Disabled-2 Is an Epithelial Surface Positioning Gene*

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The formation of the primitive endoderm layer on the surface of the inner cell mass is one of the earliest epithelial morphogenesis in mammalian embryos. In mouse embryos deficient of Disabled-2 (Dab2), the primitive endoderm cells lose the ability to position on the surface, resulting in defective morphogenesis. Embryonic stem cells lacking Dab2 are also unable to position on the surface of cell aggregates and fail to form a primitive endoderm outer layer in the embryoid bodies. The cellular function of Dab2, a cargo-selective adaptor, in mediating endocytic trafficking of clathrin-coated vesicles is well established. We show here that Dab2 mediates directional trafficking and polarized distribution of cell surface proteins such as megalin and E-cadherin and propose that loss of polarity is the underlying mechanism for the loss of epithelial cell surface positioning in Dab2-deficient embryos and embryoid bodies. Thus, the findings indicate that Dab2 is a surface positioning gene and suggest a novel mechanism of epithelial cell surface targeting.

Epithelial cells are positioned on the outer surface of organs or the inner surface of glandular structures and are involved in diverse physiological functions. Simple epithelia consist of monolayered cells that form a sheet through cell-cell adherens junctions and attach to a basement membrane that lies underneath (1). Epithelial cells are polarized with the apical surface exposed, or free from cell-cell contact and the basal side lying in contact with a basement membrane or stromal cells. The cell-free apical space and basal contact are unique hallmarks of an epithelium and are likely positioning cues for the surface localization of the epithelial cells (2), although the molecular details and genes critical for epithelial cell surface positioning are yet uncertain and undefined. The concept that cues are required for epithelial surface positioning is also reinforced by observations of the disorganized growth of carcinomas. Carcinoma cells can be viewed as epithelial cells that have lost their ability to perceive surface positioning cues, and the neoplastic cells no longer obey the constraint imposed by tissue organization (3).

The early embryos of vertebrates, especially mammalian species, have great plasticity, and significant cell dispersal, movement, and migration occur before their final positioning and acquisition of cell fates (4). Shown in the classical cell sorting experiments by Townes and Holtfreter (5), early embryonic amphibian cells of epidermis, endoderm, mesoderm, and neural plate, if dispersed, are able to segregate spontaneously upon aggregation, indicating cell positioning is an autonomous property.

The primitive endoderm of mammalian early embryos is the first typical epithelial cell type derived that is capable of producing a basement membrane (6). Recent understanding is that the primitive endoderm cells arise from the differentiation of the pluripotent cells of the inner cell mass and migrate out to the surface to form the primitive endoderm layer (7, 8). The mechanism for how the primitive endoderm cells actually perceive this cell surface positioning cue is not yet understood.

Disabled-1 is a gene recognized to have a role in “positioning” of brain cells in development (9, 10). The other mammalian ortholog, Disabled-2 (Dab2),3 is highly expressed in many epithelial cell types but is lost in cancer cells (11). Dab2 interacts with several components of the endocytic apparatus including the α-adaptin subunit of the clathrin adaptor protein AP-2, clathrin, the cytoplasmic domain of lipoprotein receptor-related protein family members, and the endocytic motor protein myosin VI (12–14). It has been proposed that Dab2 functions as a cargo-selective endocytic adaptor linking clathrin-coated endocytic vesicles to the actin-based myosin VI, traveling unidirectionally along cellular microfilaments. Although the cellular function of the endocytic adaptor Dab2 has been well studied in vitro, no significant biological phenotypes related to its role in endocytosis were identified in cultured cells (12–14). The important biological role of the dab2 gene was revealed by the early embryonic lethality of dab2(−/−) mouse embryos (15, 16). From the analysis of the dab2-deficient phenotypes in mice, embryoid bodies, and cultured cells, we realized Dab2 may be a critical determinant for the formation and maintenance of epithelial polarity, and the ability of epithelial cells to generate polarity may be the mechanism for their surface positioning.

