Tetraspanin CD151 Regulates Growth of Mammary Epithelial Cells in Three-Dimensional Extracellular Matrix: Implication for Mammary Ductal Carcinoma In situ

Vera Novitskaya, Hanna Romanska, Marwa Dawoud, J. Louise Jones, and Fedor Berditchevski

Abstract

Tetraspanin CD151 is associated with laminin-binding integrins (i.e., α3β1, α6β1, and α6β4) and regulates tumor cell migration and invasion. Here, we examined the role of CD151 in proliferation of mammary epithelial cells using in vitro and in vivo models. Depletion of CD151 suppressed growth of HB2 cells, a nontumorigenic breast epithelial cell line, in three-dimensional (3D) extracellular matrices (ECM) and in Matrigel-based xenografts. Whereas the presence of α3β1 (but not α6 integrins) was necessary to support growth of HB2 cells in 3D ECM, the pro-proliferative activity of CD151 did not require direct interaction with integrins. Furthermore, depletion of CD151 potentiated formation of the internal lumen and partial restoration of polarity when HB2 cells were cultured in 3D ECM. This correlated with a decrease in phosphorylation levels of extracellular signal-regulated kinase 1/2 and cAkt in CD151-negative cells and increase in activation of caspase-3. Accordingly, the number of CD151-positive colonies with internal lumen was increased by ~5-fold when cells were cultured in the presence of MAP/ERK kinase (U0126) and phosphoinositide 3-kinase (LY29004) inhibitors. To establish the physiologic relevance of pro-proliferative and morphogenetic activities of CD151, we analyzed the expression of this tetraspanin in ductal carcinoma in situ (DCIS), which is characterized by neoplastic proliferation of mammary epithelial cells. Strong homogeneous membrane expression of CD151 was found to be associated with a high grade of DCIS (P = 0.004). Taken together, these results strongly suggest that CD151 complexes play a crucial role in the development of hyperproliferative diseases in the mammary gland. Cancer Res; 70(11) June 1, 2010; DOI: 10.1158/0008-5472.CAN-09-4330

Introduction

Four transmembrane domain proteins of the tetraspanin superfamily are associated with integrin adhesion receptors and are known to regulate motility and invasiveness of various cell types (1). It has been proposed that tetraspanins function through a special type of microdomains on the cell surface, which are referred to as tetraspanin-enriched microdomains (TERM or tetraspanin webs; ref. 2). It is thought that the function of TERM-associated integrins (e.g., α3β1 and α6 integrins) is influenced by other proteins within TERM, including cytoplasmic enzymes and adaptors (1). In addition to their motility-dependent functions, tetraspanins regulate cell-cell fusion (3), trafficking, and processing of the associated molecules (4) and can influence the lipid composition of the plasma membrane (5).

Early studies involving anti-tetraspanin monoclonal antibodies (mAb) have shown that various members of the tetraspanin superfamily also function as costimulatory molecules in T and B cells (6). Costimulatory/pro-proliferative activities of tetraspanins were linked to their ability to interact with critical components of the T-cell receptor complex, including CD4, CD8, CD25, and others. The involvement of tetraspanins in proliferation of hematopoietic cells was confirmed more recently using various knockout models (7–10). Although the underlying molecular mechanisms have not been investigated in most of these studies, the experiments using CD37-negative T cells have suggested that this tetraspanin is involved in dephosphorylation of Lck, a Src family tyrosine kinase responsible for delivering the proliferative signal from CD3 (8).

Tetraspanin CD151 is directly associated with laminin-binding integrins (i.e., α3β1, α6β1, α6β4, and α6β4) and is known to regulate cell motility (11–14). In epithelial cells, it also controls “group cell migration” (15). The involvement of CD151 in proliferation of nonhematopoietic cells remains controversial. There were no obvious proliferative defects in CD151-deficient mice and humans (16–19). Consistent with this, deletion of CD151 did not affect proliferation of primary endothelial cells on Matrigel in vitro (13). On the other hand,
proliferation of CD151-negative primary keratinocytes on a laminin substrate was impaired (20). We and others have found that whereas depletion of CD151 diminished growth of tumor cells in immunocompromised animals, cell proliferation under standard conditions was not affected (21, 22). Taken together, these results suggest one of the following possibilities: (a) involvement of CD151 in proliferation may be cell type specific; (b) host microenvironment may have an important role in CD151-dependent cell proliferation; (c) the involvement of CD151 in proliferation of tumor cells under standard culturing conditions (i.e., growth on plastic) may be overshadowed by intrinsic activating mutations in genes that control cell proliferation and are found in most established cancer cell lines [e.g., Ras, B-Raf, and phosphoinositide 3-kinase (PI3K)].

