T-cadherin is located in the nucleus and centrosomes in endothelial cells

Alexandra V. Andreeva,1 Mikhail A. Kutuzov,1 Vsevolod A. Tkachuk,2 and Tatyana A. Voyno-Yasenetskaya1

1Department of Pharmacology, University of Illinois at Chicago, Chicago, Illinois; and 2Cardiology Research Center, Moscow, Russia

Submitted 1 June 2009; accepted in final form 31 August 2009

Andreeva AV, Kutuzov MA, Tkachuk VA, Voyno-Yasenetskaya TA. T-cadherin is located in the nucleus and centrosomes in endothelial cells. Am J Physiol Cell Physiol 297: C1168–C1177, 2009. First published September 2, 2009; doi:10.1152/ajpcell.00237.2009.—T-cadherin (H-cadherin, cadherin 13) is upregulated in vascular proliferative disorders and in tumor-associated neovascularization and is deregulated in many cancers. Unlike canonical cadherins, it lacks transmembrane and intracellular domains and is attached to the plasma membrane via a glycosylphosphatidylinositol anchor. T-cadherin is thought to function in signaling rather than as an adhesion molecule. Some interactive partners of T-cadherin at the plasma membrane have recently been identified. We examined T-cadherin location in human endothelial cells using confocal microscopy and subcellular fractionation. We found that a considerable proportion of T-cadherin is located in the nucleus and in the centrosomes. T-cadherin colocalized with a centrosomal marker γ-tubulin uniformly throughout the cell cycle at least in human umbilical vein endothelial cells. In the telophase, T-cadherin transiently concentrated in the midbody and was apparently degraded. Its overexpression resulted in an increase in the number of multinuclear cells, whereas its downregulation by small interfering RNA led to an increase in the number of cells with multiple centrosomes. These findings indicate that deregulation of T-cadherin in endothelial cells may lead to disturbances in cytokinesis or centrosomal replication.

T-cadherin is a glycoprotein that belongs to the cadherin cell adhesion family. In contrast to canonical cadherins, T-cadherin lacks many amino acids crucial for Ca2+-dependent intercellular dimerization (15, 60). As a consequence, it is monomeric in the absence and in the presence of calcium (15) and is thought to mainly function as a signaling molecule. Another unique feature of T-cadherin is the absence of the transmembrane and cytoplasmic domains [although a rare cell line-specific transmembrane form has been reported (54)]. T-cadherin is anchored within lipid rafts through glycosylphosphatidylinositol (GPI) (60, 68) and may possibly use cytoplasmic domains of several interactive partners (58) to transduce signals inside the cell. T-cadherin may operate through integrin-linked kinase (ILK), which is upstream of the Akt/GSK3β/β-catenin pathway (41, 42). It has been suggested and confirmed in an in vivo model that T-cadherin may play an antiapoptotic and protective role under hypoxic conditions (30, 42).

In confluent endothelial cells (ECs) T-cadherin, unlike vascular endothelial (VE)-cadherin (which is mainly present at the cell-cell junctions), is located over the entire cell body with only a slight enrichment at cell borders, whereas in wounded cultures it is polarized to the leading edge of migrating cells (59). Some recent reports described nuclear localization of cleaved cytoplasmic domains of canonical cadherins (21, 66) and their potential role in the modulation of gene transcription (21).

Several lines of evidence point to a potential involvement of T-cadherin in cell-cycle regulation in different cell lines. T-cadherin expression is decreased or undetectable in some tumor samples and various cancer cell lines (63, 73). In contrast, overexpression of T-cadherin was demonstrated in tumorigenic liver tissue and a hepatocellular carcinoma cell line (63). The role of T-cadherin in tumor neovascularization was recently confirmed using in vivo model (30). T-cadherin expression in T-cadherin-deficient C6 glioma cells results in G2 phase arrest and aneuploidy, which is dependent on increased expression of p21(CIP1) and is eliminated in p21(CIP1)-deficient fibroblasts (35). Ectopic expression of Cdh1, one of the substrate recognition components (E3 ligase) of the anaphase-promoting complex, stimulates T-cadherin degradation (5). In human umbilical vein endothelial cells (HUVECs), overexpression of T-cadherin leads to an increased expression of cyclin D1, a key regulator of G1 to S-phase progression (24) and to a rapid entrance into S-phase (38).