EXPERIMENTAL PROCEDURES

Dab2-deficient Mice—Two lines of dab2 knock-out mice were established previously (16), and the colonies were main-

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3 The abbreviations used are: Dab2, disabled-2; ES, embryonic stem; PAS, periodic acid-Schiff; H&E, hematoxylin and eosin; DAPI, 4',6-diamidino-2-phenylindole; siRNA, small interfering RNA.
tained in the C57BL6 background by inbreeding in the animal facility of Fox Chase Cancer Center. The two lines have given identical phenotypes and will not be distinguished here.

Genotyping—At earlier stages (earlier than E6.5), dab2 genotyping was performed by examining the morphology and/or Dab2 immunostaining of the embryos. dab2/(+)/+ or (+/−) and (−/−) genotypes were distinguished by immunostaining of Dab2 in neighboring sections. Dab2-positive littermates in the same uterus were used as positive controls.

For adult mice, DNA was extracted from tails to amplify simultaneously in PCR reactions both the wild-type (850 bp) and targeted (1100 bp) allele. The PCR reaction was set up by addition to the template DNA a common master mix of PCR reagents including three primers: a sense primer P1 (5′-CACATATGAGAGAGACGGGC-3′) in the dab2 gene, an antisense primer for the wild-type dab2 gene P2 (3′-CGGGGCAGCCT-GTTGGCCT-5′), and another antisense primer P3 (3′-CCGTGCACTTGGCAGTTTGAG-5′) specific for the recombinant mutant allele.

Histology and Histochemistry—Uteri containing embryos at different developmental stages were collected, formalin-fixed, and paraffin-embedded. Sections (5 μm) were cut and adhered to positively charged slides (Fisher). Standard H&E staining was applied. Immunohistochemistry using a primary antibody to Dab2 (BD Transduction Laboratories, Lexington, KY) diluted 1:400 was performed as described previously (16). For GATA4 staining, the sections were subjected to antigen retrieval by steaming for 20 min in citrate buffer (10 mM). Rabbit polyclonal anti-GATA4 antibodies (Santa Cruz Biotechnology, Inc.) were used at 1:800 dilution. For periodic acid-Schiff (PAS) staining, the slides were first subjected to deparaffinization and hydration, submerged in PAS solution (Sigma) for 20 min and then counterstained with hematoxylin. For lectin histochemistry, Dolichos biflorus agglutinin from Sigma was used to stain primitive endoderm cells.

Immunofluorescence Microscopy—Generally, cells were cultured on glass coverslips or in slide chambers and analyzed using CCD or laser-scanning confocal microscopy. Antibodies, both mouse monoclonals and rabbit polyclonals, for Dab2, megalin laminin (Sigma), and rat E-cadherin (Zymed Laboratories Inc.) were used. For immunofluorescence microscopy, anti-megalin and Dab2 antibodies were used at 1:200 dilution in phosphate-buffered saline containing 1% bovine serum albumin and 0.1% Tween 20 and incubated at 37 °C for 2 h. The cellular localization of the antigens was revealed by fluorescein isothiocyanate- or Texas Red-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:200 dilution. The secondary antibodies used were donkey anti-mouse IgG conjugated with Texas Red and donkey anti-rabbit IgG conjugated with fluorescein isothiocyanate. Rabbit anti-megalin antibodies were used with mouse anti-Dab2 antibodies for double labeling. Nuclei were labeled by DAPI staining. A Nikon Eclipse E 800 epifluorescence microscope with 60× oil immersion objective linked to a Roper Quantix CCD (charged-coupled device) camera was used to examine the slides.

siRNA Down-regulation of Dab2 in Cell Culture—We have established a siRNA approach to down-regulate Dab2 expression in both human and mouse cells using a sequence of 21-bp oligonucleotides 367 bp downstream from the ATG site (Dab2siRNA367) (see below) that effectively down-regulates the Dab2 protein level when transfected into cells. A Dab2-siRNA expression vector (GeneSuppressor, Imgenex) was constructed based on this sequence. The transcript is under the control of the U6 promoter, and the vector contains a neomycin resistance gene as a selectable marker.