Mammary ductal carcinoma in situ (DCIS) is the nonobligate precursor of invasive breast cancer and is characterized by proliferation of neoplastic cells within the duct lumen. Here, we found that the elevated expression of CD151 correlated with a more aggressive phenotype in DCIS. By knocking down the expression of CD151 in HB2 cells, a nontumorigenic mammary epithelial cell line, we found that this tetraspanin controls proliferation of cells in vivo (mouse xenografts) and in three-dimensional (3D) extracellular matrix (ECM). Furthermore, many of the CD151-negative colonies developed internal lumens when grown in 3D ECM. The expression of CD151 and its pro-proliferative and morphogenetic activities correlated with increased activation of extracellular signal-regulated kinase 1/2 (Erk1/2) and c-Akt. These results strongly suggest that CD151 may play an important role in the development of hyperproliferative diseases in the mammary gland and may determine behavior of DCIS.

Materials and Methods

Cells, antibodies, and reagents

All cells were grown in DMEM (Sigma) supplemented with 10% fetal bovine serum, 5 μg/mL insulin, and 10 μg/mL hydrocortisone. Plasmids encoding constitutively active MEK1 (DD-MEK) and cAkt (myr-Akt) were provided by Drs. E. Tulchinsky (Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, United Kingdom) and A. Eliopoulos (Institute for Molecular Biology & Biotechnology, Heraklion, Crete, Greece), respectively. HB2/CD151 (−) were generated after transfection of pSuperior-shRNA-CD151 (23) and subsequent cell sorting. HB2/CD151Rec and HB2/CD151-QRD cell lines were established after transfections of HB2/CD151(−) cells with constructs encoding shRNA-resistant wild-type and QRD194-196→INF mutant of CD151 (21, 23). Cell lines expressing low levels of α3β1, and α6 integrins [HB2/α3β1(−) and HB2/α6(−)] were established by infecting HB2 cells with pLVTHM-based lentivirus encoding shRNA that targets α3 or α6 integrin subunit, respectively (target sequences were α3 5′-GCTACATGGATCCTGGAAGCA-3′; α6 5′-GCTGCAACGTCATGCTGTCAC-3′). Mouse anti-CD151 mAbs used were as follows: 5C11 (24), 11B1G4 (kindly provided by Dr. L. Ashman, School of Biomedical Sciences and Hunter Medical Research Institute, University of Newcastle, Callaghan, Australia), and NCL-CD151 (Novocastra). Mouse mAb against laminin α6 chain (5D6) was kindly provided by Dr. K. Sekiguchi (Institute for Protein Research, Osaka University, Osaka, Japan). Mouse mAb against β3 integrin was from Sigma; mouse mAbs against β4 integrin subunit (3E1) and laminin γ2 subunit were from Chemicon; mouse mAb against E-cadherin was from Abcam; mouse mAb against epithelial-specific antigen (ESA) was from Dako; mouse mAb to GM130 was from BD Bioscience; mouse mAb against human MUC1 and keratins 18 and 19 were from Cancer Research UK; and mouse mAb against human keratin 5/6 was from Dako. Rabbit polyclonal antibodies to α3 and α6 integrin were generously provided by Drs. F. Watt (Wellcome Trust Centre for Stem Cell Research, University of Cambridge, Cambridge, United Kingdom) and A. Cress (Department of Pharmacology, The University of Arizona, Tucson, AZ). Goat polyclonal antibody against the α3 integrin subunit was purchased from Santa Cruz Biotechnology. Mouse mAbs to α3 (A3-IVA5), α6 (A6-ELE), and β1 (TS2/16) integrin subunits were described previously (25–27). The rest of the antibodies used in this study were purchased from Cell Signaling Technology.

