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Sun-mediated mechanical LINC between nucleus and cytoskeleton regulates βcatenin nuclear access

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ABSTRACT

βcatenin acts as a primary intracellular signal transducer for mechanical and Wnt signaling pathways to control cell function and fate. Regulation of βcatenin in the cytoplasm has been well studied but βcatenin nuclear trafficking and function remains unclear. In a previous study we showed that, in mesenchymal stem cells (MSC), mechanical blockade of adipogenesis relied on inhibition of βcatenin destruction complex element GSK3β (glycogen synthase kinase 3β) to increase nuclear βcatenin as well as the function of Linker of Cytoskeleton and Nucleoskeleton (LINC) complexes, suggesting that these two mechanisms may be linked. Here we show that shortly after inactivation of GSK3β due to either low intensity vibration (LIV), substrate strain or pharmacologic inhibition, βcatenin associates with the nucleoskeleton, defined as the insoluble nuclear fraction that provides structure to the integrated nuclear envelope, nuclear lamina and chromatin. Co-depleting LINC elements Sun-1 and Sun-2 interfered with both nucleoskeletal association and nuclear entry of βcatenin, resulting in decreased nuclear βcatenin levels. Our findings reveal that the insoluble structural nucleoskeleton actively participates in βcatenin dynamics. As the cytoskeleton transmits applied mechanical force to the nuclear surface to influence the nucleoskeleton and its LINC mediated interaction, our results suggest a pathway by which LINC mediated connectivity may play a role in signaling pathways that depend on nuclear access of βcatenin.

1. Introduction

Mechanical forces acting within the cellular environment define cellular form, and drive physiological function. βcatenin, the primary effector molecule of Wnt signaling axis (Baron and Kneissel, 2013) is central to mechanosignaling and its mechanical activation is a part of normal physiologic response (Robinson et al., 2006). In the case of musculoskeletal progenitor mesenchymal stem cells (MSC), mechanical signals generated during loading promote osteoblast differentiation (Uzer et al., 2013) and inhibit adipocyte recruitment(Sen et al., 2011). These MSC phenotypes are in part controlled by mechanically and chemically regulated βcatenin signaling (Sen et al., 2008). How βcatenin moves from cytoplasm into the nucleus after stimulation is unclear. At the cellular level, forces imposed on the cytoskeleton not only activate signaling events but promote cytoskeletal structure configurations that enhance activation of mechanosignaling pathways (Burridge and Wittchen, 2013; Uzer et al., 2016). In this way, cytoskeletal structure and connectivity may play a direct role in βcatenin nuclear trafficking.

βcatenin control of gene expression relies on its nuclear localization (Cong et al., 2003), but it does not possess a classic nuclear localization signal; instead, βcatenin's armadillo repeat sequence is believed to mediate nuclear transit through direct contact with the nuclear pore complex (NPC) (Koike et al., 2004; Tolwinski and Wieschaus, 2004). NPCs bind to nuclear lamina at the inner nuclear envelope, a highly organized structure that maintains dynamic connectivity with both chromatin and cytoplasmic cytoskeleton (Gruenbaum et al., 2005). In this way, reflecting its functionality in organizing internal structure and external connectivity, the structural component of the nucleus has been referred to as the "nucleoskeleton"(Cook, 1988). The nucleoskeleton acts as a master scaffold for regulatory proteins, transcription factors and chromatin to regulate functions of nuclear machinery (Simon and Wilson, 2011); this suggests that βcatenin might utilize the existing nucleoskeletal network to facilitate nuclear entry.

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Connectivity between the nucleoskeleton and the cytoplasmic cytoskeleton is maintained by a mechanosensitive complex called LINC (Linker of Nucleoskeleton and Cytoskeleton) (Crisp et al., 2006) which traverses the nuclear envelope. The LINC complex is made up of multiple components: actin binding giant Nesprin Proteins 1&2 which are anchored to the inner nucleus by Sun proteins 1& 2, that finally interact with the inner Lamin A/C network. We have shown that disabling LINC function via siRNA deletion of Sun-1 and Sun-2 proteins, or by overexpressing the Nesprin KASH (Klarsicht, ANC-1, Syne Homology) domain, not only decreases mechanical responsiveness to mechanical challenges but also promotes adipogenesis of MSCs (Uzer et al., 2015), a process largely dependent on nuclear βcatenin (Sen et al., 2009). βcatenin is known to be retained at cell–cell junctions(Aberle et al., 1996) and recently, KASH-less small Nesprin isoforms were shown to cause βcatenin localization to the plasma membrane (Zhang et al., 2016). Reminiscent of the disposition of βcatenin at cell–cell contacts at the plasma membrane, the Nesprin component of the LINC complex also associates with βcatenin at the nuclear envelope (Lu et al., 2012; Neumann et al., 2010). Consistent with a potential regulatory role of LINC complexes for βcatenin, progeroid mutations involving LINC and nucleoskeleton elements (Gruenbaum et al., 2005) are marked by increased adipogenic infiltration in musculoskeletal tissues indicating reductions in Wnt activity and cellular βcatenin (Hernandez et al., 2010). As such, the LINC complex may serve as a critical regulator of MSC fate through influencing βcatenin trafficking.

Here, utilizing sub-cellular fractionation and immunostaining experiments, we show that both mechanically and biochemically-induced βcatenin nuclear entry is preceded by a rapid but transient association of the molecule with the nucleoskeleton. The LINC elements Sun-1 and Sun-2 are critical in facilitating this βcatenin-nucleoskeleton interaction. When LINC connectivity is disrupted via Sun-1&2 co-depletion, basal levels of nuclear βcatenin drop and its interaction with the nucleoskeleton is impaired. Loss of LINC connectivity thus results in decreased efficiency of both mechanical and biochemical βcatenin-activating events.

