Epithelial cell-cell adhesion is mediated by E-cadherin, an intercellular N-glycoprotein adhesion receptor that functions in the assembly of multiprotein complexes anchored to the actin cytoskeleton named adherens junctions (AJs). E-cadherin ectodomains 4 and 5 contain three potential N-glycan addition sites, although their significance in AJ stability is unclear. Here we show that sparse cells lacking stable AJs produced E-cadherin that was extensively modified with complex N-glycans. In contrast, dense cultures with more stable AJs had scarcely N-glycosylated E-cadherin modified with high mannose/hybrid and limited complex N-glycans. This suggested that variations in AJ stability were accompanied by quantitative and qualitative changes in E-cadherin N-glycosylation. To further examine the role of N-glycans in AJ function, we generated E-cadherin N-glycosylation variants lacking selected N-glycan addition sites. Characterization of these variants in CHO cells, lacking endogenous E-cadherin, revealed that site 1 on ectodomain 4 was modified with a prominent complex N-glycan, site 2 on ectodomain 5 did not have a substantial oligosaccharide, and site 3 on ectodomain 5 was decorated with a high mannose/hybrid N-glycan. Removal of complex N-glycan from ectodomain 4 led to a dramatically increased interaction of E-cadherin-catenin complexes with vinculin and the actin cytoskeleton. The latter effect was further enhanced by the deletion of the high mannose/hybrid N-glycan from site 3. In MDCK cells, which produce E-cadherin, a variant lacking both complex and high mannose/hybrid N-glycans functioned like a dominant positive displaying E-cadherin extracellular domains (ECs) on adjacent cells in a calcium-dependent manner (5), whereas the intracellular domains anchor the cadherin to the actin cytoskeleton via catenins. β-Catenin and γ-catenin (plakoglobin) bind directly to the distal region of the cadherin cytoplasmic tail in a mutually exclusive manner. They also recruit α-catenin, which links the cadherin, either directly or indirectly, to the actin cytoskeleton (4–7). The linkage of AJs to the actin cytoskeleton can be further stabilized through the binding of cytoskeletal proteins such as vinculin. Although the binding between E-cadherin ECs does not require association with the actin filaments, the interaction via catenins and cytoskeletal proteins provides strength to AJs by holding together the clustered receptors at cell-cell contacts (8).

E-cadherin can be post-translationally modified by phosphorylation, O-glycosylation and N-glycosylation. Casein kinase II, a serine-threonine kinase, phosphorylates the cytosolic tail of E-cadherin and enhances binding to β-catenin (9). Cytoplasmic O-glycosylation of the E-cadherin cytosolic tail has been shown to occur in response to endoplasmic reticulum (ER) stress and inactivate E-cadherin-mediated intercellular adhesion by preventing its transport to the cell membrane (10). In addition, E-cadherin can be N-glycosylated: the mouse E-cadherin has three N-glycan addition sites, one in EC4 and two in EC5, whereas human and canine E-cadherins have an additional site, each in different parts of the extracellular region. Even though N-glycans represent the most prominent modification, contributing up to 20% of the total mass of the E-cadherin molecule, virtually nothing is known about their role in the stability of AJs.

N-Glycans modify proteins at asparagine residues within the consensus sequence NX(S/T), where X can be any amino acid with the exception of proline (11–13). However, not every potential N-glycan addition site on a given N-glycoprotein is modified, and frequently there are variations in the number of sites occupied by N-glycans, their overall sizes, and composition. This microheterogeneity reflects regulated changes in the activities of key enzymes that function in the synthesis and processing of N-glycans (11, 12, 14, 15). For instance, N-glycan site occupancy has been shown to be regulated with growth and development by changes in the expression of GPT, the enzyme that initiates the synthesis of the lipid-linked oligosaccharide precursor in the ER (14). A high level of GPT expression results in the presence of a prominent complex N-glycan addition site.

**N-Glycosylation Affects the Molecular Organization and Stability of E-cadherin Junctions**

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Epithelial cell-cell adhesion is mediated by E-cadherin, an intercellular N-glycoprotein adhesion receptor that functions in the assembly of multiprotein complexes anchored to the actin cytoskeleton named adherens junctions (AJs). E-cadherin ectodomains 4 and 5 contain three potential N-glycan addition sites, although their significance in AJ stability is unclear. Here we show that sparse cells lacking stable AJs produced E-cadherin that was extensively modified with complex N-glycans. In contrast, dense cultures with more stable AJs had scarcely N-glycosylated E-cadherin modified with high mannose/hybrid and limited complex N-glycans. This suggested that variations in AJ stability were accompanied by quantitative and qualitative changes in E-cadherin N-glycosylation. To further examine the role of N-glycans in AJ function, we generated E-cadherin N-glycosylation variants lacking selected N-glycan addition sites. Characterization of these variants in CHO cells, lacking endogenous E-cadherin, revealed that site 1 on ectodomain 4 was modified with a prominent complex N-glycan, site 2 on ectodomain 5 did not have a substantial oligosaccharide, and site 3 on ectodomain 5 was decorated with a high mannose/hybrid N-glycan. Removal of complex N-glycan from ectodomain 4 led to a dramatically increased interaction of E-cadherin-catenin complexes with vinculin and the actin cytoskeleton. The latter effect was further enhanced by the deletion of the high mannose/hybrid N-glycan from site 3. In MDCK cells, which produce E-cadherin, a variant lacking both complex and high mannose/hybrid N-glycans functioned like a dominant positive displaying increased interaction with γ-catenin and vinculin compared with the endogenous E-cadherin. Collectively, our studies show that N-glycans, and complex oligosaccharides in particular, destabilize AJs by affecting their molecular organization.

E-cadherin is an N-glycoprotein cell-cell adhesion receptor that plays pivotal roles in epithelial tissue formation, cell polarity, and differentiation (1–3). E-cadherin mediates cell-cell adhesion through the assembly of multiprotein complexes linked to the actin cytoskeleton referred to as adherens junctions (AJs) (4–8). The AJs are formed by the binding of E-cadherin extracellular domains (ECs) on adjacent cells in a calcium-dependent manner (5), whereas the intracellular domains anchor the cadherin to the actin cytoskeleton via catenins. β-Catenin and γ-catenin (plakoglobin) bind directly to the distal region of the cadherin cytoplasmic tail in a mutually exclusive manner. They also recruit α-catenin, which links the cadherin, either directly or indirectly, to the actin cytoskeleton (4–7). The linkage of AJs to the actin cytoskeleton can be further stabilized through the binding of cytoskeletal proteins such as vinculin. Although the binding between E-cadherin ECs does not require association with the actin filaments, the interaction via catenins and cytoskeletal proteins provides strength to AJs by holding together the clustered receptors at cell-cell contacts (8).

