involving Rock II kinase (Jiao et al., 2008b).

Transforming growth factor-β (TGF-β) is a multifunctional cytokine that modulates cell growth, motility, apoptosis, differentiation, matrix production, and tumorigenesis (Junn et al., 2000;

* Corresponding author at: Kimmel Cancer Center, Department of Cancer Biology, Thomas Jefferson University, Room 1050 BLSB, 233 South 10th Street, Philadelphia, PA 19107, United States. Tel.: +1 215 503 5649; fax: +1 215 503 9334.
** Corresponding author at: Blue Cross Blue Shield Endowed Professor for Cardiovascular Health, Director of SC Cardiac Signaling Center and Department of Cell Biology and Regenerative Medicine of MUSC, Charleston, SC 29401, United States. Tel.: +1 843 792 3898; fax: +1 843 792 4606.
E-mail addresses: richard.pestell@jefferson.edu (R.G. Pestell), moradm@musc.edu (M. Morad).
[1] Equal first authors.
[2] Equal senior authors.

1357-2725/$ – see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.biocel.2011.03.011

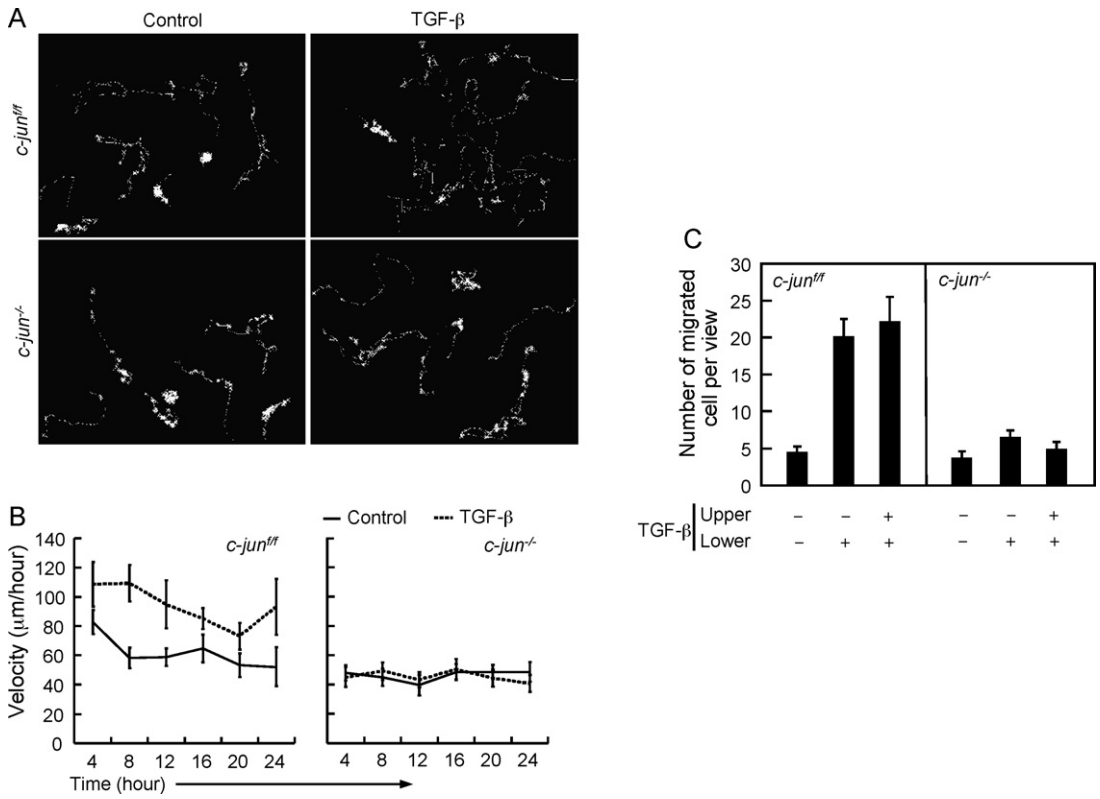

Fig. 1. c-Jun excision reduces cellular migration rates. Cell movement was monitored in c-jun$^{f/f}$ and c-jun$^{-/-}$ cells. Cells were imaged every 15 min by video microscope for 24 hrs. The trace (A) and velocity of single cell movement (B) was analyzed by metamorph software. Transwell cell migratory experiment also showed c-jun knockout inhibits TGF-β induced cell migration (C).