Here we report that in ECs a considerable proportion of T-cadherin is located in the nucleus. It is also present in the centrosomes and colocalizes with γ-tubulin. In the telophase, T-cadherin transiently concentrates in the midbody. Overexpression of T-cadherin results in an increase in the number of multinuclear cells, whereas its downregulation leads to an increased number of cells with multiple centrosomes. These findings indicate that T-cadherin is a nuclear and centrosomal protein and that its deregulation may interfere with the cell cycle via disturbance in cytokinesis or centrosomal replication.

MATERIALS AND METHODS

Materials. The antibodies used were the following: Akt1, Bcl2, calreticulin, GFP, GRP78, LAMP1, T-cadherin (sc-7940; designated here as SC), γ-tubulin, VE-cadherin, horseradish peroxidase (HRP)-conjugated anti-goat antibody (Santa Cruz Biotechnology); FLAG (M2; Sigma), histone 1 (AE–4; Millipore), GM130, heat shock protein 90 (Hsp90), PP5, Rab5, Rac1 (BD Transduction Laboratories), and ZO-1 (Zymed). HRP-conjugated anti-mouse and anti-rabbit secondary antibodies were from Amersham, AlexaFluor 488, 594, or 633 anti-mouse, anti-rabbit, or anti-goat antibodies were from Molecular Probes. The affinity-purified polyclonal T-cadherin antibody designated here as TK was described previously (70). FLAG-tagged T-cadherin constructs were kindly provided by Drs. Kalpana Ghoshal and Samson Jacob (Ohio State University) (5). T-cadherin constructs and specificity of the antibodies used in this work are depicted in Fig. 1A and Supplementary Fig. 1A.

Cell culture. Human embryonic kidney (HEK) 293A cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM), supple-
mented with 10% fetal bovine serum. Human endothelial cells were obtained at passage 3 from Lonza and cultured as described previously (4). Transient transfection of HEK 293A and ECs was performed using Lipofectamine 2000 (Invitrogen) and SuperFect (Qiagen), respectively, according to manufacturers’ instructions.

**Confocal microscopy.** Cells cultured on gelatin- or fibronectin-coated coverslips were fixed with 3.7% paraformaldehyde or with 50% methanol (for preservation of centrosomes), followed by permeabilization in 0.5% Triton X-100. Cells were incubated with primary antibodies followed by incubation with appropriate secondary antibodies, using Tris-buffered saline containing bovine serum albumin as a blocking buffer.

**siRNA-mediated depletion.** Inhibition of T-cadherin expression was performed using small interfering RNA (siRNA) designed by Dharmacon. Cells were transfected using siRNA transfection reagent (Santa Cruz Biotechnology) or Lipofectamine 2000 (Invitrogen). Control siRNA was purchased from Santa Cruz Biotechnology (cat. number sc-7940). T-cadherin siRNA was purchased from Santa Cruz Biotechnology.

**Nuclear fractionation.** Cells from a 10-cm dish were washed twice with PBS, recovered by scraping, and resuspended in 300 μl of lysis buffer containing 20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 10 mM EGTA, 5 mM MgCl₂, 0.5% Na deoxycholate, 0.5% Nonidet P-40, and protease inhibitor cocktail (1:200 dilution, Sigma). Nuclei were pelleted by centrifugation at 2,500 g for 10 min. The supernatant was underlaid with a 60% sucrose cushion (0.8 ml), and centrosomes were sedimented at 25,000 g for 10 min. The cushion and two volumes of the buffer above it (2.4 ml total) were collected, mixed, and laid on the top of a discontinuous sucrose gradient (1 ml 70%, 0.6 ml 50%, and 0.6 ml 40% sucrose) and centrifuged at 100,000 g for 1 h. Fractions of 0.45 ml were collected and diluted to reduce sucrose concentration to <20%. Centrosomes were pelleted at 25,000 g for 30 min and dissolved in the SDS sample buffer for electrophoresis.

