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Protocadherin 12 (VE-cadherin 2) is expressed in endothelial, trophoblast, and mesangial cells

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Abstract

Protocadherin 12 protein (PCDH12, VE-cadherin 2) is a cell adhesion molecule that has been isolated from endothelial cells. Here, we have used Northern and Western blots, immunohistology, and flow cytometry to examine the distribution of PCDH12 in mouse tissues. It is an N-glycosylated protein of 150-kDa mass. In the endothelium, PCDH12 immunoreactivity was variable and dependent upon the vascular bed. In both the embryo and embryonic stem cell differentiation system, signals were localized in vasculogenic rather than angiogenic endothelium. In addition, the protein was strongly expressed in a subset of invasive cells of the placenta, which were identified as glycogen-rich trophoblasts. In adult mice, strong PCDH12 signals were observed in mesangial cells of kidney glomeruli whereas expression was not detected in other types of perivascular cells. As opposed to most protocadherins, PCDH12 is not expressed in early embryonic (day 12.5) and adult brains. As a first approach to obtain insight into PCDH12 function, we produced transgenic mice deficient in PCDH12, which were viable and fertile. They did not display any obvious histomorphological defects. We conclude that PCDH12 has a unique expression pattern and that its deficiency does not lead to conspicuous abnormalities. Moreover, PCDH12 is the first specific marker for both glycogen-rich trophoblasts and mesangial cells.

Keywords: Cadherin; Endothelium; Mouse; Placenta; Trophoblast cells; Glomeruli; Mesangial cells; Knockout

Introduction

Cadherins constitute a superfamily of transmembrane glycoproteins mediating calcium-dependent cell–cell adhesion [1]. These molecules are characterized by a variable number of cadherin ectodomains located in the extracellular part, a single transmembrane segment, and a cytoplasmic domain. The cadherin superfamily includes several groups: the classical cadherins, such as E-, N-, or VE-cadherin, the protocadherins, the desmocollins, the desmogleins, and other related proteins including Fat, T-cadherin, and Ret [2]. Classical cadherins have been extensively studied. These molecules are involved in cell cohesion, tissue morphogenesis, and cell behavior [3–6]. The cytoplasmic domain of classical cadherins interacts with catenins, allowing association with the cytoskeleton and cadherin–cytoskeleton association is required for strong cell–cell interaction.

Although recently discovered, protocadherins constitute the largest group of the superfamily [7,8]. They were first identified a decade ago by a PCR strategy using primers amplifying the cadherin ectodomain [9]. Full-length cDNA sequencing revealed that their cytoplasmic domains had no significant homology between each other and with those of classical cadherins. Hence, protocadherins do not possess

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catenin-binding sequences, and for those that have been tested, are not or only weakly associated with the cytoskeleton [8]. Recently, 52 novel protocadherins have been identified [10]. They are organized in three large clusters, α, β, and γ, on mouse chromosome 18. The vast majority of protocadherins, including those from the three clusters, are mostly or exclusively expressed in the central nervous system [9,11,12]. Their specific distribution in distinct neuronal populations may reflect their function in the establishment of neuronal circuits [8]. In contrast, protocadherin LKC, 12, 18, and μ were not detected in the central nervous system, but in various visceral organs [13–18].

In general, protocadherins display weak homotypic adhesion properties [9]. It is thus likely that these molecules are not involved in tissue cohesion. Additionally, heterophilic interactions have been suggested for some of them, for example with matrix proteins, suggesting functions independent of cell–cell connection [7,17,19,20]. Little is known about the in vivo biological activities of protocadherins. The first cues on protocadherin properties suggest diverse roles in tissue organization, cell survival, and proliferation [7,8].