Kawabata and Miyazono, 1999). The receptors for the TGF-β super family are transmembrane serine kinases that phosphorylate members of the Smad family (Abdollah et al., 1997; Feng and Derynck, 2005) that in turn signal the nucleus, activating diverse and cell type-specific responses that, in part, depend on the type of activated Smad, the complexing of Smad with other transcription co-activators, and the availability of DNA sequence-specific transcription factors at the promoter DNA (Feng and Derynck, 2005). The activation of TGF-β type I receptor/Smad3C pathway by TGF-β inhibits the growth of normal epithelial cells, while activation of the JNK/pSmad3L pathway promotes invasion by mesenchymal cells, thus providing, respectively, for the tumor suppressor and tumor promoter characteristics of TGF-β (Matsuzaki and Okazaki, 2006). Smads are also shown to bind directly to the Jun family of AP-1 transcription factors (Liberati et al., 1999) and thus regulate TGF-β induced transcription (Zhang et al., 1998).

A variety of studies have demonstrated an important role for TGF-β in regulation of calcium signaling, but the role of c-Jun in this process remains unknown. Ca^{2+} controls cellular proliferation by activating transcription factors either in the cytoplasm or the nucleus (Berridge et al., 2000). The route of Ca^{2+} entry, the cytosolic Ca^{2+} levels, and the time course of Ca^{2+} elevations all affect the expression of transcription factors, providing for fine tuning by Ca^{2+} signaling (Clapham, 1995). Prolonged effects of TGF-β treatment include the induction of the ryanodine receptor in mink lung epithelial cells (Cheifetz et al., 1988) and elevation of inositol phosphate in Rat-1 fibroblasts (Muldoon et al., 1988a,b). Acute effects of TGF-β, which occur within seconds, include influx-dependent elevation of cytosolic calcium in MIN6 cells (Ishiyama et al., 1996). Ca^{2+} regulation is also thought to play a role in TGF-β signaling as calmodulin physically interacts with Smads and calcium functions as a second messenger for TGF-β-dependent insulin secretion (Ishiyama et al., 1996; Zimmerman et al., 1998).

As c-jun is overexpressed in a variety of human tumors and is found at the leading edge of tumors, we investigated the mechanism by which c-jun mediates cellular migration. Given the important role for TGF-β in human tumor pathogenesis and cellular migration and the prior findings that c-Jun is a downstream target of TGF-β, we investigated the potential role of endogenous c-Jun in TGF-β mediated cell migration and calcium signaling. We find that TGF-β-induced calcium signaling and migration is c-Jun dependent. The characteristics of the c-Jun dependent calcium signaling pathway appear to involve PIP_2 signaling.

2. Materials and methods

2.1. Transgenic mice, cells and reagents

Transgenic mice carrying a floxed *c-jun* allele, *c-jun*$^{f/f}$, were maintained as previously described (Jiao et al., 2008b). Experimental procedures with transgenic mice were approved by the ethics committees of Georgetown and Thomas Jefferson Universities. Mouse embryo fibroblasts (MEFs) were isolated from *c-jun*$^{f/f}$ mice as previously described (Albanese et al., 1999). 3T3 cells were derived from *c-jun*$^{f/f}$ MEFs (Li et al., 2006) by standard protocol. Excision of the *c-jun*$^{f/f}$ allele was monitored by identification of the recombinant 600 bp fragment as previously described (Katiyar et al., 2007a). A retroviral expression plasmid encoding Cre was cloned through the insertion of the cDNA from the vector pMC-Cre-PGKHyg as an *EcoRI* fragment into the retroviral expression plasmid pMSCVIRES-GFP (Neumeister et al., 2003). Expression of Cre from the pMSCV-IRES-GFP vector was confirmed by Western blot using an antibody directed to Cre (MMS-106). The c-Jun cDNA from pGEM c-Jun was inserted as an *EcoRI* fragment into the pMSCV-IRES-DsRed.