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The atypical cadherin Dachsous1 localizes to the base of the ciliary apparatus in airway epithelia

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ABSTRACT

Mucociliary clearance requires the distinct orientation and coordinated movement of airway cilia, which is established through planar cell polarity signaling (PCP). The atypical cadherin Dachsous1 (Dchs1) is a transmembrane protein that regulates PCP in *D. melanogaster*. However, little is known about Dchs1 expression and its potential role in PCP in mammalian adult tissues. Here, we show that Dchs1 is ubiquitously expressed in mouse embryos, but exhibits a highly restricted expression to lung tissues in the adult stage. Strikingly, human Dchs1 localized exclusively to the base of the ciliary apparatus in cultured human respiratory epithelial cells with differentiated motile 9 + 2 cilia. This localization could be functionally important as we observed aberrant *DCHS1* mRNA expression in human non-small cell lung cancer tissue. In sum, we establish Dchs1 as a component of the membrane domain surrounding the ciliary base. This suggests a specific role of Dchs1 in PCP-dependent organization of ciliary function and a possible role in lung disease.

1. Introduction

Planar cell polarity (PCP) describes the polarized organization of a multicellular structure within a plane, for example in epithelial tissues. A main characteristic of PCP is the asymmetric distribution of diverse PCP signaling proteins within a cell [1]. In the human lung, a PCP-dependent polarization of multiciliated respiratory epithelial cells has been observed recently [2–4]. One of the major PCP pathways, thoroughly characterized in *Drosophila,* involves the atypical giant cadherin Dachsous [1]. The human orthologue Dachsous1 (Dchs1) interacts transcellularly with Fat4 and both proteins are widely expressed in an overlapping manner during development [5]. Targeted deletion of either *Dchs1* or *Fat4* in mice leads to postnatal growth failure and early lethality. Mutant animals are characterized by smaller lung and kidney sizes, renal cysts, defects of vestibulo-cochlear ciliary orientation [5] and impaired neuronal migration [6,7]. In humans, mutations in *DCHS1* cause the Van Maldergem syndrome (VMS; MIM601390) [6], which is characterized by neuro-cranial defects. A subset of patients also suffer from respiratory phenotypes such as tracheomalacia. The precise molecular mechanism underlying the diverse pathologies seen in mice and humans is currently unknown.

Dchs1 mRNA distribution was previously analyzed in mice, showing generalized expression in embryonic tissues and a more restricted expression in the adult mostly to the lung and to a lesser extent to the brain and kidney [8]. The tissue-specific protein expression and subcellular localization of Dchs1 in adult mammalian tissue is currently unknown. However, this is important for gaining an entry point into the molecular function of Dchs1 in cells.

2. Materials and methods

2.1. DCHS1 full-length clone

Human full-length *DCHS1* cDNA (NM_003737) (3298 bp) was

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constructed in a multistep approach from three fragments using RT-PCR introducing cleavage sites (*Eco*RI and *Xho*I) and by subsequent ligation. Primers are listed in Table 1. Constructs bearing an additional FLAG-tag $_{3285}$ESGLEDYKDDDDKPPDDTELHI$_{3298}$ or lacking the cytoplasmic domain (Dchs1ΔC) were also generated. *DCHS1* full-length cDNA was subcloned in pCR-Blunt II-TOPO (No. K2800-40, Invitrogen/ThermoFisher) or pcDNA3.1 (+)I (No. V790-20, Invitrogen/ThermoFisher).

2.2. Dchs1-specific antisera

Following immunization of rabbits with two different peptide sequences from the cytoplasmic domain of human Dchs1 (NP_003728.1), $_{3081}$CEPPAPDTWYKGRK$_{3094}$ and $_{3232}$PASHRSPISHEGSLSC$_{3246}$, two antisera, α-Dchs1-C1 (α−DS-60) and α-Dchs1-C2 (α−DS-40), were generated and purified by ammonium sulfate precipitation. α-Dchs1-C2 was further purified by standard affinity chromatography according to Harlow and Lane.

2.3. Cell culture

NRK-52E cells (a rat renal proximal tubular cell line), Cos-7 cells (a fibroblast-like cell line derived from monkey kidney) and L cells (a murine fibroblast cell line lacking E-cadherin) were investigated. Cell culture was carried out in DMEM with 10% fetal calf serum and 1% Penicillin/Streptomycin. Transfection of cell lines was performed using Fugene®6 (Roche Diagnostics) or Lipofectamine® 2000 (Invitrogen/ThermoFisher) as described [9].

