Available online at www.sciencedirect.com
ScienceDirect
Cellular Signalling 20 (2008) 1061–1072
CELLULAR SIGNALLING
www.elsevier.com/locate/cellsig

Cadherin-11 interacts with the FGF receptor and induces neurite outgrowth through associated downstream signalling

Cécile Boscher [a,b,c], René-Marc Mège [a,b,c,*]

[a] INSERM, U839, Paris, F-75005 France
[b] Université Pierre et Marie Curie-Paris 6, Paris, F-75005 France
[c] Institut du Fer à Moulin, Paris, F-75005 France

Received 21 September 2007; received in revised form 18 December 2007; accepted 16 January 2008
Available online 19 January 2008

Abstract

Cadherin-11 is a cell–cell adhesion molecule whose expression is often correlated with cellular migratory phenomena. We recently demonstrated that cadherin-11 activation by immobilized cad11–Fc (cadherin-11 ectodomain fused to Fc fragment) promotes axonal extension of spinal cord explants. Here, we show that this induced neurite outgrowth is dependent on the FGF receptor (FGFR) activity. Downstream, DAG lipase/CAM kinase and PI3 kinase pathways are required, but not the MAP kinase signalling. We also demonstrate that a tagged form of FGFR1 co-immunoprecipitates with β-catenin containing cadherin-11 immunocomplexes. FGFR1 and β-catenin show colocalization and enhanced association during cadherin-11 engagement, suggesting that FGFR1 interaction with cadherin-11 adhesion complexes is reinforced during cell contact formation. *In vitro* pull-down experiments using recombinant ectodomains suggest that cadherin-11/FGFR interact directly through their extracellular domains. Altogether, we propose that cadherin-11 recruits the FGFR upon adhesive engagement at nascent contacts, triggering the activation of downstream pathways involved in growth cone progression.

Keywords: Cadherin-11; FGF receptor; N-cadherin; Neurite outgrowth; Adhesion; Migration

1. Introduction

Classical cadherins are transmembrane receptors that mediate calcium dependent cell–cell adhesion through homophilic interaction of their extracellular region composed of five ectodomains (EC1-5) [1–3]. Their intracellular juxta-membrane domain interacts with the catenin p120, and their C-terminal tail recruits catenins β or γ [4,5]. α-catenin can interact with β-catenin and actin, therefore provides a dynamic link between cadherins and the cytoskeleton [6]. In addition, cadherins recruit numerous extracellular and intracellular partners at the site of interaction between adjacent cells, likely forming a mechano-chemical signalling adhesion plaque [7]. In particular, cadherins associate with various receptor and non-receptor tyrosine kinases and phosphatases linking them to other signalling pathways [8,9].

Cadherins participate to embryonic development in correlation with their regulated spatio-temporal expression. Their spheres of action are cell segregation, migration, differentiation, proliferation and survival [10,11]. Combinatory expression of cadherins plays a crucial role during neuronal development in precursor cell aggregation and sorting [12]. For instance the cadherins code may define the partition of the neural tube, as in the telencephalon where differential expression of cadherins 6 and R delimits the subsequent cortico-striatal subdivisions [13]. Cadherins are expressed according to longitudinal and lateral segmental subdivisions of the neuroepithlium which correspond to future functional structures of the brain [14]. In addition

Abbreviations: Cad11–Fc or Ncad–Fc, cadherin-11 or N-cadherin extracellular domain fused to the human-Fc fragment of immunoglobulin; N-CAM, Neural Cell Adhesion Molecule; CAM kinase, Calmoduline kinase; DAG lipase, Diacyl glycerol lipase; ERK1/2, extracellular signal-regulated kinase 1 and 2; FGF, Fibroblast growth factor; FGFR, FGF receptor; PI3 kinase, Phosphatidyl inositol 3 phosphate kinase; PLCγ, Phospholipase Cγ; PKA, Protein Kinase A; PO, Poly-ornithine.

* Corresponding author. Institut du Fer à Moulin, Paris, F-75005 France. Tel.: +33 1 45 87 61 36; fax: +33 1 45 87 61 32.
E-mail address: mege@fer-a-moulin.inserm.fr (R.-M. Mège).

0898-6568/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellsig.2008.01.008

axons from chicken embryo tectofugal projections were shown to be segregated in function of expressed cadherins, strongly suggesting that these cell adhesion molecules participate to axonal guidance in vivo [15].

The cadherin cytoplasmic domain is highly conserved, but divergences in the extracellular domain lead to divide classical cadherin family into two subgroups: type I (E, N, P and R) and type II (VE, 6–12, 15, 18 and 19) [16]. Both types of cadherins share common structural and functional features – they all induce adhesion between cells and interact with catenins – but their adhesive properties differ. Cells expressing cadherins 7 or 11 aggregate more slowly and weakly than E- or N-cadherin expressing cells [17]. Differences in adhesive strength were shown to be due to intrinsic properties of the extracellular domain. These observations correlate with differences found in the structure of the EC1 domain that confers the adhesive specificity [18]. For instance two tryptophan residues are present in positions 2 and 4 in type II EC1 domains, while only one is found in position 2 in type I [18]. Their mutation or blockade with antibodies highlighted their importance for adhesion [19]. Thus, the adhesive interfaces of type I and type II cadherins would be very different.