RESULTS

Dab2 Is Required for Endoderm Organization in Early Mouse Embryos—The early embryonic lethality of dab2(−/−) mouse embryos (15, 16) is not well explained by the lack of a prominent cellular phenotype related to its role in endocytosis, which is revealed by studies in cultured cells (12–14). We therefore further characterized the dab2(−/−) embryos to determine the link between the known cellular function of Dab2 and the mechanism leading to the phenotype presented in knock-out mice. Dab2 is expressed in the peripheral endoderm layer of E4.5–5.5 embryos. In all E4.0–4.5 embryos from the crossing of dab2/(+)/− parents, primitive endoderm cells indicated by positive D. biflorus agglutinin staining were located throughout the inner cell mass, which is relatively small at this stage (Fig. 1A). This is consistent with the reported “salt and pepper” pattern of GATA6 staining of the embryos at this stage (8). Thus, the phenotype of Dab2 loss is not apparent at E4.0–4.5 since Dab2-positive and -negative embryos are indistinguishable by primitive endoderm markers. By E5.5, the effect of Dab2 loss is clear: the dab2(−/−) embryos, identified by the absence of Dab2 staining, appear disorganized and lack a recognizable endoderm layer (Fig. 1B). In wild-type embryos, the Dab2-expressing primitive endoderm cells form a polarized epithelium and further differentiate into visceral (Fig. 1B, arrow) and parietal (Fig. 1B, double arrows) endoderm cells at this stage. The visceral endoderm cells are organized into an epithelium at the periphery of the egg cylinder (Fig. 1B, arrow). These cells are polarized with a microvillous apical surface facing the yolk sac cavity (17, 18). The parietal endoderm cells migrate to cover the inner surface of the trophoblast layer and are responsible for producing the thick, multilayered basement membrane (Reichert’s membrane) found between parietal endoderm and trophectoderm cells (17, 18). GATA4 is a specific endoderm marker in early embryos (19), and its expression is restricted to the visceral endoderm cells forming an epithelial layer covering the surface of the primitive egg cylinder (Fig. 1C, arrow) and the parietal endoderm cells outlining the embryo (Fig. 1C, double arrows), as shown in an example of an E5.5 embryo. In the dab2(−/−) embryos, however, GATA4-positive cells (nuclei stained dark brown, arrowhead) are present but are disorganized and distributed throughout the mass of the embryonic cells (Fig. 1C, arrowhead).

One property of parietal endoderm cells is the ability to actively express and produce laminin and collagen IV for the
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FIGURE 1. Loss of surface positioning of endoderm cells in E5.5 dab2(−/−) embryos. A, fixed and paraffin embedded E4.5 embryos in utero from timed matings of dab2(+/-) mice were sectioned and stained with D. biflorus agglutinin to identify primitive endoderm cells. Two representative embryos are shown. B, fixed and paraffin embedded E5.5 embryos in utero from timed matings of dab2(+/-) mice were sectioned and examined for morphology following H&E staining. The dab2(−/−) embryos were identified by the lack of staining. Representative wild-type and dab2(−/−) embryos are shown. C, visceral endoderm cells were identified by GATA-4 immunostaining. Representative GATA-4 immunostainings of E5.5 embryos from timed-matings of dab2(−/−) mice are shown. D, a parietal endoderm defect in Dab2 deficient mouse embryos was revealed by laminin staining. A representative laminin staining of E5.5 mouse embryos is shown. Laminin staining mainly associates with the parietal endoderm cells in the outer layer (arrow) of the wild-type embryos, but in the dab2(−/−) embryo shown, the laminin-stained, presumptive parietal endoderm cells (arrowhead) are disorganized or remain in the interior of the embryos.