**Immunoprecipitation and Western blotting.** Cells were lysed in 50 mM HEPES, pH 7.5, 50 mM NaCl, 5 mM MgCl₂, and 1% Triton X-100. Proteins were immunoprecipitated with appropriate antibodies (as specified in figure legends) and protein A/G agarose (Santa Cruz Biotechnology) for 4 h at 4°C. Immunoprecipitates were washed three times with the lysis buffer. Proteins were separated on 5–20% gradient SDS gels and transferred onto polyvinylidene fluoride (Osmonics) or nitrocellulose (Protran, Schleicher & Schull) membranes. Membranes were probed with appropriate antibodies and developed using Dura reagents (Pierce). Densitometry of protein bands was performed on scanned images using NIH Image 1.63 software.

**RESULTS**

**T-cadherin is located in the nucleus in ECs.** In accordance with a report that T-cadherin is only slightly enriched at the cell borders (59), we only occasionally observed cell surface staining in the primary cultures of ECs (Fig. 1B, indicated with arrowheads at the top). In contrast, a robust staining of the nuclei was observed using two different polyclonal T-cadherin antibodies, designated here as TK [raised against synthetic hexasector}
peptides of the first extracellular subdomain (70)) and SC (raised against a peptide overlapping with CD2 and CD3 domains, Fig. 1A). Nuclear staining was observed in three different cell lines (Fig. 1B): human pulmonary artery endothelial cells (HPAECs), human umbilical vein endothelial cells (HUVECs), and human microvascular endothelial cells (HMVECs). It was not due to the secondary antibody used in these experiments, since omitting primary antibodies resulted in the absence of nuclear staining (Fig. 1B, bottom).

To confirm the nuclear location of T-cadherin, we fractionated ECs into a nuclear fraction and a fraction containing other cellular compartments and cytoplasmic proteins. The fractionation was confirmed using histone H1 as a nuclear marker (Fig. 2, A and B). Approximately one-half of the total endogenous T-cadherin was found in the nuclear fractions in HPAECs, as revealed by Western blot analysis with the TK antibody, which recognizes 105- and 130-kDa forms (Fig. 2A). A lower proportion (10–20%) of endogenous T-cadherin was found in the nuclear fraction of HUVECs (Fig. 2B). The absence of contamination of the nuclear fractions by proteins present in the postnuclear supernatant was evident from reprobing the membranes for a number of markers for different subcellular compartments: Bcl2 (mitochondria), calreticulin (endoplasmic reticulum, ER), GRP78 [ER/plasma membrane (PM)], LAMP1 (lysosomes), VE-cadherin (adherens junctions), GM130 (Golgi), Hsp90 (cytoplasm), Rab5 (early endosomes), ZO-1 (tight junctions), postnuclear supernatant. It is worth noting that some of the examined proteins have been reported to either be present in the nucleus {PP5 (9), ZO-1 (27), Akt1 (56), Rac1 (50, 51)} or to be functional or interacting partners of T-cadherin {Akt1 (42), Rac1 (57), GRP78 (58)}.