Cadherin-11, a type II cadherin, is considered as a mesenchymal adhesion receptor widely expressed in mesoderm derived cells [20]. In particular, cadherin-11 is highly expressed in osteoblastic cells and is required for their differentiation, from hence its original name of OB-cadherin [21,22]. As a consequence, cadherin-11 null transgenic mice display defects in bone structures [23]. Cadherin-11 is also expressed in the neural tube, the developing brain and spinal cord during nervous system development [24–27]. Cadherin-11 is strongly expressed in motoneurons during axonal elongation, in cell bodies and their extensions [28]. In the hippocampus, cadherin-11 might participate to the organization and stabilization of synaptic connections [29]. Electrophysiological analysis of cadherin-$11^{-/-}$ mice hippocampus revealed an increased long-term potentiation. Finally, increased expression of cadherin-11 has also been related to the migratory phenotype of some tumorigenic cells [30]. Altogether different studies converge to the hypothesis that cadherin-11 plays an important role in cell differentiation and migration during development and tumour invasion.

In contrast to cadherin-11, N-cadherin is a well-known type I cadherin widely expressed in neuronal cells and various other cells. N-cadherin is present during neurectoderm formation and throughout the nervous system development, playing different important roles [12,31]. Of note, N-cadherin is involved in neuronal circuit maturation probably through contribution in axonal extension. Indeed numerous neurons are known to respond to N-cadherin activation by extending neurite [32–34]. Among the mechanisms proposed to explain N-cadherin induced neurite outgrowth, the activation of intracellular biochemical signalling cascades has been explored, involving the FGF receptor (FGFR) activity and downstream PLCγ/DAG lipase/CAM kinase/Ca^{2+} pathway [35–37].

Therefore the present study aims at understanding the mechanisms associated to cadherin-11 induced neurite outgrowth. We show that cadherin-11 engagement promotes neurite outgrowth of E12.5 ventral spinal cord explants in a dose-dependent manner, although the effect is weaker than the one observed for N-cadherin. We further demonstrate for the first time that the FGFR and the associated pathways are required for cadherin-11-driven induction of neurite outgrowth. We also show that cadherin-11 and FGFR interact directly through their extracellular domains and this interaction is enhanced at nascent cell contacts. Altogether, these results allow proposing for the first time a putative signalling cascade involved in cadherin-11 induced neurite outgrowth. They provide new important information on the role of cadherins/receptor tyrosine kinases cross-talk in cell migration.

2. Experimental procedures

2.1. Cell culture and transfections

HEK-293 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10% fetal calf serum (FCS) at 37 °C in a 5% CO_2 atmosphere. For biochemical studies, cells were cultured at 10^4 to 10^5 cells/cm^2; after 24 h they were transfected with Fugen reagent (Roche). Different ratios of encoding vectors were tried; we used 4 μL of Fugen for 4 μg of vectors with a ratio 3:1 for FGFR1 (a gift from Dr. D. Ornitz; [38]) and cadherin-11 (a gift from Dr. S.W. Byers; [30]) or 3:1:1 for FGFR1, cadherin-11 and N-cadherin-GFP [39] or cadherin-6-HA (kindly provided by Dr. T. Inoue).

2.2. Purification and characterisation of the recombinant cadherin-Fc and FR1AP proteins

Ncad–Fc and cad11–Fc recombinant proteins were obtained as previously described [40,41]. Briefly EBNA-1/HEK-293 cells (Invitrogen) were electroporated with pREP/cad11–Fc or pCEP4/Ncad–Fc expression vectors, cells were cultivated for 24 h in DMEM medium containing 10% FCS, and switched into serum free AIMV synthetic medium (Invitrogen). This medium was recovered after two days and the secreted cad11–Fc and Ncad–Fc proteins were purified using protein G-coupled beads (Amersham). The chimera concentrations were evaluated against a Fc standard solution by Coomasie blue staining, and the activity was checked thanks to recombinant protein coated beads (Dynabeads) in aggregation assays. The cad6-Fc chimera was obtained from R&D Systems.

For production of FR1AP, EBNA-1/HEK-293 cells were transfected with Fugen reagent (Roche) and a vector encoding FGFR1 ectodomain fused to alkaline phosphatase (FR1AP, a gift from Dr. Ornitz; [42]). Cells were then maintained in DMEM with 10% FCS; conditioned medium was recovered after 48 h and tested by dot-blot for its content in recombinant protein.

2.3. Recombinant protein coating

For explants cultures, coverslips were prepared as previously described [40]. Briefly sterile glass coverslips were incubated