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2 The abbreviations used are: AJ, adherens junction; EC, extracellular domain; ER, endoplasmic reticulum; GPT, dolichol phosphate-dependent N-acetylglycosaminidase; PNGaseF, PNGaseF; EndoH, endoglycosidase H; CHO, Chinese hamster ovary; MDCK, Madin-Darby canine kidney; DSA, Datura stramonium agglutinin; GNA, Galanthus nivalis agglutinin; SNA, Sambucus nigra agglutinin.
in abundant protein N-glycosylation during cell proliferation and tissue morphogenesis, and down-regulation of GPT leads to diminished N-glycosylation in growth-arrested cells and cytodifferentiated tissues (14, 16).

N-Glycans have a documented role in protein folding, and they have been shown to inhibit protein aggregation, increase protein solubility, and influence the binding of chaperones (11, 12). In addition, N-glycans function in protein secretion, turn-over, and bioactivity, and increasing evidence points to their role in intracellular signaling events (17–21). Recent studies have also revealed that N-glycans are important players in cell adhesion. Ablant E-cadherin-mediated cell-cell interactions (22). Increased β(1,6)glycan branching on β1 integrin subunit leads to diminished α5β1 integrin clustering and increased cell migration (21). A similar increase of β(1,6)glycan branching on N-cadherin has been shown to lead to a marked decrease of cell-cell adhesion in human fibrosarcoma HT1080 and mouse NIH3T3 cells (20). In the yeast Saccharomyces cerevisiae, attenuation of protein N-glycosylation causes increased cell aggregation (23).

We have been interested in examining how the presence of, and changes in N-glycan structures on E-cadherin ECs affected its adhesive function. Previously, inhibition of protein N-glycosylation with tunicamycin had been shown not to interfere with the formation of E-cadherin-mediated AJs, indicating that N-glycans themselves were not the adhesive structures (24). Our earlier studies with the postnatally developing hamster and mouse submandibular glands, however, provided evidence that, during tissue morphogenesis, when GPT expression was high, E-cadherin was primarily in unstable cell-cell contacts, whereas during cytodifferentiation, which was accompanied by down-regulation of GPT expression, there was increased association of E-cadherin with stable AJs (16, 25). These data suggested that N-glycans might function in modulating E-cadherin adhesive stability. Here, we provide evidence that reduced N-glycosylation of E-cadherin with complex N-glycans leads to its preferential association with the actin cytoskeleton. We further support this finding by showing that, in Chinese hamster ovary (CHO) cells, an E-cadherin N-glycosylation variant lacking the major complex N-glycan exhibited greater interaction with vinculin and increased association with the actin cytoskeleton. Likewise, in MDCK cells, a variant missing both complex and high mannose/hybrid N-glycans displayed an enhanced association with γ-catenin and vinculin compared with either the wild-type or endogenous E-cadherin. Collectively, our data show that N-glycans influence the stability of AJs by affecting their molecular organization. We propose that extensive modification of E-cadherin with complex N-glycans renders the formation of dynamic but weak AJs, whereas diminished N-glycosylation of E-cadherin promotes the establishment of stable AJs.

EXPERIMENTAL PROCEDURES

Reagents—Monoclonal antibody to the cytoplasmic region of human E-cadherin, as well as monoclonal antibodies α-catenin, β-catenin, and γ-catenin, were obtained from BD Transduction Laboratories. Monoclonal antibody to the Myc tag (clone 9B11) was purchased from Cell Signaling, to vinculin (clone V284) from Upstate Biotechnology, to actin (pan Ab-5, clone ACTN05) from NeoMarkers, and to α-tubulin (clone B-5-1-2) from Sigma. Rhodamine-tagged phalloidin, used for visualization of F-actin, and 4',6-diamidino-2-phenylindole, for visualization of nuclear acids, were obtained from Molecular Probes. Secondary antibodies for immunostaining included goat anti-mouse IgG(Fc), which was derivatized with fluorescein isothiocyanate (Molecular Probes). For Western blot analyses horseradish peroxidase-conjugated secondary antibodies were obtained from Amer sham Biosciences. Lectin blot analyses were performed using the DIG glycan differentiation kit (Roche Applied Science).

Vector Construction and in Vitro Mutagenesis—Human E-cadherin (GenBank™ accession no. Z13009) was cloned into pCMV/5Bmyc vector (Stratagene). To produce the E-cadherin N-glycan site mutations singly and in different combinations, a PCR-based site-directed mutagenesis was carried out using a QuikChange XL site-directed mutagenesis kit (Stratagene). The site-directed mutagenesis primers for replacement of the asparagine for glutamine in the N-glycosylation sites (Asn-404 → Gln, Asn-468 → Gln, and Asn-483 → Gln) were created as follows: for E-cadherin Asn-404 → Gln variant: 5'-GAGCA-CGTGAAAGCAGACGTACACG; and 3'-TAGG-GCTGTGACATGCTGCTCACGTCGTC'; for E-cadherin Asn-468 → Gln variant: 5'-GACCTTTCCACAGACATCTC-CCTTCAACAGCA and 3'-TGCTGTAGGGAGGATGTCTG-GGGAGAAGGTC; and for E-cadherin Asn-483 → Gln variant: 5'-GGGGCGATGTCGCCAGTTGACATCTC and 3'-GTGTAAGAGTGGATGTCTGTGGAGAAGGTC'. All mutations were verified by sequencing.

