Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Cell Research 312 (2006) 457 – 467

Experimental Cell Research

www.elsevier.com/locate/yexcr

Research Article

E-cadherin modulates Wnt-dependent transcription in colorectal cancer cells but does not alter Wnt-independent gene expression in fibroblasts

Felix Kuphal, Jürgen Behrens*

Department of Experimental Medicine II, Nikolaus-Fiebiger-Center for Molecular Medicine, University of Erlangen, Glueckstr. 6, 91054 Erlangen, Germany

Received 29 July 2005, revised version received 29 September 2005, accepted 8 November 2005

Abstract

E-cadherin mediates homophilic adhesion of epithelial cells and is a major determinant of epithelial differentiation during embryonic development and tumor progression. At cell junctions, E-cadherin associates with β-catenin, which also functions as a transcriptional co-activator of the canonical Wnt signaling pathway by interacting with TCF transcription factors. Here, we have analyzed whether E-cadherin plays a role in the control of gene expression in Wnt-dependent and -independent cellular systems. In DLD-1 colorectal cancer cells, which show constitutive activation of Wnt signaling and exhibit E-cadherin-based cell contacts, the siRNA-mediated knock-down of E-cadherin led to the disturbance of cell junctions, translocation of β-catenin to the nucleus and an enhancement of β-catenin/TCF-dependent reporter activity. In L929 fibroblasts, which are deficient in Wnt signaling and E-cadherin-mediated cell adhesion, ectopic expression of E-cadherin induced the stabilization of β-catenin at the cell junctions and caused marked alterations in cellular morphology and phenotype. However, E-cadherin did not significantly change the transcriptional program of these cells as revealed by DNA microarray analysis. Our data indicate that E-cadherin may modulate Wnt-dependent gene expression by regulating the availability of β-catenin but has a surprisingly small impact on gene expression in the absence of Wnt signaling.

Keywords: E-cadherin; Wnt signaling; Gene expression; siRNA; Knock-down; DNA microarray

Introduction

E-cadherin is a well-characterized cell–cell adhesion molecule which connects neighboring epithelial cells in a specialized structure termed the adherens junction. Classical cadherins are transmembrane glycoproteins that interact homophilically with cadherin molecules on opposing cell surfaces in a calcium-dependent manner. In order to provide physical rigidity to an epithelial tissue, interacting cadherin molecules are linked to the actin cytoskeleton via proteins of the catenin family [1]. The cytoplasmic portion of cadherins harbors binding sites for β-catenin or γ-catenin/plakoglobin and p120 catenin. β-Catenin in turn binds to α-catenin, which mediates the anchorage of the E-cadherin/β-catenin complex to the underlying cytoskeleton, either by binding directly to F-actin or indirectly via interaction with several F-actin binding proteins [2].

The functional significance of E-cadherin expression during embryonic development is highlighted by the fact that E-cadherin knock-out mice die very early after the morula stage due to a decompaction of the preimplantation embryo [3,4]. In the adult, E-cadherin is an important determinant of the differentiated phenotype of epithelial cells. Mice with a conditional knock-out of E-cadherin display disruption of adherens junctions and severely disturbed differentiation of epidermal keratinocytes and hair follicles [5,6] or show impaired terminal differentiation of alveolar epithelial cells in the mammary gland [7]. Furthermore, a deregulation of the cadherin-based cell adhesion system is frequently observed in highly aggressive and invasive carcinomas [8]. Reintroduction of E-cadherin into invasive cancer cells restores cell–cell adhesion and

* Corresponding author. Fax: +49 9131 8529111.
E-mail address: jbehrens@molmed.uni-erlangen.de (J. Behrens).

0014-4827/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2005.11.007

causes a reduction of invasion, which established E-cadherin as a suppressor of invasion [9,10]. In addition, a causal role for the loss of E-cadherin in the progression from adenoma to carcinoma has been demonstrated in vivo [11].

Formation and maintenance of cadherin-mediated cell–cell adhesion is a highly dynamic process, and a number of physiological or pathological conditions alter the stability of the cadherin/catenin complex. Prominent examples are epithelial-to-mesenchymal transitions during gastrulation, or the process of wound healing, in which cells have to loosen their intimate cell–cell contacts and acquire a more mobile phenotype [12]. The downregulation of E-cadherin can occur as a result of the action of transcriptional repressors, e.g., proteins of the Snail/Slug family, which bind to E-box elements within the promoter region of E-cadherin [13,14]. Also, tyrosine phosphorylation of E-cadherin or β-catenin can lead to the destabilization and internalization of adherens junction components [15,16]. In malignant tumors, E-cadherin is frequently downregulated by the aforementioned transcriptional repressors, hypermethylation of the promoter region or mutations in the E-cadherin gene [12,17,18].