Culturing of cells in 3D Matrigel

Culturing of cells in 3D ECM was performed as previously described (21). For morphologic analyses in 3D culture experiments, representative pictures were taken using Axioplan 2 Zeiss microscope. Immunofluorescence staining in 3D ECM was carried out as described in (28) and analyzed using Zeiss LSM510 META confocal system.

Analysis of activation of FAK, PKB/cAkt, and extracellular signal-regulated kinase 1/2 in 3D ECM

Cells were released from 3D ECM by mechanical pipetting. Released cells were washed once with PBS and lysed directly into Laemmli sample buffer supplemented with 2 mmol/L phenylmethanesulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 100 μmol/L Na3VO4, 10 mmol/L NaF, and 10 mmol/L Na2P2O7. Cellular proteins were resolved on 10% to 12% SDS-PAGE, transferred to the nitrocellulose membrane, and probed with appropriate phosphospecific or conventional antibodies.

Xenograft experiments

All experiments were performed in accordance with institutional and national animal research guidelines. Cells (1 × 105/0.2 mL Matrigel/PBS) were injected s.c. Four to six animals per group were used in the experiments. The experiments were terminated 4, 8, or 14 weeks after injection.

Immunohistochemistry

After antigen retrieval, immunostaining of paraffin sections was performed using a standard avidin-biotin-peroxidase complex method. The sections counterstained with hematoxylin were analyzed using the Olympus DP70 camera and the Olympus DP-Soft software. Quantification of glandular structures in xenografts was performed by counting structures in
nine high-power (400×) fields corresponding to 0.0625 mm². The numbers represent the average from nine counts. For analysis of DCIS tissue samples, sections of formalin-fixed paraffin-embedded tissue were taken from 87 cases of DCIS (39 pure DCIS and 48 with associated invasion) and 14 normal breast samples and stained as described above. The use of the samples for the study was approved by Local NHS Research Ethics Committee (COREC ref 06/Q0403/182).

Analysis of staining
The staining intensity of CD151 was scored in tumor cells of DCIS cases on an individual duct basis to be able to study the relationship between pattern and expression intensity as well as grade and expression intensity. Only membranous staining was considered positive. Staining was scored as heterogeneous when the staining intensity was different in the tumor cells located at the periphery of the duct from those located at the center. Statistical analysis of the results was done by Pearson χ² test, with P < 0.05 considered as significant, and by using SPSS software version 11.

Results
Increased expression of CD151 correlates with a higher grade of DCIS
We have recently found that elevated expression of CD151 correlates with advanced stage in patients with invasive ductal carcinoma of the breast (21). To examine whether deregulation of CD151 expression could be observed earlier during breast cancer evolution, we analyzed the expression of this tetraspanin in DCIS, a preinvasive form of breast cancer that is characterized by filling of the ductal luminal space. Eighty-seven cases of DCIS (39 pure DCIS and 48 with associated invasive disease) were included in this study. In normal breast tissue, CD151 is restricted to the myoepithelial cell layer, with no or very weak staining of luminal epithelial cells (Fig. 1A). Because DCIS can exhibit significant

Figure 1. Expression of CD151 in DCIS and characterization of HB2 xenografts. A, in normal breast, staining is limited to the myoepithelial–basement membrane interface (top left, arrows), with sparing of the luminal cells. In DCIS, staining was either homogeneously positive (top right), with strong membrane staining in all the neoplastic luminal cells, or heterogeneous (bottom panels), with strong membrane staining of neoplastic cells at the periphery of the duct, but without CD151 expression in the cells toward the center of the ducts, particularly seen as the cells started to form small lumen. B, cells were injected s.c. into nude mice as described in Materials and Methods, and xenografts were grown for 8 weeks. The morphology (H&E staining) of representative glandular structures is shown.
heterogeneity of grade and pattern within a case, the expression was analyzed on a duct-by-duct basis (a total of 394 ducts). The luminal neoplastic cells in 104 ducts were negative for CD151 expression. More than two thirds of the remaining ducts showed strong, homogeneous membrane expression of CD151 by all the DCIS cells (Fig. 1A). The expression of CD151 was found to be associated with a high grade of DCIS ($P = 0.004$; Supplementary Table S1); however, there was no association with disease pattern. Furthermore, the frequency of positivity of DCIS was similar in the pure cases to those with established invasion, although the pattern of staining with CD151 expressed by cells at the periphery of the duct and not centrally was more frequent in those cases with invasion.