Cell Culture, Transfection, and Preparation of Cell Lysates—CHO cells were grown in minimum essential medium α medium (Invitrogen) supplemented with 10% fetal calf serum, penicillin, and streptomycin. MDCK cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal calf serum, penicillin, and streptomycin in 5% CO₂ at 37 °C. CHO cells were transiently transfected with cDNA encoding E-cadherin and its N-glycosylation variants using PolyFect transfection reagent (Qiagen), whereas MDCK cells were transfected using Lipofectamine 2000 (Invitrogen). Transfectants were cultured for 48 h, after which they were collected and extracted with 600 μl of ice-cold Triton X-100/β-octylglucoside buffer (10 mM imidazole, 100 mM NaCl, 1 mM MgCl₂, 5 mM Na₂EDTA, 1% Triton X-100, 0.87 mg/ml β-octylglucoside) containing 50 μg/ml aprotinin, 25 μg/ml soybean trypsin inhibitor, 100 μM benzamidine, 5 μg/ml leupeptin, and 0.5 μM phenylmethylsulfonyl fluoride). For determination of cytoskeletal association of E-cadherin and its N-glycosylation variants, CHO cells were extracted with ice-cold Triton extraction buffer (10 mM imidazole, 100 mM NaCl, 1 mM MgCl₂, 5 mM Na₂EDTA, 1% Triton X-100, pH 7.4, containing 50 μg/ml aprotinin, 25 μg/ml soybean trypsin inhibitor, 100 μM benzamidine, 5 μg/ml leupeptin, and 0.5 μM phenylmethylsulfonyl fluoride). The Triton-soluble and -insoluble fractions were separated by centrifugation at 12,000 × g for 10 min. The resulting Triton-insoluble pellet was re-extracted with β-octylglucosidase (0.87 mg/ml) in the above buffer in the absence of Triton. Protein
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concentrations were determined by using a BCA protein assay (Pierce).

**PNGaseF and EndoH Digestions**—Total cell lysates were digested with 1 unit of PNGaseF and/or EndoH (New England Biolabs) for 1 h at 37 °C, loaded onto a 7.5% SDS-PAGE, and examined by Western blotting. For controls, samples were incubated without the enzymes.

**Western Blotting**—Total cell lysates were fractionated on either 4–12% or 7.5% SDS-PAGE and blotted onto nitrocellulose membranes (Invitrogen). For comparison of expression levels of the wild type E-cadherin and its N-glycosylation variants in CHO and MDCK cells, typically 10 μg of total cellular protein was used. For immunoblot analyses of Triton-soluble fractions, samples were loaded at equal amounts of protein (10 μg). Their respective insoluble fractions were loaded at volumes equal to the soluble fractions so that direct comparisons could be made. The samples were blocked in PBS-Tween (PBST) (20 mM Tris/137 mM NaCl/0.1% Tween 20, pH 7.6) with 10% milk, and membranes were incubated with primary antibodies at appropriate dilutions in PBST with 5% milk for 2 h at room temperature. Next, membranes were washed four times with PBST solution, followed by incubation with horseradish peroxidase-linked secondary antibody (1:3000) in PBST with 5% milk. The results were visualized with ECL Plus Detection Reagents (Amersham Biosciences).

**Immunoprecipitation**—Equal amounts of total protein (200 μg) were precleared with 30 μl of protein G (Sigma) and incubated 2 h at 4 °C. After centrifugation at 12000 rpm for 1 min, supernatant was incubated for 2 h at 4 °C with 2.5 μg of antibodies against either E-cadherin, or β-catenin, or the myc tag and 30 μl of protein G. Next, the beads were washed three times with the lysis buffer (10 mM Tris HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, and 0.5% Triton X-100). Samples were resuspended in 100 μl of 2× SDS sample buffer and boiled 5 min at 95 °C prior to analyses by Western and lectin blotting.

**Lectin Blotting**—Polyvinylidene difluoride membranes were incubated overnight in blocking reagents (Roche Applied Science) at 4 °C. Next the blots were washed twice with TBS (50 mM Tris, 150 mM NaCl, pH 7.5) and once with lectin buffer 1 (TBS, 1 mM MgCl₂, 1 mM MnCl₂, pH 7.5). Subsequently, the blots were incubated for 1 h with digoxigenin-labeled lectins Galanthus nivalis agglutinin (GNA), Sambucus nigra agglutinin (SNA), and Datura stramonium agglutinin (DSA) (1 μg/ml). Membranes were washed three times with TBS and incubated with anti-digoxigenin-AP conjugate (diluted 1:1000 in TBS). Reactivities to specific lectins were detected using ECL Plus Detection Reagents and 3,7-diamine-tagged phalloidin, and 4’,6-diamidino-2-phenylindole. Next, cells were rinsed four times with PBS and mounted in Vectashield (Vector Laboratories, Inc.). The immunostained samples were analyzed with a Nikon Eclipse TE300 epifluorescence microscope.

**RESULTS**

**N-Glycosylation of E-cadherin Is Cell Density-dependent**—Previous studies have shown that protein N-glycosylation is proliferation-dependent, being abundant in dividing cells and diminished in dense cultures (26). Similarly, the stability of AJs in cultured endothelial cells was shown to depend on cell density: sparse endothelial cells formed unstable AJs, whereas contact-inhibited cells produced AJs stably associated with the actin cytoskeleton (27). To determine whether the formation of E-cadherin-containing AJs in epithelial cells was also dependent on cell density, we first examined the association of E-cadherin with the actin cytoskeletal membrane fraction in sparse and dense MDCK cultures using Triton solubility criteria (25, 27, 28). We used this approach because Triton insolubility of cadherins has been correlated with linkage to the actin cytoskeleton and association with lipid rafts (29, 30). As shown in Fig. 1A, the majority of E-cadherin from sparse cultures was found in the actin-unassociated (Triton-soluble) membrane fraction. Also, in dense cultures, E-cadherin in the actin-unassociated fraction from sparse cultures migrated as a large and diffuse band and with an overall higher molecular size compared with E-cadherin in the cytoskeletal fraction (Fig. 1B). This was most probably due to the presence of extensively N-glycosylated E-cadherin glycoforms in the actin-unassociated fraction. In contrast, in dense cultures, the abundance of E-cadherin in the actin-unassociated fraction was decreased while the level of E-cadherin in the cytoskeletal membrane fraction became augmented. Similarly, levels of β- and α-catenins in the cytoskeletal pools increased in dense cultures (Fig. 1A). Moreover, E-cadherin in the actin-unassociated fraction from sparse cultures was less diffuse and of a smaller size compared with E-cadherin in the cytoskeletal fraction. These data suggested that scarcely N-glycosylated E-cadherin was preferentially associated with the actin-cytoskeletal membrane fraction.