Fig. 2. Distribution of T-cadherin (105 kDa, mature glycosylated form; 130 kDa, partially processed precursor) between nuclear and nonnuclear fractions of HPAECs (A) and HUVECs (B) and assessment of the purity of respective nuclear fractions (C) are shown. Untransfected cells or cells transfected with FLAG-T-cadherin were fractionated into the nuclear (N, 1,000 g pellet) and soluble (S, 1,000 g supernatant) fractions. Proteins were separated on SDS-PAGE, transferred onto a polyvinyldifluoride membrane, and probed with the TK or FLAG antibodies. Positions of molecular mass markers (PageRuler, Fermentas) are indicated. In A and B, quantitation of the immunoblotting data for endogenous T-cadherin forms was performed using ImageJ software. Data shown are means of three separate experiments (error bars ± SD). H1, histone H1. C: fractions shown in A and B were probed for various markers for indicated subcellular structures and compartments (AJ: adherens junctions; ER, endoplasmic reticulum; PM, plasma membrane; TJ, tight junctions). Proteins that have been reported to either be present in the nucleus {PP5 (9), ZO-1 (27), Akt1 (56), Rac1 (50, 51)} or to be functional or interacting partners of T-cadherin {Akt1 (42), Rac1 (57), GRP78 (58)}.

The predicted molecular mass of the mature protein without signal peptide is ~65 kDa (60). In ECs, there are two major forms of T-cadherin, mature 105 kDa and a partially processed precursor 130 kDa, both of them are presumably glycosylated (70). The results of subcellular fractionation clearly indicate that both forms of T-cadherin are present in the nucleus. Both T-cadherin antibodies gave similar nuclear patterns in immunofluorescence experiments (Fig. 1B). The SC antibody has not been used in fractionation experiments (Fig. 2), because on Western blots it failed to recognize full-length T-cadherin, although it recognized truncated T-cadherin mutant ΔC3-4 with high efficiency (Supplementary Fig. 1, A–C). Because T-cadherin has several potential glycosylation sites, this might be due to conformation sensitivity or epitope masking by glycosyl group(s). Since the presence of T-cadherin in the nucleus was highly unexpected and of a considerable potential interest, we confirmed this finding using polyclonal antibodies against CD1 and CD5 domains of T-cadherin (40), respectively (Supplementary Fig. 1D).

Analysis of T-cadherin primary structure did not reveal any canonical nuclear localization sequences, although it is enriched in basic residues and is predicted to be a nuclear protein by the ESLpred (8) and SubLoc (28) algorithms. We also found that T-cadherin contains a Leu-rich sequence LRFSLPSVLLLSLFSLACL within its COOH-terminal recognition sequence (CSR) that perfectly matches a nuclear export signal consensus Lx2–3Lx2–3LxL (46). Before mature GPI-anchored proteins are delivered to the cell surface, they undergo processing in the endothelial reticulum (ER). Their CSRs are removed by proteolytic cleavage in the ER simultaneously with the attachment of the GPI anchor to the newly exposed COOH-terminus (22). We examined the localization of FLAG-tagged
T-cadherin construct, where the epitope is placed after the CSR for GPI-attachment (see Supplementary Fig. 1A). COOH-terminal epitope tags attached after CSRs are not expected to impede the proper protein processing and are removed when the protein is processed in the ER (2, 7, 65), thus only the molecules that are not (yet) processed are detectable with the epitope-specific antibody. The nonprocessed FLAG-tagged T-cadherin could be exclusively detected in the nonnuclear fractions (Fig. 2B, Supplementary Fig. 1C), presumably in the ER, taken into account a characteristic reticular pattern observed with FLAG-tagged T-cadherin (Supplementary Fig. 1C).

We also examined the effect of leptomycin B (LMB, an inhibitor of CRM1-dependent nuclear export) on distribution of endogenous T-cadherin in HUVECs. The proportion of both endogenous T-cadherin forms increased by 1.5- to 2-fold in the nucleus upon LMB treatment (Supplemental Fig. 1E), indicating that the presence of T-cadherin in the nucleus is dynamically regulated. Because it is unlikely that mature T-cadherin possesses this CSR sequence, it is possible that it interacts with a partner with functional nuclear export signal.