Loss of Apical Localization of Glycoproteins in Visceral Endoderm Cells of Dab2-deficient Embryos—The glycoprotein/transmembrane receptor megalin, which binds Dab2 (22), is located on the apical surface of epithelial cells and is a marker for apical-basal polarity (23). In an immunostaining of an E5.5 embryo, megalin is detected only at the apical edge of visceral endoderm cells (Fig. 2A and B, arrow). In a representative dab2(−/−) E5.5 embryo, megalin-positive, presumptive visceral endoderm cells are found in the interior of the embryonic cell mass. Close inspection indicates that megalin is no longer restricted to one side of the cell surface but is distributed diffusely along the entire cell surface as well as in the cytoplasm (Fig. 2A, arrowhead). The distribution of megalin and Dab2 in E5.5 embryos was further characterized by immunofluorescence microscopy (Fig. 2B). In the Dab2-positive E5.5 embryo, megalin (green) is restricted to the apical membrane of the surface endoderm epithelial cells, and Dab2 (red) is distributed throughout the cytoplasm of the visceral endoderm cells (Fig. 2B). The polarized distribution of megalin is lost in Dab2 knock-out embryos (Fig. 2B). Thus, the Dab2 protein is required to establish the polarity of megalin distribution in the visceral endoderm cells.

The PAS reaction stains carbohydrates and carbohydrate-rich macromolecules, including megalin, and stains the apical surface of the visceral endodermal cells (Fig. 2C, arrow). As shown in a higher magnification of the indicated areas (Fig. 2D), the PAS staining of the visceral endoderm cells in the normal embryos is restricted to the glycoproteins of the brush border, the outermost layer of the cells on the apical side of the membranes that enclose the vacuoles. In contrast, although PAS-stained cells are present in E5.5 embryos identified as dab2(−/−) by the absence of Dab2 staining (Fig. 2, C and D), no endoderm layer with a distinct brush border can be observed. The PAS staining is more diffuse or cytoplasmic (Fig. 2C, arrow, also shown in a higher magnification in Fig. 2D), and the staining in some cells (arrow) appears to be in cellular structures located more interior than the vacuoles of the cells. Thus, the loss of Dab2 influences apical localization not only of megalin but also of the majority of the apical glycoproteins.

Dab2 Is Required for the Surface Positioning of Endoderm Epithelial Cells in Embryoid Bodies—We used embryoid bodies from the aggregation of embryonic stem (ES) cells as models (24, 25) to investigate the role of Dab2 in the formation of the endoderm structure. The siRNA approach was used to down-regulate Dab2 expression in cells in cultures. A siRNA targeting sequence for strong Dab2 suppression was found following testing of three mouse dab2 cDNA sequences in transient transfection experiments, and this optimal sequence (Dab2siRNA367) was inserted into a neo-selectable siRNA expression vector. Dab2 expression in pooled G418-selected clones was greatly suppressed compared with vector-transfected controls (Fig. 3A). The Dab2-suppressed ES cells were no longer capable of forming embryoid bodies with a primitive endoderm outer layer as seen in wildtype ES cells following aggregation (Fig. 3B, arrow). GATA4 and Dab2 staining indi-
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Dab2 Is Required for Apical Localization of Megalin in Cultured Cells—An obvious question is whether the observed loss of endoderm cell polarity in dab2 knock-out embryos is the cause or the consequence of the disorganization of the embryos and embryoid bodies. Thus, we examined the phenotype of Dab2 loss in cultured cells. When treated with retinoic acid for 4 days in monolayer culture, the majority of mouse ES cells differentiated into primitive endoderm-like cells (19, 25). These cells are an epithelial type and express collagen IV, laminin, megalin, and Dab2. In the endoderm-like cells derived from wild-type ES cells, megalin is largely confined to the outer ring of multiple cell aggregates, defining a compartment in these cultured cells corresponding to the apical surface of an epithelium (Fig. 4A, arrow), and megalin is absent in the cell-cell contact surface (arrowhead). In Dab2 down-regulated cells, the megalin-stained outer rim disappears and the megalin staining displays a punctate cytoplasmic pattern (Fig. 4B). Generally in the wild-type cells, the cytoplasmic Dab2 and the apical membrane megalin have minimal colocalization. Likely the megalin-containing endocytic cargos recycle back to the apical membrane rapidly and linger in the cytoplasm only in the absence of Dab2. It has been reported, however, that Dab2 is required for internalization of megalin (26). Such a discrepancy may due to different systems and remains to be resolved.