We next evaluated E-cadherin N-glycosylation from sparse and dense cultures using sensitivity to PNGaseF and EndoH. Both enzymes remove N-glycans from proteins, although they differ in glycan specificities: EndoH is an endoglycosidase that hydrolyzes only high mannose and some hybrid N-glycans at N-glycosylation from proteins, whereas PNGaseF is an amidase that differs in glycan specificities: EndoH is an endoglycosidase that hydrolyzes only high mannose and some hybrid N-glycans at the chitobiose core, whereas PNGaseF is an amidase that cleaves most N-glycans, including high mannose, hybrid, and complex structures, at the asparagines residues. A noted exception to PNGaseF sensitivity includes a class of complex N-glycans modified by fucose at the chitobiose core (26). We used this approach because Triton insolubility of cadherins has been correlated with linkage to the actin cytoskeleton and association with lipid rafts (29, 30). As shown in Fig. 1A, the majority of E-cadherin from sparse cultures was found in the actin-unassociated (Triton-soluble) membrane fraction. Also, in dense cultures, E-cadherin in the actin-unassociated fraction from sparse cultures was less diffuse and of a smaller size compared with E-cadherin in the cytoskeletal fraction. These data suggested that scarcely N-glycosylated E-cadherin was preferentially associated with the actin-cytoskeletal membrane fraction.
herin from dense cultures displayed detectable EndoH and some PNGase F sensitivity providing evidence that it was decorated with high mannose/hybrid and some complex N-glycans. The finding that E-cadherin from sparse cultures was more extensively modified with complex N-glycans than in dense cultures was confirmed by the reactivity of immunoprecipitated E-cadherins with SNA and DSA lectins (Fig. 1C). Both lectins recognize complex N-glycans, with SNA being specific for terminal sialic acid linked \( \alpha(2-6) \) to galactose, and DSA interacting with terminal galactose linked \( \beta(1-4) \) to GlcNAc residues in complex and hybrid structures. There was no significant difference in the reactivity of E-cadherins from sparse and dense cultures with GNA, a lectin specific for high mannose/hybrid N-glycans (Fig. 1C). This may reflect the presence of core mannose residues on complex N-glycans in sparse cells and high mannose/hybrid N-glycans as well as a few core mannose residues in complex type structures in dense cultures. Collectively, these data show that there were differences between the sizes and types of N-glycans that modified E-cadherin in sparse and dense cultures. Notably, E-cadherin from sparse cultures that formed unstable AJs was modified with substantial complex N-glycans, whose abundance diminished in dense cultures. Also, there was a notable decrease in the overall levels of E-cadherin in dense compared with sparse cultures (Fig. 1, A and B), suggesting that changes in N-glycosylation affected its stability. We concluded that E-cadherin N-glycosylation status is cell density-dependent.

**FIGURE 1. N-Glycosylation of E-cadherin in MDCK cells is density-dependent.** A, comparison of E-cadherin mobilities and differential partitioning of E-cadherin into Triton-soluble (S) and -insoluble (I) fractions from sparse and dense MDCK cultures. MDCK cells were extracted with the Triton extraction buffer, and Triton-insoluble pellets were solubilized with \( \beta \)-octylglucoside. Equal amounts of protein (10 \( \mu \)g) from the Triton-soluble fractions and corresponding volumes from the Triton-insoluble fractions were analyzed for E-cadherin, \( \beta \)-catenin, and \( \alpha \)-catenin expression by Western blotting. B, N-glycosylation of E-cadherin in sparse and dense cultures. Total cell lysates from sparse and dense cultures were treated with either EndoH (H) or PNGaseF (F) prior to analysis on Western blots. E-cadherin from sparse cultures underwent a significant shift in mobility following PNGaseF treatment, indicating that it was modified primarily with complex N-glycans. E-cadherin from dense cultures exhibited decreased overall N-glycosylation and modification with EndoH- and some PNGaseF-sensitive N-glycans. C, analysis of N-glycans on E-cadherin from sparse and dense cultures using reactivity to lectins. Cell extracts (1 mg of total protein) from sparse and dense cultures were immunoprecipitated with anti-E-cadherin antibodies (10 \( \mu \)g), immunoprecipitates were fractionated on 4–12% SDS-PAGE, blotted onto polyvinylidene difluoride membranes, and probed with lectins SNA, DSA, and GNA. The amount of E-cadherin in each sample was assessed by reprobing of the blots with E-cadherin antibodies. Bar graph, extent of lectin reactivity was determined as ratio of density after normalization to E-cadherin. Sparse cells produced E-cadherin that displayed 50 and 20% more reactivity to SNA and DSA lectins, respectively, than E-cadherin from dense cultures. SNA recognizes sialic acid linked \( \alpha(2-6) \) to galactose, whereas DSA is specific for Gal[\( \beta(1-4) \)GlcNAc in complex and hybrid N-glycans. Sparse and dense MDCK cells have similar reactivity to GNA, which recognizes terminal mannose, \( \alpha(1-3) \), \( \alpha(1-6) \), or \( \alpha(1-2) \) linked to mannose.
Generation of E-cadherin N-Glycosylation Variants—To examine the role of N-glycans and their site occupancy in the stability of AJs, we generated seven N-glycosylation variants lacking selected potential N-glycan addition sites singly and in different combinations (Fig. 2A; “Experimental Procedures”). Specifically, we focused on the three N-glycosylation sites located in EC4 and EC5 that are shared by the human, canine, and mouse E-cadherins. To avoid interfering with E-cadherin structure and/or function, individual potential N-glycan sites at Asn-404, EC4, and at Asn-468 and Asn-483, EC5, were deleted and substituted with Gln, where least perturbation was expected (Figs. 2A). The cytomegalovirus (CMV) promoter was used to drive transient expression of these myc-tagged E-cadherins (Fig. 2B). First, we examined expression of E-cadherin structure and/or function, individual potential N-glycan sites at Asn-404, EC4, and at Asn-468 and Asn-483, EC5, were deleted and substituted with Gln, where least perturbation was expected (Figs. 2A). The cytomegalovirus (CMV) promoter was used to drive transient expression of these myc-tagged E-cadherins (Fig. 2B).

FIGURE 2. Construction of E-cadherin N-glycosylation variants lacking selected N-glycan addition sites. A, wild-type human E-cadherin cDNA was used as a template for the generation of mutations in potential N-glycan addition sites singly and in combinations, by substituting asparagine (Asn) within the N-glycan addition consensus sequences with glutamine (Gln), at indicated sites in EC4 and EC5, as described under “Experimental Procedures.” 1–5, E-cadherin ECs 1–5; filled box, transmembrane domain; C, cytoplasmic domain; myc tag at the C-terminal region; three N-glycans at EC4 and EC5 are indicated. B, schematic representation of E-cadherin N-glycosylation variants with myc tags at their carboxyl-terminal regions. E-cad, wild-type E-cadherin; V1, lacking Asn-404; V2, lacking Asn-468; V3, lacking Asn-483; V12, lacking Asn-404 and Asn-468; V13, lacking Asn-404 and Asn-483; V23, lacking Asn-468 and Asn-483; V123, lacking Asn-404, Asn-468, and Asn-483.