**T-cadherin is present in the centrosomes.** In dividing HPAECs, T-cadherin was associated with two punctate structures located on the opposite sides of chromatin and thus resembling centrosomes (see Fig. 1B, lower HPAEC panel). Z-sectioning of individual cells confirmed that each mitotic cell contained exactly two T-cadherin-positive structures (Supplementary Fig. 2A), consistent with their identification as centrosomes. Therefore, we examined whether T-cadherin would colocalize with a centrosomal marker γ-tubulin. We found a clear colocalization between the two proteins throughout the cell cycle (Fig. 3; methanol fixation was used in this experiment to optimize centrosome preservation, which is not optimal for visualization of the nuclei). This indicated that T-cadherin is indeed present in the centrosomes. In the telophase it was often transiently concentrated in the midbody, with reduced or undetectable presence in the centrosomes (note that the brightness of the green channel is increased in the telophase panel compared with other panels in Fig. 3). In HUVECs, these T-cadherin-positive centrosomes could be observed throughout the cell cycle, including mitosis. In HPAECs, a qualitatively similar pattern was observed; however, centrosomal T-cadherin staining was most obvious in metaphase and anaphase.

Centrosome-associated T-cadherin could also be detected using the TK antibody (Supplementary Fig. 2B), although less efficiently than with the SC antibody (while both antibodies stain the nuclei similarly, Fig. 1B). Lower efficiency of the TK antibody might be due to partial unavailability of its epitope (CD1). In PC12 cells, T-cadherin was reported to be ubiquitinated by Cdh1, a component of a major ubiquitination system that controls the proteasome-dependent destruction of cell-cycle regulators (5). Proteasomes are located in both the nucleus and the centrosomes (see references in 16). Cdh1 localizes to the nucleus during interphase and to the centrosomes during metaphase and anaphase (78). This prompted us...
to test whether the levels of T-cadherin in HUVECs would be affected by proteasome inhibition. After 4-h incubation with proteasome inhibitors I1 and MG132, the levels of T-cadherin were significantly increased, whereas incubation with I2 (calpain inhibitor that does not affect proteasome) resulted in only marginal, if any, increase in T-cadherin levels (Fig. 4A). These data indicate that T-cadherin levels in HUVECs are under control of proteasome-dependent degradation.

To confirm the centrosomal location of T-cadherin, we isolated centrosomal fractions from HUVECs by differential centrifugation using a 20–70% discontinuous sucrose gradient. The fractionation was confirmed by using γ-tubulin as a centrosomal marker. Immunoblotting showed a biphasic distribution of γ-tubulin (Fig. 4B). The lower molecular mass form, but not the 130-kDa form, of T-cadherin could be detected in the centrosomal fractions using the TK antibody and was mainly associated with the first of the two γ-tubulin peaks (Fig. 4B). Reprobing the same blot with the SC antibody [which does not recognize full-length glycosylated T-cadherin] revealed the presence of a ~60-kDa band, associated with the second γ-tubulin peak, and also found in a denser fraction (Fig. 4B). Thus the results of centrosome isolation indicate that only the mature processed form, but not the 130-kDa form, of T-cadherin is associated with a subfraction of the centrosomes. Our data are also compatible with a truncated form or nonglycosylated T-cadherin present in a distinct centrosomal subfraction.

To examine whether T-cadherin and γ-tubulin might physically associate, we performed immunoprecipitation from HUVECs. We first attempted to assess whether endogenous T-cadherin might associate with γ-tubulin. However, endogenous T-cadherin could not be detected in γ-tubulin immunoprecipitates, whereas reciprocal immunoprecipitation proved inconclusive due to the nearly identical molecular weight of γ-tubulin and the heavy immunoglobulin chain (data not shown). Taking into account that only a small proportion of endogenous T-cadherin is located in the centrosomes compared with the nucleus in ECs, we next attempted to use overexpressed T-cadherin to assess the possibility of its interaction with γ-tubulin. Since the SC antibody recognizes the ΔC3-4 T-cadherin construct, but not full-length constructs, on Western blots (as discussed above and shown in Supplementary Fig. 1B), HUVECs were transfected with the latter construct or empty vector. Immunoprecipitation was performed using γ-tubulin- and GFP-specific antibodies. The presence of a band of the expected molecular size (see Supplementary Fig. 1B) recognized by the SC antibody could be detected in the material precipitated from T-cadherin transfected cells with the γ-tubulin antibody but not in the negative controls (Fig. 4C). No corresponding signal could be detected in the immunoprecipitates using FLAG antibody, consistent with a form that has been COOH terminally processed in the ER.