2. Results

2.1. Mechanical inactivation of GSK3β leads to a non-monotonic increase of nuclear βcatenin

Mechanical strain application activates Focal Adhesion Kinase (FAK) and, through Fyn mediated recruitment of mTORC2 (Thompson et al., 2013), activates Akt (Ser 473). This leads to phosphorylation (Ser 9) and inhibition of GSK3β, preventing βcatenin proteolysis (Case et al., 2010). Similar to strain, low intensity vibration (LIV), also activates FAK/Akt, promotes MSC osteogenesis and inhibit adipogenesis (Uzer et al., 2015). Here, using marrow derived MSCs, we tested whether LIV inhibits GSK3β. We probed for Akt and GSK3β phosphorylation immediately following LIV (0.7 g, 90 Hz, 20 min). LIV increased both p-Akt (277%, $p < 0.001$) and p-GSK3β (246%, $p < 0.001$) consistent with decreased βcatenin proteolysis (Fig. 1a). Application of strain (2%, 0.17 Hz, 20 min) also increased p-Akt (218%, $p < 0.001$) and p-GSK3β (181%, $p < 0\,0\,1$) when compared to non-strained controls (Fig. 1b).

βcatenin enters the nucleus to activate its gene targets (Cong et al., 2003). We separated the soluble from the insoluble nuclear fraction and probed for localization of active (non-phosphorylated) βcatenin via western blot analysis. As indicated in Fig. 1c and d, 180 min after loading both LIV (177%, $p < 0.01$) and strain (173%, $p < 0.001$) samples showed increased βcatenin localization to the soluble nuclear protein fractions. PCR analysis further indicated that both LIV (205%, $p < 0.05$) and strain (198%, $p < 0.05$) application increased expression of Axin-2 mRNA, a positive transcriptional target of βcatenin (Leung et al., 2002).

To better understand βcatenin localization prior to nuclear entry, we probed nuclear βcatenin levels both immediately and 120 min after a single application of LIV or strain. Similar to our findings at 180 min post-load, we found increased βcatenin levels in the soluble nuclear fraction 120 min post-LIV (Fig. 1e, 140%, $p < 0.05$) and at 120 min post-strain (Fig. 1f, 158%, $p < 0.05$). This long-term increase of βcatenin in the soluble nuclear fraction was non-monotonic and was preceded by a significant decrease in the soluble nuclear βcatenin immediately following both LIV (Fig. 1e, 20 min, 49%, $p < 0.05$) and strain (Fig. 1f, 20 min, 45%, $p < 0.05$). These findings suggest that assay of the soluble nuclear fraction may reflect only a subset of total nuclear βcatenin, and indicate that βcatenin interacts with distinct nuclear compartments that are excluded in assays which capture only the soluble nuclear fraction.

2.2. Mechanical signals cause a rapid and transient βcatenin association with the nucleoskeleton

As βcatenin lacks a classical nuclear localization signal, it is thought to directly interact with Nuclear Pore Complexes (NPCs) during nuclear entry (Sharma et al., 2012). βcatenin forms complexes with LINC component Nesprin at the nuclear envelope (Markiewicz et al., 2006; Neumann et al., 2010) suggesting that the cell cytoskeleton interacts with the LINC complex to provide a scaffold to localize βcatenin in close proximity of NPCs. Depicted in Fig. 2a, we tested this possibility by extracting the insoluble nucleoskeletal (Nsk) fraction. The Nsk fraction was found to be free of the soluble nuclear protein marker PARP, but rich in the nuclear envelope proteins, Sun-1, Sun-2, Nesprin-1, as well as structural proteins LaminA/C and nucleoporin Nup358. Emerin was found in both soluble and insoluble fractions. We probed for a βcatenin-Nsk interaction immediately following the application of either LIV (0.7 g, 90 Hz, 20 min) or strain (2%, 0.17 Hz, 20 min), times when βcatenin levels in the soluble nuclear fraction were decreased; LaminA/C was used as a referent. Immediately after LIV, the βcatenin-Nsk association was increased to 179 ± 8.4% that of control cells (Fig. 2b, $p < 0.05$). Application of strain similarly increased βcatenin-NsK association to 189 ± 17% (Fig. 2c, $p < 0.05$).

Akt signal activation resulting from both LIV and strain is transient, returning to baseline within 120 min (Uzer et al., 2015). We thus tested if the mechanically directed interaction between βcatenin and Nsk was also transient: at the 140 min time point, the βcatenin-Nsk association dropped below baseline (82 ± 16%, NS) (Fig. 2b). This drop in the baseline association corresponds to the time when the soluble nuclear βcatenin fraction rises (Fig. 1e and f). At this point, if LIV was reapplied, βcatenin-Nsk association again rose to 176 ± 19% of the baseline ($p < 0.05$). Similarly, with strain, the stimulated association of βcatenin-Nsk returned to baseline levels 120 min after the first strain application (73 ± 26%, NS) (Fig. 2c). A second strain bout once again increased the βcatenin-Nsk association to 200 ± 4.3% ($p < 0.05$). These findings confirm that βcatenin's association with the nucleoskeleton is transient and indicate that mechanically directed association of βcatenin with the nucleoskeleton precedes translocation of βcatenin into the soluble nuclear fraction.

2.3. Co-depletion of LINC elements Sun-1 and Sun-2 disrupts βcatenin traffic into the soluble nuclear fraction

The mechanically-induced association of βcatenin with the nucleoskeleton suggests that nucleo-cytoskeletal connectivity might be necessary for βcatenin trafficking. To answer this question,