Given its potent functional role in epithelial differentiation, the question arises whether E-cadherin exerts its effects in part by altering gene expression. In particular, E-cadherin might be involved in regulating its binding partner β-catenin, which holds a second function as a transcriptional co-activator of the canonical Wnt signaling pathway (for a review, see [19]). When Wnt signaling is inactive, cytosolic β-catenin is constantly degraded by a multiprotein complex. Activation of the pathway results in stabilization of β-catenin in the cytoplasm and its translocation to the nucleus, where it associates with T cell factor (TCF) proteins [20,21] and drives the transcription of Wnt target genes [22]. The action of the Wnt signaling pathway is fundamental during embryonic development and for maintaining the stem cell compartment in the adult, for instance in the crypts of the intestine [23]. Deregulation of the pathway is causally involved in the development of several types of cancer, the most prominent example of which is colorectal cancer [23]. Aberrant Wnt signaling arises in the majority of colorectal tumors from truncating mutations of the adenomatous polyposis coli (APC) tumor suppressor protein, which lead to the stabilization of cytosolic β-catenin, or in a smaller fraction of tumors from stabilizing mutations in the β-catenin gene itself [24]. In several experimental systems, forced expression of E-cadherin or the cadherin cytoplasmic tail is able to sequester β-catenin away from the nucleus and thereby attenuates β-catenin-dependent transcription [25–27]. Vice versa, E-cadherin knock-out mouse embryonal stem cells showed higher nuclear localization of β-catenin and higher activity of a β-catenin/TCF reporter system (TOPflash) compared to wild-type cells, which is an indication for increased Wnt signaling if E-cadherin expression is lost [27]. These results indicate that changes in the level of E-cadherin can modulate the activity of the Wnt signaling pathway by altering the amounts of free β-catenin. In contrast to this, in several well-differentiated (E-cadherin positive) and dedifferentiated (E-cadherin negative) breast cancer cell lines, E-cadherin deficiency does not correlate with increased nuclear signaling activity of β-catenin [28]. Thus, it is still not completely clear whether the loss of E-cadherin can directly activate β-catenin/TCF signaling [29].

In this study, we addressed the question whether altering the levels of E-cadherin can lead to changes in gene transcription, either by influencing Wnt signaling or by changing global gene expression. We found that loss of E-cadherin by siRNA-mediated knock-down augments Wnt-dependent transcription in DLD-1 colorectal cancer cells in which the Wnt pathway is activated but has no effect in HaCaT keratinocytes that do not display Wnt signaling activity. Furthermore, forced expression of E-cadherin in L929 fibroblasts, which are devoid of Wnt-dependent transcription, strongly influences cell morphology and behavior but does not alter gene expression.

Materials and methods

Plasmids and cloning

Cloning of the E-cadherin cDNA into the doxycycline-regulatable expression vector pUHC10-3 [30] was performed as follows. A 650-bp fragment comprising the 5′-part of the mouse E-cadherin cDNA was PCR-amplified from the full-size E-cadherin expression vector pBATEM2 [31] using the oligonucleotides 5′-AAAATCTAGAGCCGCCATGGGAGCCCGGTG-3′ and 5′-ATGATGAAACGCCAACGGG-3′, thereby adding an *Xba*I site upstream of the translation start site. The PCR fragment was digested with *Xba*I and *Eco*RI (internal restriction site) and cloned into pBSK II as cloning vector (BD Biosciences, Heidelberg, Germany). To obtain the 3′ part of the E-cadherin cDNA, pBATEM2 was restricted with *Eco*RI and *Bam*HI and the resulting fragment ligated in frame to the 5′-fragment in pBSK II. Subsequently, the full-size E-cadherin cDNA was released by *Xba*I restriction and transferred into *Xba*I restricted pUHD10-3, generating pUHD E-cad. The doxycycline-inducible transactivator (rtTA2^{S}-M2) was expressed from pWHE146 which carries the neomycin resistance gene on the same transcript linked by an internal ribosome entry site [32]. pUHC13-3 carries a doxycycline-dependent luciferase gene and pUHD16-1 a constitutively expressed β-galactosidase gene [30]. pTK Hyg was used for selection of hygromycin-resistant clones (BD Biosciences). pTOPflash or pFOPflash contains three optimal TCF binding sites (TOP) or mutated sites (FOP), respectively, upstream of the luciferase gene which is under the control of the minimal c-Fos promoter [33]. pcDNA-β-catenin S33A was used to express degradation-resistant β-catenin S33A [34]. The target sequences of the siRNA oligonucleotides are siE-cad #1: 5′-GAUUGCACCGGUCGACAAAdTdT-3′ (Dharma-