To assess whether the centrosomal and nuclear location of T-cadherin is specific for ECs, we examined localization of endogenous T-cadherin in HEK 293A cells. Immunoblotting analysis showed that HEK 293A (as well as COS-7) cells express considerably lower levels of T-cadherin relative to total protein than ECs (data not shown). We found that in HEK 293A cells, most of endogenous T-cadherin colocalized with γ-tubulin and thus was associated with the centrosomes [which are unusually large structures in this cell line (55, 76)], with only very faint nuclear and cell surface staining (Supplementary Fig. 3A). In an attempt to detect association of endogenous T-cadherin with γ-tubulin, we performed immunoprecipitation experiments from HEK 293A cells. A ~60-kDa band could be detected in γ-tubulin immunoprecipitates but not in GFP antibody immunoprecipitates used as a negative control (Supplementary Fig. 3B). The molecular mass of this band is close to that expected for nonglycosylated T-cadherin without prepeptide (65 kDa).

**Interference with T-cadherin expression leads to defects in cell division.** As described above, T-cadherin was often transiently concentrated in the midbody, which plays a key role in cytokinesis (6). This observation suggested that T-cadherin might be involved in cytokinesis. Cytokinesis failure leads to the appearance of cells containing more than one nucleus. To assess whether overexpression or downregulation of T-cadherin might affect the probability of failed cytokinesis, we examined the proportion of bi- or multinuclear cells among HUVECs transfected with either T-cadherin cDNA or T-cadherin siRNA. A small proportion of ECs is naturally bi- or multinuclear, and an increase in the proportion of such cells has been associated with some physiological or pathological conditions (19, 36, 69). In line with these reports, we observed
that primary HUVEC cultures contained 4–5% cells with more than one nucleus (of which absolute majority were binuclear cells).

T-cadherin could be efficiently overexpressed in ECs (Fig. 5A; Supplementary Fig. 1C), resulting in an overall increase in T-cadherin content of up to 10- to 20-fold (ECL quantification data not shown). Immunofluorescence microscopy revealed that in some cells T-cadherin was present in a reticular pattern (Fig. 5B, top), probably reflecting its predominant presence in the ER, whereas in other cells punctate cytoplasmic as well as nuclear staining was observed (Fig. 5B, middle). The proportion of cells containing more than one nucleus was increased among the cells overexpressing T-cadherin (Fig. 5, B and C). Similar results were obtained using overexpression of FLAG-tagged T-cadherin in HUVECs (data not shown) and in HEK 293A cells (Supplementary Fig. 3, C–E).

We also depleted endogenous T-cadherin in ECs using the siRNA approach and examined the cells for any apparent defects associated with cell division. Both 105- and 130-kDa forms of T-cadherin could be efficiently downregulated in HPAECs and in HUVECs (Fig. 6A). It was reported that the levels of different cadherins may influence each other, for example, VE-cadherin expression is reduced in N-cadherin-deficient endothelium (48). VE-cadherin levels remained unchanged in the cells with 80–90% T-cadherin depleted (Fig. 6A). In some experiments, 95–98% T-cadherin depletion was achieved, which was accompanied by a 30–40% decrease in VE-cadherin levels (data not shown). T-cadherin depletion also resulted in a considerable decrease in T-cadherin-positive staining in immunofluorescence experiments (Fig. 6, B and C).

