Acta Biomaterialia 8 (2012) 2963–2968

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

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

Actin cytoskeleton controls activation of Wnt/β-catenin signaling in mesenchymal cells on implant surfaces with different topographies

C. Galli [a,b,*], M. Piemontese [b,c], S. Lumetti [a], F. Ravanetti [d], G.M. Macaluso [a], G. Passeri [b]

[a] Sez. Odontostomatologia, University of Parma, Via Gramsci 14, 43100 Parma, Italy
[b] Department of Internal Medicine, University of Parma, Via Gramsci 14, 43100 Parma, Italy
[c] Department of Experimental Medicine, University of Parma, Via Gramsci 14, 43100 Parma, Italy
[d] Department of Animal Health, University of Parma, Via Gramsci 14, 43100 Parma, Italy

ARTICLE INFO

Article history:
Received 7 November 2011
Received in revised form 15 April 2012
Accepted 26 April 2012
Available online 4 May 2012

Keywords:
Titanium
Topography
ROCK
Cytoskeleton
Wnt

ABSTRACT

Surface topography affects cell function and differentiation. It has been previously shown that rough surfaces can enhance the activation of canonical Wnt signaling, an important pathway for osteoblast differentiation and bone maintenance, but the underlying mechanisms are still poorly understood. The present paper investigates whether cytoskeletal organization contributes to regulating this pathway. Rho-associated protein kinase (ROCK), an important controller of actin microfilaments, was inhibited with 2 mM specific antagonist Y-27632 in mesenchymal and osteoblastic cells growing on titanium discs with a polished or acid-etched, sand-blasted (SLA) surface. Y-27632 subverted the morphology of the cytoskeleton on polished and, to a lesser extent, on SLA surfaces, as evidenced by fluorescence microscopy. Although ROCK inhibition did not affect cell viability, it increased activation of Wnt signaling in uncommitted C2C12 mesenchymal cells on polished surfaces but not on SLA discs upon reporter assay. Consistently with this, real-time polymerase chain reaction analysis showed that MC3T3 cells on polished surfaces expressed higher mRNA levels for β-catenin and alkaline phosphatase, a known Wnt target gene, and for the osteoblastic differentiation marker osteocalcin after ROCK inhibition. Taken together, these data demonstrate that cytoskeletal organization mediates activation of Wnt canonical signaling in cells on titanium surfaces with different topographies.

1. Introduction

Osseointegration is a prerequisite for successful implant rehabilitation. Implant surfaces should therefore promote the formation of sound bone along the implant body [1]. Several studies have convincingly shown that rough surfaces are more effective in stimulating osseointegration than smoother ones [2,3]. In vitro research supports this finding, as it is well known that rough surfaces promote cell commitment to the osteoblastic lineage and support greater expression of phenotype-specific markers [4–8]. It has been recently demonstrated that rough topography enhances the activation of Wnt/β-catenin signaling in mesenchymal cells [9–11]. The Wnt/β-catenin-mediated pathway, also known as the canonical pathway, is one of the possible alternative signaling cascades that can be activated by binding of Wnt growth factors to specific membrane receptor complexes. While non-canonical pathways do not require β-catenin, the canonical pathway is mediated by nuclear translocation of this protein, which binds to Lymphoid enhancer factor/Transcription factor (TFC-Lef) and acts as a co-transcription factor. Although the canonical pathway plays a fundamental role in embryogenesis, it has also been shown to be necessary for osteoblast differentiation, and genetic ablation of β-catenin leads to a lack of osteoblasts in mice [12]. Moreover, Wnt canonical signaling controls the expression of important osteoblastic genes such as alkaline phosphatase and osteoprotegerin, which inhibits osteoclastogenesis by antagonizing RANKL [13], and thus controls bone homeostasis. The mechanisms underlying the topographical control of such an important and far-reaching pathway, however, are still poorly known. A recent publication by Kilian et al. [14] linked cell shape and fate through the activation of Wnt-related pathways by cytoskeletal components by using stereolithographic patterned surfaces. Cells growing on implant surfaces display different morphologies because they must adapt to the topographic differences of the substrate, and their cytoskeleton conforms to the surface pattern to maintain an adequate adhesion [15]. Some Wnt factors can activate non-canonical Wnt pathways that are not dependent on β-catenin and can also control the organization of the cytoskeleton by regulating the activity of Rho-associated protein kinase (ROCK) through the planar cell polarity pathway [16]. This pathway, in turn, has been shown to be able to affect Wnt canonical, β-catenin-dependent signaling. It is thus possible to envisage a situation where the surface topography of

* Corresponding author at: Sez. Odontostomatologia, University of Parma, Via Gramsci 14, 43100 Parma, Italy. Tel.: +39 0521 986722; fax: +39 0521 292955.
E-mail address: carlo.galli@unipr.it (C. Galli).

1742-7061/$ - see front matter © 2012 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2012.04.043