Figure 2. Effect of CD151 depletion on growth of HB2 cells in vivo and in 3D ECM. A, sections of representative HB2/CD151(+) and HB2/CD151(-) xenografts (8 weeks). B, quantification of structures formed by CD151-positive and CD151-negative HB2 cells in vivo. Means of the number of structures are shown. Error bars represent SEM. C, Ki-67 labeling index. Results of quantification of Ki-67-immunopositive cells [average from 10 high power fields (×400), ∼500 cells/sample are shown]. D, HB2/CD151(+) and HB2/CD151(-) cells were grown for 10 days in 3D collagen (top) or 3D Matrigel (bottom). Representative fields are shown.
Characterization of HB2 cellular aggregates in vivo and in 3D ECM

To examine whether CD151 can contribute to the proliferative phenotype associated with the development of DCIS, we used HB2 cells as a model system (29). HB2 cells express various markers of luminal cells (30), and therefore more closely resemble DCIS (>90% of which exhibit luminal phenotype; ref. 31) than the more widely used DCIS.com cell model, which have marker profile of myoepithelial cells (32). When injected into nude mice, HB2 cells formed glandular-like structures of ~30 to 70 µm in diameter 8 weeks after the injection (Fig. 1B). These structures were of different degree of morphogenic organization, with most of the structures having appeared as cysts with a single layer of epithelium. None of the xenografts have developed into a tumor mass, and most of them regressed by 12 to 14 weeks (results are not shown). Immunohistochemical analysis showed that CD151 and α3β1 were abundant at cell-cell contacts (Supplementary Fig. S1). When cultured in vitro in 3D ECM, HB2 cells formed compact round colonies with well-defined contours (Supplementary Fig. S2A). Further characterization of these structures has shown that in contrast to HB2 xenografts, most of the colonies in 3D ECM did not develop internal lumens even after prolonged culturing. Immunofluorescent staining of 10-day structures revealed that cells inside colonies formed tight cell-cell contacts (as indicated by staining with anti-E-cadherin and anti-ESA mAbs; Supplementary Fig. S2B). We found that α3β1 integrin was also abundant at cell-cell contacts (Supplementary Fig. S2B).

Depletion of CD151 suppresses growth of HB2 xenografts and proliferation of cells in 3D ECM

To establish the role of CD151 in growth of HB2 cells in vivo and in 3D ECM, we generated HB2/CD151(−) cells in which expression of this tetraspanin was decreased by >90% (Supplementary Fig. S3). Similar to the control, HB2/CD151(−) cells formed glandular structures when grown as subcutaneous xenografts in nude mice. However, detailed quantification of xenografts revealed that the total number of glandular structures formed by CD151-negative cells was ~7-fold lower when compared with HB2/CD151(+) cells (Fig. 2A and B). Accordingly, we found that the Ki-67 index was higher in CD151-positive xenografts (Fig. 2C). In contrast, there was no apparent difference between HB2/CD151(+) and HB2/CD151(−) xenografts when sections were stained with the antibody recognizing activated caspase-3.

Figure 3. The role of CD151-associated integrins in pro-proliferative and morphogenetic activities of the tetraspanin. A, morphologic appearance of colonies formed by the indicated HB2 sublines grown for 10 days in 3D Matrigel. Insets show magnified images of representative colonies. Note that rare colonies formed by HB2/α3β1(−) cells have the appearance of the CD151(+) aggregates. B, Western blots (WB) showing expression of CD151 and integrin subunits in cells used in A.
These results suggested that differences in growth of HB2/CD151(+) and HB2/CD151(−) cells in vivo are due to the effect of CD151 on cell proliferation rather than apoptosis. There were also differences in the total number of CD151-positive and CD151-negative colonies when cells were cultured in 3D ECM (Fig. 2D). Importantly, when expression of CD151 protein was reconstituted in HB2/CD151(−) (HB2/CD151rec cells), the “CD151-negative phenotypes” reverted to that of the control cells (Supplementary Fig. S4). Taken together, these results showed that tetraspanin CD151 controls the proliferative potential of mammary epithelial cells in vivo and in 3D ECM.