Dab2 Is Required for Apical Exclusion of E-cadherin in Cultured Cells—In the Dab2-down-regulated cells, we observed a dramatic loss of polarized distribution of surface E-cadherin. The cell adhesion molecule E-cadherin is a principal component of the epithelial adherens junction, and its function is critical for cell organization and the development of blastocysts (27). Targeting and restricting E-cadherin molecules to the apical domain where adherens junctions are organized is an early and necessary step in establishing epithelial polarity (28, 29). In undifferentiated ES cells, E-cadherin, though present in the junction surfaces between cells that make contact, distributed in the cytoplasm in a speckled pattern (Fig. 5A). In retinoic acid-treated/differentiated cells, E-cadherin was restricted to the cell-cell junctions with little cytoplasmic staining. Presumably this E-cadherin staining pattern reflects the apical-basal cates the lining of primitive endoderm cells on the surface of embryoid bodies derived from wildtype ES cells (Fig. 3C, arrow). However, the GATA4-positive presumptive primitive endoderm cells are not localized on the surface but are distributed throughout the interior of the spheroids derived from Dab2-suppressed ES cells (Fig. 3C, arrowhead). In two independent experiments, of over 50 embryoid bodies derived from Dab2-suppressed ES cells examined, all lack an endoderm structure on the surface. Among these, two embryoid bodies were observed to contain a few Dab2-positive cells (presumably those lost siRNA suppression). Another two examples of the random distribution of GATA4-positive cells in spheroids formed from Dab2-suppressed ES cells are shown in supplemental Fig. 1. Thus, Dab2 is required for the surface positioning of endoderm epithelial cells in embryoid bodies.
polarity of an epithelium, with E-cadherin expression restricted to bilateral domains (Fig. 5A, arrowhead) and absent from the apical surface (Fig. 5A, arrow). When Dab2 was down-regulated in the cells, however, the polarized cellular distribution of E-cadherin was abolished: although most of the protein was still membranous, E-cadherin aligned along the entire cellular membrane including the apical surface (Fig. 5A, arrowhead).

This apical localization of E-cadherin depends on cell-cell contacts. In Dab2-suppressed cells that were isolated and lacked contact with neighboring cells (Fig. 5B), E-cadherin had a spotted distribution in the cytoplasm (arrowhead). No surface E-cadherin staining was ever observed in single cells, and apical E-cadherin staining (arrow) was only observed in a group of two or more Dab2-down-regulated cells (Fig. 5B). The dotted E-cadherin cellular staining pattern likely represents E-cadherin localized in endocytic vesicles. In Dab2-positive cells, such E-cadherin-containing endocytic vesicles often could be observed in single/isolated cells or in a group of two contacting cells, as shown in an example in Fig. 5C and supplemental Fig. 2. Most of the E-cadherin-containing vesicles are also Dab2-positive (Fig. 5C, arrow), indicating the localization to the same population of endocytic cargos. The loss of E-cadherin polarized surface distribution in Dab2-suppressed cells suggests that Dab2 is involved either in the bilateral targeting or more likely in the apical removal of E-cadherin. In mouse embryos, E-cadherin is also present in dab2−/− cells, although presumably the distribution is not polarized (supplemental Fig. 3). Thus, Dab2 has a crucial role in regulating the cellular trafficking and polarized distribution of E-cadherin. Although E-cadherin is known to traffic in clathrin-coated cargos (30), with which...
Dab2 also associates (12, 14), it is not known if additional direct or indirect interactions exist between E-cadherin and Dab2 for the recruitment of E-cadherin into Dab2-positive cargos. The finding from the Dab2-suppressed ES cells is that removal of E-cadherin from the apical domain, in addition to bilateral targeting, is also important in the polarized distribution of E-cadherin. These cell culture experiments indicate that loss of Dab2 influences targeting cell surface proteins such as megalin and E-cadherin to the apical cell surface.