2.4. Immunoblotting

Wild type mice were euthanized in accordance with local government regulations and perfused with PBS. Harvested tissues were snap frozen in liquid nitrogen and protein lysates were prepared using standard 8 M urea buffer containing protease inhibitors. Cell lysates of cultured cells were generated using standard RIPA buffer containing protease inhibitors. Protein concentration was assessed as described by Bradford. For immunoblotting (IB) 20 μg of total protein were loaded per lane onto 5% gels (SDS-PAGE), processed and detected as described [9]. Protein concentrations and corresponding loading volumes employed for the IBs shown in Fig. 2 are listed in Table 2. Table 3 provides information on primary antibodies.

2.5. Immunostaining

Immunofluorescence (IF) [9] of Cos-7 cells and IF or immunohistochemistry (IHC) [10] of mouse tissue (paraformaldehyde-fixated, paraffin-embedded, 4 μm sections) were performed as described before. Human respiratory epithelial cells were obtained by transnasal brush biopsy and processed as specified [11]. Primary antibodies are listed in Table 3.

2.6. Quantitative reverse transcription polymerase chain reaction of human lung tumor samples

DCHS1 mRNA expression was investigated in a cDNA biobank from a tissue collective of patients with primary lung neoplasia and adjacent normal tissue (Centralized biomaterial bank of RWTH Aachen University, http://www.cbmb.rwth-aachen.de, ethical review board ref no. EK-206/09, informed consent obtained from all patients). Real-time PCR (ABI Prism 7300 sequence detector, Applied Biosystems), with primers listed in Table 4, was performed by applying the ΔΔCT method with reference to the housekeeping gene *PPIA (Cyclophilin A)*, which has been previously used to normalize mRNA expression in lung and lung carcinoma cells [12]. To ensure a selection of samples containing >80% of tumor cells, the used tissue had been verified for tumor cell content by hematoxylin-eosin-stained histologic sections at the time of generation of the cDNA biodatabase. Total RNA was prepared using TRIzol® reagent (ThermoFisher). cDNA was synthesized by a standard reverse transcription protocol employing 1 μg of total RNA and random primers.

2.7. Analysis of publicly available microarray data

A previously published data set [13] was obtained via the Gene Expression Omnibus (GEO) database, http://www.ncbi.nlm.nih.gov/geo/, profile accession ID 69684677, and analyzed for *DCHS1* transcriptional information. The sample cohort stemmed from 60 female non-smoking patients diagnosed with non-small cell lung cancer (NSCLC) (93% adenocarcinoma) and consisted of matched tumor and adjacent normal lung tissue. The array data for the *DCHS1*-specific probe set (No. 218892_at; Affymetrix GeneChip Human Genome 133 plus 2.0) were available following background-adjustment and a three-step normalization process consisting of Robust Multichip Analysis, median polish method and quantile normalization algorithm. Expression data were converted from log2 to linear representation and normalized to the normal lung sample group.

2.8. Statistical analysis

Prism 6.0b software (Graph Pad, La Jolla, CA, USA) was applied.

3. Results

3.1. Dchs1 localizes to cell–cell contacts

To investigate the subcellular localization of the 360 kDa-sized human Dchs1, we generated a full-length cDNA with or without a C-terminal FLAG-tag for transient transfection into cells (Fig. 1A). Dchs1 comprises an N-terminal cadherin-like domain, a membrane spanning middle domain and a C-terminal domain, which is predicted to be largely unstructured. The cadherin-like domain is

Table 1
Primers for cloning of DCHS1.

Primer	Sequence	Method
AhDHS fw	CATTCTAGACACCATGCAGAAGGAGCTGGGC	Conventional PCR for subsequent cloning
AhDHS rev	ACGAATTCGCCAGGCAGCGCAACCTCA	
BhDHS fw	CGAATTCGTAGTGCGGGTGACTGCT	
BhDHS rev	CCTCGAGAGAGAGATGGGCACTCCCA	
ChDHS fw	TCTCGAGGTGCCTGAGGGCCAGGA	
ChDHS rev	CTCGAGCTAGATGTGCAGCTCCGTGT	
DhscytoHa rev	CGTCGTATGGGTAGGGTGCTGCCTGTGACAT	
DhscytoHa fwd	TCCCTGACTATGCGCTGGAGCCACCTGATGAC	