The role of CD151-associated integrins in 3D ECM growth of HB2 cells

It has been postulated that CD151 functions via integrins in nonhematopoietic cells (33, 34). To examine whether the pro-proliferative activity of CD151 is dependent on its association with integrins, we analyzed the behavior of HB2/CD151-QRD cells. These cells express the QRD194-196→INF mutant of CD151 (CD151-QRD), which is unable to interact with laminin-binding integrins directly (35). Interestingly, although the expression of the CD151-QRD mutant in CD151-negative cells has effectively restored their colony-forming potential in 3D ECM, HB2/CD151-QRD aggregates appeared...
distinctly different from either CD151(+) or CD151(−) colonies. In contrast to typically compact structures, a large proportion of HB2/CD151-QRD colonies appeared as aggregates of loosely associated cells (Fig. 3). These experiments suggested that (a) the pro-proliferative activity of CD151 does not depend on its association with integrins and (b) the formation of compact colonies in 3D ECM relies on the CD151-integrin complexes. Integrins α3β1, α6β1, and α6β4 are principal partners of CD151 in epithelial cells. For further assessment of the roles played by CD151-associated integrins, we analyzed the growth of HR2 cells deficient in expression of either α3β1 or α6β1/α6β4 [HB2/α3β1(−) and HB2/α6(−) cells, respectively]. Although the ability of HB2/α3β1(−) cells to grow in 3D ECM was severely compromised, depletion of

Figure 5. Depletion of CD151 causes redistribution of αβ, integrin and apical relocalization of Golgi. A and B, cells were grown in Matrigel for 10 days. The colonies were fixed with paraformaldehyde and stained with the indicated mAb. In B, actin filaments were visualized using Alexa 594–conjugated phalloidin, and nuclei were stained with 4′,6-diamidino-2-phenylindole. Note the fragmented nuclei inside the HB2/CD151(−) colony. Representative images are shown. Scale bars represent 50 μm.
α₆(−) integrins had no apparent effect on the plating efficiency and morphology of the colonies formed by HB2 cells (Fig. 3). Although these results were somewhat surprising given an important role of α₆ integrins in regulating the growth of epithelial cells in Matrigel (28, 36), we found that HB2 cells deposited laminin-332 at the perimeter of growing colonies (see below). Thus, it is likely that in the absence of α₆ integrins, HB2 cells fully rely on the interaction between α₃β₁ integrin and cell-produced laminin-332. Collectively, these results indicated that (a) proliferative and morphogenetic activities of CD151 are dependent on α₃β₁ and (b) α₆β₁/α₃–laminin-332 interactions cannot compensate for the absence of α₃β₁ in 3D ECM.