DISCUSSION

This study leads to the identification of dab2 as a gene required for positioning of epithelial cells on surface. We propose that the activity of Dab2 in the coupling of selective cargos to unidirectional cellular trafficking results in the genesis and maintenance of epithelial polarity. Subsequently, we suggest that a consequence of epithelial apical-basal polarity may be the surface positioning and monolayered structure of epithelium. The finding provides a mechanistic explanation for the early embryonic phenotype of Dab2 knock-out embryos. Thus, the study reveals a novel mechanism for the positioning of cells on the surface, as demonstrated for the development of the first embryonic epithelium, the primitive endoderm, in mammals.

Role of Dab2 in Generation and Maintenance of Epithelial Polarity—The generation and maintenance of epithelial polarity is a fascinating question in cell biology (2). Cell adhesion, cytoskeleton arrangement, and endocytic trafficking of cellular proteins coordinate to establish cell polarity (2, 28, 29, 31–33). Two components, namely the restriction of surface domains by the formation of tight junctions and adherens junctions, and the sorting of cell surface proteins to specific surface domains, are critical to achieve and maintain polarity in an epithelium.

Based on the analysis of E5.5 dab2-deficient embryos and studies of Dab2 suppression in cultured cells, we propose that Dab2 couples selective endocytic cargos to the myosin VI motor attached to microfilaments in unidirectional trafficking to establish apical and basal polarity, and the loss of polarity in Dab2-deficient embryos subsequently compromises the restriction of the endoderm cells to a surface position (Fig. 6).

Since Dab2 is known to mediate directional trafficking of endocytic vesicles (13, 14), a role for Dab2 in epithelial polarity

FIGURE 5. Loss of polarized distribution of E-cadherin in endoderm-like cells in culture following Dab2 down-regulation by siRNA. A, undifferentiated (−RA), differentiated (+ RA), or differentiated ES cells transfected with siRNA for Dab2 suppression were used for immunofluorescence staining for E-cadherin (red) and DAPI (blue). B, examples of the differentiated Dab2-siRNA ES cells are shown for either an isolated single cell or a group of three cells in contact stained with E-cadherin (green) and DAPI (blue). Note that E-cadherin staining in the single cell exhibits a cytoplasmic dotted pattern (arrowhead) without surface staining. Apical surface staining (arrow) is present in cells that are in contact. C, E-cadherin (green), Dab2 (red), and DAPI (blue) immunofluorescence staining is shown in two contacting, differentiated wild-type ES cells. The area indicated by the box is shown at a higher magnification in the right panel. The two layers were shifted slightly (right lower panel) to reveal the pairs of red (Dab2) and green (E-cadherin) dots representing endocytic vesicles containing both E-cadherin and Dab2. Overlapping dots are indicated by arrows, and non-overlapping dots are indicated by arrowheads.
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FIGURE 6. Schematic illustration of the role of Dab2 in cellular transport and cell surface positioning. A, Dab2-mediated unidirectional transport of specific endocytic cargos enables the establishment of epithelial apical-basal polarity. Dab2 mediates the removal of E-cadherin from and targeting of megalin to the apical surface. B, Dab2-deficient cells are unable to establish an apical-basal polarity. The loss of a polarized distribution of cell adhesion molecules eliminates the inhibition of cells interacting at the apical surface, and thus the cells are no longer restricted to a surface position. The loss of surface positioning of the epithelial cells is the secondary event and the consequence of the inability to generate polarity in Dab2-deficient endoderm cells. During the formation of the primitive endoderm in the blastocyst stage, loss of polarity due to the absence of Dab2-mediated cargo sorting subsequently compromises the surface positioning restriction of the endoderm cells in Dab2-deficient embryos. Intriguingly, the other mammalian ortholog, Disabled-1, is a gene recognized to have a role in positioning of brain cells in development (9, 10).