Evaluation of the remaining N-glycans on E-cadherin variants V1, V3, and V13 using sensitivities to EndoH and PNGaseF confirmed that site 1 in EC4 was modified with the largest N-glycan that was complex in type. As shown in Fig. 3B, variant V1 migrated as a smaller species than the wild-type E-cadherin. After the treatment of V1 with EndoH, its molecular size
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FIGURE 3. Characterization of E-cadherin N-glycosylation variants in CHO cells. A, expression of E-cadherin N-glycosylation variants in CHO cells. CHO cells were transiently transfected with E-cadherin N-glycosylation variants, and expression levels were examined by Western blot using E-cadherin antibody specific to the cytoplasmic domain. B, characterization of the N-glycosylation status of E-cadherin variants V1, V3, and V13 in transiently transfected CHO cells. Total CHO cell lysates from selected transfectants were treated with EndoH (H) and PNGaseF (F) and analyzed for mobility shifts by Western blot. Although the wild-type E-cadherin displayed some sensitivity to EndoH, it was mostly PNGaseF-sensitive, indicating that it was modified with a major complex N-glycans and some high mannose/hybrid structures. Treatment of V1 with EndoH resulted in a substantial mobility shift that was not enhanced further by PNGaseF, indicating that site 1 in EC4 was modified with a large complex N-glycan and its absence in V1 rendered the remaining high mannose/hybrid oligosaccharide EndoH-sensitive. V3 was primarily PNGase-F sensitive and produced a mobility shift that was greater in magnitude compared with EndoH- or PNGaseF-treated V1, indicating that site 3 in EC3 was modified with a smaller high mannose/hybrid oligosaccharide. As expected, removal of N-glycans from both sites 1 and 3 (V13) produced no detectable mobility shift with either EndoH or PNGaseF, indicating that N-glycans 1 and 3 were the major contributors to E-cadherin N-glycosylation. Shown is one of two independent experiments. C, immunofluorescence localization of E-cadherin and variants V1 and V13 in CHO cells. Transiently transfected CHO cells with E-cadherin N-glycosylation variants V1 and V3 were grown to 80–90% confluence, fixed with 3.7% paraformaldehyde, and immunostained for E-cadherin using an antibody that recognizes its cytoplasmic domain. The images shown reflect 60% of cells expressing E-cadherins from a total population of 5 × 10^6. The results represent one of three independent experiments.

became comparable to deglycosylated E-cadherin with no additional effect of PNGaseF. This shift in response to EndoH reflected the contribution of the remaining N-glycan at site 3 in ectodomain 5. Indeed, variant V3 migrated between the wild-type and deglycosylated E-cadherin and displayed EndoH-resistance but a detectable shift in mobility after the treatment with PNGaseF, although not as substantial as the wild-type E-cadherin (Fig. 3B). This is consistent with V3 being modified with the complex N-glycan at site 1 and lacking the high mannose/hybrid oligosaccharide at site 3. Variant V13 exhibited no detectable shift in mobility after either EndoH or PNGaseF treatments indicating that removal of N-glycans from sites 1 and 3 produced E-cadherin with either a minor or no N-glycan remaining at site 2 and whose removal did not have a pronounced effect on E-cadherin mobility (Fig. 3B, V13). Taken together, these results indicated that in CHO cells E-cadherin was modified with N-glycans whose sizes and types ranged from the largest complex N-glycan at site 1, intermediate high mannose/hybrid oligosaccharide at site 3, and minor if any N-glycan at site 2. It is interesting to note that, although human E-cadherin has an additional N-glycan site (not shared with murine and canine E-cadherins) located nine amino acids downstream from site 1 and which was not removed in any of the variants, this site did not become utilized to a significant extent upon the removal of site 1.

To validate that scarcely N-glycosylated E-cadherins could be targeted to the plasma membrane in CHO cells, we examined cellular distribution of variants V1 and V13 by immunofluorescence localization using an antibody to the cytoplasmic domain of E-cadherin. As shown in Fig. 3C, similar to the wild-type E-cadherin, variants V1 and V13 were well organized at cell-cell contact sites. This suggested that different E-cadherin glycoforms are likely to participate in the formation of intercellular junctions.

N-Glycans Affect the Composition of E-cadherin Protein Complexes and Linkage to the Cytoskeleton in CHO Cells—The stability of AJs has been shown to depend on the interaction of the E-cadherin cytoplasmic domain with cytoskeletal proteins. To determine whether N-glycans affected these associations, the wild-type E-cadherin and variants V1, V3, and V13 were immunoprecipitated from total CHO cell lysates with an antibody to E-cadherin and analyzed for associated proteins by immunoblot (Fig. 4A). The efficiency of E-cadherin immunoprecipitations was assessed by stripping and reprobing blots with an antibody to E-cadherin. Variant V1 bound β-catenin similar to the wild-type E-cadherin, whereas variants V3 and especially V13 exhibited decreased association with β-catenin. Diminished interaction between variant V13 and β-catenin was further confirmed by immunoprecipitating total cell lysates with an antibody to β-catenin followed by blotting for E-cadherin (Fig. 4B). Because β-catenin binds E-cadherin in the ER and promotes its transport to the cell membrane (32), its decreased association with V13 may reflect the effect of N-glycans on E-cadherin–β-catenin interaction early in the secretory pathway. No major changes in the association with α-catenin were detected between the wild-type and E-cadherin variants (data not shown). Therefore, we next examined whether removal of N-glycans affected the interaction between E-cadherin–catenin complexes with the cytoskeletal protein vinculin, because this protein has been shown to have a stabilizing effect on AJs (4, 33, 34). As shown in Fig. 4A, variants V1 and V13 displayed a 4- and 3.5-fold increase, respectively, in the amount of associated vinculin, whereas variant V3 had a much lesser effect (1.3-fold increase) over the wild-type E-cadherin (Fig. 4A, bar graph). The binding of vinculin to E-cadherin variant-containing complexes was verified with reverse immunoprecipitation.
using the antibody to vinculin and blotting for E-cadherin and the catenins (data not shown). These results indicated that modification of site 1 on ectodomain 4 with complex N-glycans interfered with the recruitment of vinculin to AJs. In contrast, the high mannose/hybrid N-glycan on inner-most site 3 in ectodomain 5 had no significant effect on the interaction of E-cadherin catenin complexes with vinculin.