T-cadherin depletion led to an only marginal increase in the proportion of multinucleated cells, which was not statistically significant (Fig. 6E). However, we noticed that downregulation of T-cadherin was accompanied by an increased proportion of mononuclear ECs with multiple centrosomes (Fig. 6, C, D, and F), which suggests a specific role of T-cadherin in the control of centrosomal organization.

**DISCUSSION**

We report here that a considerable proportion of T-cadherin is located in the nucleus, as evidenced by immunofluorescence and by immunoblotting upon cell fractionation using antibodies raised against different epitopes. Nuclear localization of cleaved intracellular domains of some other members of the cadherin family, such as protocadherins α (18), γ (29), and Fat1 (33, 49), E-cadherin (21) was reported. However, T-cadherin has no intracellular domain and is present in the nucleus as a full-length molecule; therefore trafficking pathways suggested for other cadherins may not be relevant in the case of T-cadherin. Full-length cell surface receptors, both G protein-coupled receptors and tyrosine kinase receptors, were reported to translocate to the nucleus (17, 20, 25, 53, 62, 72), including LPA1, for which caveolin-mediated endocytosis was suggested (26). Nucleus is rich in lipid environments, which contain lipid raft components, including caveolin-1 (1). However, several lines of evidence argue against a role of caveolin in the nuclear trafficking of T-cadherin: 1) Pertussis toxin inhibits LPA1 trafficking to the nucleus (26) but does not affect the nuclear localization of T-cadherin (our unpublished observations), which makes a common trafficking mechanism unlikely. 2) Although caveola-dependent endocytosis was originally considered for GPI proteins (3), later studies suggested a caveola-independent pinocytotic pathway (44, 45, 64) or macropinocytotic pathways triggered by a clustering agent or being ligand independent (14, 74). 3) In ECs, caveolin is not involved in T-cadherin-mediated signaling (59). However, a recently described T-cadherin interaction with GRP78 (58), which is reportedly involved in caveola-dependent internalization and nuclear localization of extracellular matrix protein DMP1 (61), reintroduces the question about a possible role of caveola.
In some cell lines, T-cadherin may be not GPI modified but rather expressed as a protein with a transmembrane domain (54). Thus it cannot be excluded that precise sites of processing of T-cadherin in ECs might differ from those assumed on the basis of canonical processing of GPI-anchored proteins.

If T-cadherin is processed as a canonical GPI-anchored protein, the questions arise whether it is targeted to the nucleus after its delivery to the PM or directly from the ER. Some models for the nuclear translocation of cell surface receptors have been suggested (47), and although these questions are beyond the scope of this work, T-cadherin would be an excellent model to study this process.

Apart from the question of how T-cadherin is imported into the nucleus, another question is what functional role the nuclear T-cadherin may play. Cleaved intracellular domain of E-cadherin may modulate gene transcription (21). Some other transmembrane receptors may also act as transcriptional factors or modulators of expression (17, 20, 25, 34, 53, 62, 72). As T-cadherin upregulates cyclin D1 (41) and may activate the serum-response element (our unpublished data), a similar function seems conceivable for T-cadherin.

We also found that T-cadherin is located in the centrosomes. A quantitative difference between venous (HUVECs) and arterial (HPAECs) endothelial cells could be observed, because T-cadherin was present in the centrosomes uniformly throughout the cell cycle (except telophase) in HUVECs but was most noticeable during metaphase in HPAECs. The mechanisms of protein delivery to the centrosomes are poorly understood. For some proteins, such as RGS14 (13), nuclear-cytoplasmic shuttling may be important. In this respect, it may be relevant that the two lines of ECs studied here also differed in the proportion of nuclear T-cadherin. Since our data suggest that T-cadherin may play a role in cytokinesis, it is also worth noting that some proteins required for cytokinesis are sequestered into the nucleus during interphase (6). Centrosomal T-cadherin may have a longer half-life than T-cadherin in other compartments, since the centrosomes were the last location where it could be detected upon siRNA-mediated depletion. This is similar to the observations for centrosomal γ-tubulin (67). In line with these data, in HEK 293A cells (where T-cadherin expression is low), the endogenous T-cadherin could only be observed in the centrosomes and to a minimal extent in the nucleus.