Once the ability to generate polarity is lost (such as by the loss of Dab2) in an epithelial cell located in a monolayer epithelium, neighboring cells may invade and occupy the apical surface of the cells. Ultimately, cell organization is compromised and the epithelium is disrupted (Fig. 6B). The phenotype of endoderm disorganization in dab2(−/−) embryos thus is likely secondary and the consequence of the inability to generate polarity in Dab2-deficient primitive endoderm cells. During the formation of the primitive endoderm in the blastocyst stage, loss of polarity due to the absence of Dab2-mediated cargo sorting subsequently compromises the surface positioning restriction of the endoderm cells in Dab2-deficient embryos. Intriguingly, the other mammalian ortholog, Disabled-1, is a gene recognized to have a role in positioning of brain cells in development (9, 10).

Role of Dab2-interacting Proteins in Extraembryonic Endoderm Development—The early embryonic lethal phenotype suggests that Dab2-mediated directional endocytic trans-
port involving clathrin, α-adaptin, megalin, and myosin VI may be functionally important in establishing the polarity of extraembryonic endoderm cells around E5.5. The primitive endoderm is one of the earliest epithelial structures in development and few genes are known to be required for the extraembryonic endoderm development. Clathrin and α-adaptin have additional diverse cellular roles in endocytosis and protein trafficking, and their function is likely autonomous and critical for cell survival. A fraction of megalin knock-out mice were reported to survive to adult (40, 41), thus megalin is dispensable for extraembryonic development. Likely, in endoderm cells there are a large number of apical surface glycoproteins which can replace the role of megalin in maintaining the apical polarity. Also, myosin VI deficiency in Snell’s waltzer mice leads to the degeneration of inner ear epithelium (42) and does not resemble the dab2 knock-out phenotype (16). Dab2 may interact with one or more myosin VI-like protein motors present in endoderm cells since there are a large number of myosin genes involved in various cellular transport functions (43, 44). Indeed, a non-muscle myosin molecule was found to be required for establishing cellular polarity in C. elegans embryos (45).

Interactions of Dab2 with other proteins also suggest additional roles in epithelial to mesenchymal transition (46), Wnt signaling (47), and kidney transport (48).

Epithelial Neoplastic Morphological Transformation as Consequence of the Loss of Polarity—The association between carcinomas, malignancies of epithelia, with the loss of epithelial polarity and organization is well known, although the underlying mechanism is not clear. Recently, gene knock-out studies suggest loss of polarity may be causative for neoplastic transformation, such as the Lethal giant larvae gene (49) and the Peutz-Jeghers cancer syndrome gene LKB1 (Peutz-Jeghers cancer syndrome gene 1) (50).

Not unexpectedly, a reduction of dab2 gene dosage also results in the development of uterine tumors and ovarian epithelial hyperplasia and neoplasia (51). The finding of the uterine tumor prone phenotype of dab2(+/−) mice adds to the accumulating evidence between the link of epithelial polarity and cancer.

Based on the known cellular function of Dab2, we suggest that a role for epithelial polarity is to create a non-adherent apical surface, which is critical for maintaining surface positioning and a monolayer structure. Once an epithelial cell loses polarity, neighboring cells can occupy the apical surface, leading to formation of a multilayered or stratified, dysplastic lesion. Thus, loss of epithelial polarity is a required step in the neoplastic morphological transformation of a simple monolayered epithelium to adenomas and carcinomas (51–53). The loss of polarity in knock-out mice most likely accelerates the selection of compromised cells with genetic and epigenetic changes favoring the formation of neoplastic lesions.

In summary, our study leads to the identification of dab2 as a gene required for the establishment of epithelial polarity and underscores the importance of polarity in the surface positioning and monolayered structure of epithelium. The finding also provides genetic evidence and a mechanism supporting the newly proposed model of mosaic lineage differentiation followed by segregation into primitive endoderm layer in the development of early mouse embryos (7, 8).

Here we favor the proposed model that the ability of the cell to generate apical polarity is the determinant for surface localization of endoderm cells. Potentially, however, Dab2 may also influence cell migration or selective cell adhesion, which might affect cell positioning. Future experiments will be needed to examine further the current model and rule out any of these possibilities.

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