Increased interaction of E-cadherin N-glycosylation variants with vinculin suggested that they were in a greater association with the actin cytoskeleton compared with the wild-type E-cadherin. Thus, we examined the extent of Triton insolubility of wild-type E-cadherin and variants V1, V3, and V13 in CHO cells. All E-cadherins were found primarily in the actin-unassociated pools. Nonetheless, variants V1 and V13 displayed increases of 3- and 5-fold, respectively, in their association with the cytoskeleton over the wild-type E-cadherin (Fig. 4C, bar graph). This suggested that greater amounts of vinculin found in complexes with variants V1 and V13 (Fig. 4A) promoted
FIGURE 5. Expression of E-cadherin N-glycosylation variants in MDCK cells. A, comparison of exogenous wild-type E-cadherin (Ecad) and variants V1, V3, and V13 with the endogenous E-cadherin (Mock) in transiently transfected MDCK cultures. MDCK cells were extracted with Triton/β-octylglucoside extraction buffer, and whole cell homogenates were examined for expression levels of exogenous E-cadherins by Western blot using an antibody to Myc and compared with the expression of total E-cadherin with the antibody to E-cadherin. B, expression of E-cadherin, β-catenin, and γ-catenin in total cell lysates (TCL) of MDCK cells transiently transfected with the empty vector (Mock), the wild-type E-cadherin (Ecad), and variant V13. C, composition of wild-type E-cadherin (Ecad) and variant V13 protein complexes in transiently transfected MDCK cells. Cells were extracted with Triton extraction buffer, and E-cadherins were immunoprecipitated from whole cell lysates with the Myc antibody and fractionated on 4–12% SDS-PAGE, and their association with catenins and vinculin was assessed by Western blotting. Bar graph, quantification of catenins and vinculin associated with the wild-type E-cadherin and variant V13. Amounts of association were determined from the ratios of densities after normalization to E-cadherins. Complexes containing variant V13 exhibited increased amounts of associated γ-catenin and vinculin compared with the exogenous wild-type E-cadherin. D, composition of total E-cadherin-containing protein complexes in MDCK cells transiently transfected with the wild-type E-cadherin and variant V13. Based on the amount of total E-cadherin in the complex, variant V13 had more associated γ-catenin and vinculin compared with either the wild-type (Ecad) or endogenous (Mock) E-cadherin. E, E-cadherin N-glycosylation variant V13 localizes to the plasma membrane in MDCK cells. MDCK cells, transiently transfected with the wild-type E-cadherin or variant V13, were processed for indirect immunofluorescence staining using a Myc antibody to detect exogenous E-cadherins. Cells were counterstained with rhodamine-phalloidin for F-actin and with 4′,6-diamidino-2-phenylindole for nuclei to assess architecture and organization. Typically, 10% of cells were c-Myc-positive. Results represent one of two independent experiments.
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cytoskeletal association. Indeed, variant V3 was the least
cytoskeletal-associated, further indicating that interaction with vinculin was a determinant of Triton insolubility. Therefore, modification of site 1 with complex N-glycan inhibited the association of E-cadherin with the cytoskeleton, whereas the presence of high mannose/hybrid structures at site 3 did not. Removal of both complex and high mannose/hybrid N-glycans from sites 1 and 3 resulted in a greater association with the actin cytoskeleton compared with the removal from site 1 alone, suggesting additional conformational effects that impact AJ stability. It should be noted that expression of β-catenin in the Triton-soluble fraction was elevated to a similar extent in cells transfected with the wild-type E-cadherin and with variants V1, V3, and V13 (Fig. 4C, Soluble), indicating that the removal of N-glycans did not negatively effect β-catenin expression (32).

E-cadherin N-Glycosylation Variant V13 Functions as a Dominant Positive in MDCK Cells—Because scarcely N-glycosylated E-cadherins could drive the assembly of AJs in CHO cells, we asked whether they could compete with the endogenous E-cadherin for the formation of AJs in MDCK cells. Total cell lysates from MDCK cultures transiently transfected with wild-type and E-cadherin N-glycosylation variants V1, V3, and V13 were analyzed by immunoblot either with an antibody to the Myc tag, to assess exogenous E-cadherin expression, or with an antibody to E-cadherin to determine total E-cadherin expression. As shown in Fig. 5A, E-cadherin variants displayed mobility shifts that reflected the absence of specific N-glycans. In addition, they were expressed at levels lower than the wild-type E-cadherin, similar to the scenario in CHO cells (see Fig. 3, A and B). Interestingly, blotting with an antibody to E-cadherin for total E-cadherin expression revealed that the exogenous wild-type E-cadherin and variant V3 migrated as higher molecular weight species compared with the endogenous E-cadherin. This difference in the mobility of the exogenous human E-cadherin from the endogenous canine E-cadherin was unlikely to reflect variations in the fourth potential N-glycan addition site, because in CHO cells this site was not significantly utilized (Fig. 3, A and B). Rather, the endogenous E-cadherin was less N-glycosylated because of the dense status of MDCK cells at the time of harvest, and transfection of MDCK cells with exogenous E-cadherins led to an up-regulation of cellular N-glycosylation (Fig. 5A, total E-cadherin). Compared with the exogenous wild-type E-cadherin, transfected variants V1 and V13 migrated as smaller molecular weight species, indicating that they had fewer and/or smaller N-glycans. As expected, expression of exogenous wild-type E-cadherin in MDCK cells resulted in up-regulation of β-catenin and γ-catenin levels (Fig. 5B). Although transfection with variant V13 also caused an increase in β-catenin abundance, albeit less substantial than the wild-type E-cadherin, it did not result in an up-regulation of γ-catenin (Fig. 5B).