The role of T-cadherin in the centrosomes may be linked to the control of their duplication, since siRNA-mediated T-cadherin depletion resulted in an increased fraction of cells with multiple centrosomes. Since the data were collected for the cells that have an increased number of centrosomes without nuclear duplication, this reflects a defect in centrosomal duplication per se. Notably, depletion of most centrosomal proteins leads to cell cycle arrest and defects in centrosome structure (52).

Our data also suggest that T-cadherin may play a role in cytokinesis, since it transiently concentrates in the midbody during telophase, and its overexpression leads to an increased proportion of cells with more than one nucleus. Notably, both the expression of T-cadherin (39) and the proportion of bi- and...
multinucleated ECs (69) are elevated in atherosclerotic lesions. Aneuploidy, which may be caused by different mitotic aberrations, including errors in centrosome duplication or defective cytokinesis, is a characteristic feature of many cancerous cells. At the same time, T-cadherin expression is known to be either down- or upregulated in many cancer cell lines (e.g., Ref. 71 and references therein). From our findings, it would be interesting to examine whether T-cadherin expression is affected in tumors of endothelial origin, which are also characterized by centrosome abnormalities (31). We did not detect statistically significant differences in the percentage of multinucleated cells upon T-cadherin depletion. However, since T-cadherin promotes cell-cycle progression in vascular cells (38), T-cadherin depletion may “freeze” the culture in the same state it was before siRNA transfection.

Overall, our findings reported here point to novel role(s) of T-cadherin in the nucleus and/or in cell division. Although localization of T-cadherin described here is rather unexpected, its links to the cell cycle have been reported previously in endothelial and other cell types: 1) T-cadherin has been identified among cell cycle-related genes upregulated in G1 phase (37). 2) T-cadherin increases the expression of cyclin D1 in ECs (41). 3) Ectopic expression of Cdh1 (which localizes dynamically to the nucleus during interphase and to the centrosomes during metaphase and anaphase and is involved in the destruction of cell cycle regulators (75)), stimulates T-cadherin degradation in PC12 cells (5). Our data, which indicate that T-cadherin is under control of proteasome-dependent degradation in HUVECs and may be degraded in telophase, are compatible with a role of Cdh1 in T-cadherin degradation in ECs as well. 4) Enforced expression of T-cadherin induces cell cycle arrest in hepatocellular carcinoma cells (12). 5) Three kinases that act downstream of T-cadherin (41) and supposedly transduce T-cadherin signals from the PM are also implicated in the control of centrosomes and/or mitotic spindles: 1) GSK-3β regulates localization of the γ-tubulin ring complex to the spindle poles and thereby controls the formation of proper mitotic spindles (32). 2) Akt may localize to the centrosomes in a manner dependent on its phosphorylation at specific sites (43), and its reduced levels result in incomplete centrosome migration around the nucleus and in bent misoriented mitotic spindles (10, 11). 3) ILK has recently been found to localize to mitotic centrosomes and to be essential for spindle pole organization and mitosis (23).

Since our data suggest that either depletion or overexpression of T-cadherin results in defects in cell division, findings reported here suggest a mechanistic insight into a previously described correlation between either downregulation or upregulation of T-cadherin with cancers. Possible contributions of diverse pathways to the function of T-cadherin in cell division will be the subject of future studies.

ACKNOWLEDGMENTS

We thank Drs. Kalpana Ghoshal and Samson Jacob (Ohio State University) for the FLAG-tagged T-cadherin constructs, Aleksandar Kranjecvic for plasmid purification, and Dr. Jasmina Proﬁrovic for critical reading of the manuscript.

REFERENCES