In this paper, we focused on vascular endothelial-cadherin 2, also called PCDH12, in the systematic protocadherin nomenclature [14,15]. This molecule was first identified by RT-PCR in endothelioma cells using degenerated primers amplifying the cadherin ectodomain [14]. By Northern blot, PCDH12 was specifically detected in endothelioma cell lines and in highly vascularized organs. In transfected Chinese hamster ovary cells, PCDH12 was localized at intercellular junctions where it promoted calcium-dependent homophilic adhesion. PCDH12 did not co-immunoprecipitate with catenins and was only weakly associated with the cytoskeleton. Furthermore, junctional localization of PCDH12 did not modify the paracellular permeability of a confluent monolayer, suggesting that this molecule is not involved in the control of endothelial permeability. Moreover, in contrast to VE-cadherin, PCDH12 did not modify cell migration and density-dependent cell growth.

In this paper, we showed that PCDH12 expression in the endothelium was variable and dependent upon the vascularization process or the vascular bed. Surprisingly, we noticed high expression levels in glycogen-rich trophoblasts and glomerular mesangial cells. Additionally, we produced PCDH12-deficient mice, which were viable, fertile, and did not harbor obvious morphological defects.

Materials and methods

RNA extraction and analysis

RNAs were extracted from cells or embryoid bodies with Tri Reagent from Molecular Research Center. For RT-PCR analysis, RNA was reverse transcribed with Superscript II from Invitrogen. PCR amplification was performed with PCDH12-specific oligonucleotides: 5′-GGCCCCAGAAA-CACATTCAGA-3′ and 5′-TCTGTGGGGCCTCCT-CCTTGT-3′. CD31, VE-cadherin, and HPRT primers were previously described [21,22]. Amplification products were visualized either by ethidium bromide staining (Fig. 6B) or after transfer onto nylon membrane (Oncor) and hybridization with internal oligonucleotides (Fig. 7B). For Northern blot analysis, a Mouse Adult Tissue Blot containing 20 μg of RNA of various tissues was purchased from Seegene and successively hybridized with two 32P-labeled probes: a PCR fragment corresponding to nucleotides 2589–3000 of mouse PCDH12 cDNA [14] and a 1.7-kb *Eco*RI–*Sph*I fragment from mouse VE-cadherin cDNA [23].

Gene targeting, ES cell manipulation, and mouse breeding

A PCDH12 genomic clone was isolated from a λ phage 129/Sv mouse genomic library (Stratagene). For the targeting vector, a 2-kb *Sac*I–*Xmn*I fragment located upstream of the initiation ATG was inserted upstream of a promoterless *LacZ* gene followed by a phosphoglycerate kinase-neomycine resistance gene. A 2.9-kb *Xmn*I–*Nco*I genomic fragment, located downstream of the initiation codon, was inserted in the targeting vector, downstream of the neomycine resistance unit. AT1 ES cells [24] were electroporated with the linearized vector using established procedures [22]. Clones were selected with G418 (250 μg/ml) and ganciclovir (2 μM). Resistant clones were screened by Southern blot analysis. DNAs were digested with *Hin*dIII and hybridized with a probe external to the recombination fragment (probe A, Fig. 2). Homologous recombination events were confirmed by digestion with *Eco*RI and hybridization with probe B (Fig. 2). Five clones out of 336 screened were targeted at the *PCDH12* locus. Mouse chimeras derived from two clones with correct recombination event were crossed with CD1 mice (Charles River). Heterozygous mice were intercrossed to give homozygous mice. Animals from independent ES clones were not mated together.

Wild-type animals and tissue preparation

All protocols in this study were conducted in strict accordance with the “Ministère de l’Education Nationale, de la Recherche et de la Technologie” Guidelines for the Care and Use of Laboratory Animals. Experiments were performed with tissues from CD1 mice (Charles River). The animals were allowed to acclimatize for a period of 1 week before experimental manipulation. For embryo and placenta production, mice were mated and the day on which a vaginal plug was found was designated 0.5. Pregnant females were killed by cervical dislocation and conceptuses were dissected in PBS. Isolation of glomeruli from kidneys