To compare the composition of AJs formed by the exogenous wild-type E-cadherin with AJs formed by variant V13, total cell lysates from the respective MDCK transfectants were immunoprecipitated with an antibody to the Myc tag and analyzed for the associated catenins and vinculin (Fig. 5C). Although variant V13 exhibited little change in its association with β-catenin, it showed a marked increase (>2-fold) in the recruitment of γ-catenin. Because β-catenin and γ-catenin bind E-cadherin in a mutually exclusive manner, this suggested that V13 interacted with γ-catenin independently of β-catenin. Moreover, although no significant changes in the levels of α-catenin were detected (data not shown) variant V13 displayed greater (4-fold) association with vinculin than the wild-type E-cadherin (Fig. 5C). Thus, it is likely that the increased association between V13 and vinculin occurred through complexes involving γ-catenin.

To further evaluate how variant V13-driven AJs compared with the endogenous E-cadherin-mediated complexes, we examined the association of immunoprecipitated total E-cadherin from mock, wild-type, and variant V13-transfected MDCK cells with catenins and vinculin using an antibody to E-cadherin (Fig. 5D). All three E-cadherins, the endogenous, the exogenous wild-type, and variant V13, showed robust interaction with β-catenin (Fig. 5D). In contrast to the endogenous and exogenous wild-type E-cadherins, however, variant V13 exhibited greater association with γ-catenin and vinculin (Fig. 5D). Therefore, we conclude that diminished N-glycosylation of E-cadherin leads to an increased recruitment of γ-catenin and vinculin to AJs in MDCK cells. Moreover, exogenous wild-type E-cadherin and its N-glycosylation variants were competent to form AJs independent of the endogenous E-cadherin. Because variant V13 displayed increased association with stabilizing and cytoskeletal proteins, it had the ability to enhance intercellular adhesion and thus functioned as a dominant positive.

Indirect immunofluorescence staining of the wild-type E-cadherin- and V13-harboring MDCK cells using an antibody to myc validated that variant V13 localized to the plasma membrane in MDCK cells. In both transfectants, the exogenous E-cadherins were targeted to cell borders (Fig. 5E). Moreover, concomitant staining of F-actin and V13 showed regions of colocalization, as was found for the wild-type E-cadherin (Fig. 5E). We conclude that, in MDCK cells, variant V13 localized to the cell membrane where it formed stable AJs.

DISCUSSION

N-Glycosylation is one of the fundamental metabolic pathways with profound effects on cell physiology. Cellular N-glycosylation is highly regulated in response to developmental, physiological, and environmental cues, in part through changes in the expression of glycosyltransferases that function in the ER and the Golgi (11, 13, 35, 36). These changes in cellular N-glycosylation are reflected in the N-glycosylation status of glycoproteins (20, 37, 38). One N-glycoprotein whose status is modulated by cell context is E-cadherin, an intercellular adhesion receptor with a pivotal role in a wide array of morphogenetic and physiological processes. E-cadherin-mediated AJs are dynamic in nature, subject to changes in composition and stability. Here, we were interested in determining if and how changes in the N-glycosylation status of E-cadherin are linked to AJ stability. Our studies show for the first time that both the amount and composition of N-glycans affect AJ stability by influencing the organization of E-cadherin-containing protein complexes and their association with the cytoskeleton.

One criterion for the determination of AJ stability has been Triton insolubility of cadherins and associated catenins. Such
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Triton insolubility has been correlated with cadherin linkage to the actin cytoskeleton and association with lipid rafts (29, 30). Lipid rafts, however, were shown to stabilize cadherin complexes at cell-cell contact sites (30). Our results show that unstable AJs in sparse cells contained E-cadherin that was primarily modified with complex N-glycans, whereas increased amounts of Triton-insoluble E-cadherin in dense cultures correlated with its modification with high mannose/hybrid oligosaccharides (Fig. 1). This diminished overall N-glycosylation of E-cadherin and preferential modification with high mannose/hybrid-type N-glycans, coincident with increased Triton insolubility of E-cadherin in dense cultures, indicate the need for a reduced number of complex N-glycans for E-cadherin junction stabilization. Indeed, characterization of E-cadherin N-glycosylation variants V1 and V3 in CHO cells confirmed that complex N-glycans at site 1 inhibit the formation of stable AJs (Figs. 3 and 4).

Because E-cadherin functional diversity relies on its ability to form distinct protein scaffolds with catenins, cytoskeletal, and signaling proteins, co-immunoprecipitation of E-cadherin with components of its protein ensembles has been used to align AJ composition with stability (39). Whereas the minimum requirement for AJ stabilization involves the interaction between the E-cadherin-β-catenin complex and the actin cytoskeleton through α-catenin (40), this cytoskeletal association can be further stabilized and enhanced by the recruitment of cytoskeletal proteins (4). Vinculin is one of the cytoskeletal proteins that forms scaffolds with AJs and promotes their stability (33, 34, 41, 42). The stabilizing effect of vinculin stems from its ability to interact with actin directly and to bind actin via vasodilator-stimulated phosphoprotein (4). Most recently, vinculin has been also reported to stabilize PTEN (phosphatase and tensin homologue deleted on chromosome 10), the lipid phosphatase that acts as a tumor suppressor, through the regulation of the interactions of β-catenin with a scaffold protein, membrane-associated guanylate kinase inverted-2 (43). Indeed, cancer cells that lack vinculin are migratory and highly metastatic, and transfection of exogenous vinculin into these cells diminishes migration (41). Our data demonstrate that when E-cadherin N-glycosylation variants V1 and V13, lacking prominent complex N-glycans, were expressed in CHO cells they bound more vinculin than the wild-type E-cadherin. A similar scenario was observed for variant V13 in MDCK cells, where it bound more vinculin than either the wild-type or the endogenous E-cadherin (Figs. 4 and 5). Moreover, greater recruitment of vinculin to variant V1- and V13-containing complexes in CHO cells correlated with increased Triton insolubility. Taken together, these data provide evidence for a link between diminished modification with complex N-glycans and stabilization of E-cadherin scaffolds. It is possible that similar to its role in stabilizing PTEN via AJ scaffolds in mouse F9 cells (43), vinculin promotes the recruitment of phosphatase(s) to V1- and V13-containing complexes. This scenario appears plausible, because an unwarranted increase in N-cadherin β(1,6)-oligosaccharides in HT1080 and NIH3T3 fibroblasts led to an increase in β-catenin phosphorylation and weakened cell-cell adhesion (20). In addition, protein tyrosine phosphatase 1B-like phosphatase was shown to stabilize N-cadherin junctions via dephosphorylation of β-catenin in the embryonic chick retina (44).

In MDCK cells, variant V13 formed AJs that comprised γ-catenin and vinculin (Fig. 5C). Although γ-catenin has a documented role as a component of desmosomes, it can compete with β-catenin and bind to the cytoplasmic tail of E-cadherin. Similar γ-catenin- and vinculin-containing AJs have been reported in lens fiber cells (45). Our recent studies with the developing mouse submandibular gland have also shown that stabilization of AJs during cytodifferentiation of salivary epithelial cells involves preferential recruitment of γ-catenin to E-cadherin complexes.3 It is possible therefore, that increased association of γ-catenin with E-cadherin ensembles reflects maturation and stabilization of epithelial AJs under specific developmental and physiological conditions. Alternatively, in endothelial cells, γ-catenin has been shown to provide a means to couple VE-cadherin complexes via desmoplakin to the intermediate filaments, in addition to their anchorage through β-catenin and α-catenin to the actin cytoskeleton (46). Thus, it

3 A. S. Menko, J. Walker, S. Khalil, M. P. Hoffman, J. A. Kreidberg, and M. A. Kukuruzinska, submitted for publication.
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cannot be excluded that the increased association of γ-catenin with variant V13 reflects yet another unidentified E-cadherin ensemble.

Numerous studies have shown that N-glycans affect protein turnover (11, 35). Indeed, the seven variants generated in this study were expressed in CHO cells at reduced levels (Fig. 3A). Likewise, expression of exogenous E-cadherin variants V1, V3, and V13 in MDCK cells was diminished compared with the wild-type E-cadherin (Fig. 5A). Moreover, the steady-state level of E-cadherin was lower in dense than in sparse cultures (Fig. 1A). The observed reduction in the expression levels of E-cadherin with decreased N-glycosylation status is likely to reflect the role of N-glycans in E-cadherin stability. This can be a consequence of variations in the synthesis and folding of E-cadherin variants in the ER. In light of the recent report showing the relationship between high mannose N-glycans, protein folding, and degradation (47), it is tempting to speculate that preferential modification of E-cadherin with high mannose N-glycans in dense cultures may play a role in regulating its turnover (Fig. 1B). Also, because variant V13 bound less β-catenin compared with the wild-type E-cadherin (Figs. 4 and 5), it is possible that N-glycans affect E-cadherin stability, in part, by modulating its interaction with β-catenin in the ER. Our recent studies show that cytodifferentiation of the submandibular gland is accompanied by both diminished N-glycosylation of E-cadherin and its increased cleavage, suggesting that N-glycosylation status of E-cadherin is directly related to its turnover.4

Lastly, it is unclear how the modification of E-cadherin ECs with N-glycans affects its homotypic interactions. Similar to other classic cadherins, the extracellular region of E-cadherin comprises five tandemly arranged domains. To date, several different models have been proposed to describe the adhesive bond(s) formed in trans by cadherin extracellular regions. Based on the crystal structures of the amino-terminal domains of N-cadherin and C-cadherin, two versions of the “linear zipper” model involve interactions between the outermost EC1 domains but via different adhesive binding interfaces (48, 49). In other models, two outermost ectodomains, EC1 and EC2, have been implicated in homophilic adhesion but via different mechanisms. In one case, the Ca2+ binding site between EC1 and EC2 domains of E-cadherin is involved in the cis-dimer formation, which then promotes another surface of EC1 to participate in adhesive binding (50). In contrast, recent biophysical quantitative adhesion measurements provided evidence for three types of adhesive interactions that mediate overlaps between either all five domains EC1–EC5, or the three outermost domains, EC1–EC3, or just between the EC1 domains (51). Because our data show that site 1 in EC4 is modified with the most substantial complex N-glycan, whereas site 3 in EC5 contains another prominent high mannose/hybrid oligosaccharide, it is possible that in addition to affecting the composition of protein complexes bound to E-cadherin cytoplasmic tails, these oligosaccharides adversely affect the interactions between the ECs through stearic hindrance. Studies are in progress to compare the strength of adhesion between the ECs of variants V1 and V13 with those from the wild-type E-cadherin. Previous studies have shown that aberrant N-glycosylation of cadherins leads to changes in cadherin-mediated cell-cell adhesion (20, 38). Overexpression of N-acetylgalactosaminyltransferase V, the Golgi glycosyltransferase that functions in the addition of complex N-glycans, caused increased modification of N-cadherin with β(1,6)-branched oligosaccharides and weakened cell-cell adhesion (20). Consistent with this finding, increased expression of N-acetylgalactosaminyltransferase III, another Golgi glycosyltransferase that competes with N-acetylgalactosaminyltransferase V by decreasing modification with β(1,6)-branched oligosaccharides, was shown to enhance E-cadherin-mediated cell-cell adhesion (38). Both studies linked increased presence of complex β(1,6)-N-glycans on cadherins with a greater phosphorylation status of β-catenin and diminished cell-cell adhesion. Nonetheless, they did not directly address how changes in cadherin N-glycosylation affected the composition of cadherin junctional complexes and their association with the cytoskeleton.

Collectively, our studies show that N-glycans affect the molecular organization of AJs by influencing their composition and association with the actin cytoskeleton. These changes are likely to facilitate a dynamic assembly/disassembly of cell–cell contacts during proliferation and, on the other hand, promote the formation of stable AJs in contact-inhibited cells. A model representing our current understanding of how N-glycosylation drives intercellular adhesion is shown in Fig. 6. Because transition from a proliferative to a quiescent state correlates with altered N-glycosylation of E-cadherin and enhanced cell-cell adhesion, the significance of modification of cadherin receptors with N-glycans extends to tissue development, homeostasis, and cancer. For instance, an unwarranted increase in core fucosylation of E-cadherin has been linked to an increased metastatic potential of lung cancer cells (52). Also, aberrant N-linked β(1,6)-branching is a feature of oncogenesis (15), and increased modification of N-cadherin with these glycans has been recently aligned with weakened adhesion in human fibrosarcoma HT1080 cells (20). Our own investigation of human oral squamous cell carcinomas provides evidence that cellular disorganization and tumor invasion are associated with highly N-glycosylated E-cadherin in the regions of the afflicted oral epithelium.5 Further work is needed to decipher how the site occupancy, size, and composition of N-glycans affect the organization of AJs and their anchorage to the cytoskeleton in development and disease. Studies are in progress to determine how N-glycosylation-induced changes in E-cadherin junctional complexes affect signaling pathways that converge on cell-cell adhesion.

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