Depletion of CD151 induces lumen formation in 3D ECM cultures of HB2 cells

We noticed that despite the apparent deficiency in their ability to grow in 3D ECM, a large proportion of CD151-negative colonies that did grow developed internal lumens (Fig. 4A). Formation of the lumen in CD151-negative colonies also correlated with the appearance of apoptotic cells inside the colonies and increased activation of caspase-3 (Fig. 4B). In other experiments, we found that activation of caspase-3 in cells denied attachment was more pronounced in the absence of CD151 (Fig. 4C). These data strongly suggest that CD151 regulates lumen formation through signaling pathways linking integrins with activation of caspase 3. Thus, we examined the effect of CD151 depletion on the distribution of associated integrins in cells cultured in 3D ECM. Lumen formation in HB2/CD151(−) cells correlated with redistribution of α₃β₁ integrin from cell-cell contacts to the peripheral, cell-ECM interface (Fig. 5A). In contrast, depletion of CD151 had no effect on localization of α₆ integrins. The redistribution of integrins in CD151-depleted cells toward the cell-ECM interface was specific as both E-cadherin and ESA retained their localization in cell-cell contacts (Fig. 5A). Next, we investigated whether CD151-dependent relocalization of α₃β₁ can be linked to changes in secretion and/or deposition of Ln-332 or Ln-511/Ln-521—known laminin substrates for this integrin. We found that both cell lines secreted small quantities of Ln-332, which were deposited at the cell-ECM interface (Fig. 5A); Ln-511/Ln-521 was not produced by these cells as judged by the lack of immunostaining with mAb to α₅ laminin subunit (results are not shown). The membrane matrix metalloproteinase MT1-MMP/MMP14 is known to control α₃β₁-mediated interactions with Ln-332 (37). Importantly, CD151 is coprecipitated with MT1-MMP/MMP14 and regulates its subcellular distribution (38). We found that removal of CD151 did not affect the distribution of MT1-MMP/MMP14 in 3D cultures: weak MT1-MMP/MMP14 staining was diffusely spread over the cell surface and in the cytoplasm, with no apparent differences observed between the HB2/CD151(+) and HB2/CD151(−) colonies (Fig. 5A). We also found that depletion of CD151 did not affect the total levels of LN-332 and MT1-MMP/MMP14 in cells grown in 3D Matrigel (not shown). Having considered all
these results, we concluded that it is unlikely that redistribution of α3β1 integrin in CD151-depleted cells is driven by deposition (or reorganization) of its ECM ligands. Depletion of CD151 can change the organization of actin filaments at cell-cell junctions in cells grown in 2D (15). Therefore, it is plausible to suggest that the distribution of actin filaments was not affected by CD151 removal when cells were cultured in 3D Matrigel (Fig. 5B). Finally, more restricted and polarized distribution of α3β1, in HB2/CD151(-) cells prompted us to analyze whether depletion of CD151 has a more general effect on cell polarity. Thus, we examined the position of Golgi in cells grown in 3D Matrigel and the distribution of MUC-1, a well-established apical marker. In most HB2/CD151(-) cells, Golgi was positioned between the nucleus and surface facing the lumen (Fig. 5B). By contrast, distribution of the Golgi marker GM130 was more random in HB2/CD151(+) colonies (Fig. 5B). Despite such a pronounced effect of CD151 depletion on Golgi, we found no evidence for apical localization of MUC1 in HB2/CD151(-) colonies (Fig. 5B). In fact, in both CD151(-) and CD151(+) colonies, the most prominent staining was concentrated at the cell-ECM (i.e., basal) interface. Taken together, these results indicate that there was a partial restoration of the polarized phenotype in CD151-depleted cells.

**Pro-proliferative function of CD151 in 3D Matrigel is linked to the activation of Erk1/2 and c-Akt**

To establish which of the signaling pathways may be relevant to pro-proliferative and morphogenetic activities of CD151, we compared the activation of various integrin-dependent signaling pathways in HB2/CD151(+) and HB2/CD151(-) cells grown in 3D ECM. Deficiency in CD151 correlated with the decrease in the levels of phosphorylated Erk1/2 (Fig. 6A). The effect of CD151 depletion was more pronounced at days 4 and 5 after embedding. We also observed a decrease in the level of active c-Akt in HB2/CD151(-) cells. To examine whether the decrease in the levels of active Erk1/2 and c-Akt were sufficient to inhibit growth in 3D ECM and to change the phenotypic pattern of colonies, we cultured control HB2/CD151(+) cells in the presence of U0126 and LY29004, widely used inhibitors of Erk1/2 and PI3K/c-Akt signaling pathways, respectively. These experiments showed that both chemicals had a strong inhibitory effect on proliferation of the cells in 3D Matrigel (Fig. 6B). Collectively, these results showed that the pro-proliferative function of CD151 in 3D ECM is dependent on activation of Erk1/2 and c-Akt.

Importantly, growing HB2/CD151(+) cells in the presence of Erk1/2 or PI3K inhibitors facilitated lumen formation in ~50% of the colonies (Fig. 6C). Conversely, when constitutively active MEK and c-Akt were expressed in HB2/CD151(-) cells, the effect of CD151 depletion on lumen formation was reversed (Supplementary Fig. S5). These results provided further evidence for the role of CD151-dependent activation of PI3K and Erk1/2 in 3D ECM growth and lumen formation via caspase-3. A slight discordance between the kinetics of CD151-dependent activation of caspase-3 and decrease in phosphorylation of c-Akt and Erk1/2 suggests the contribution of additional pathways leading to caspase-3 cleavage (e.g., involvement of PKA, Ca2+, and/or PKCζ refs. 39–41).

**Discussion**

In this report, we described a new cellular model for mammary DCIS and showed that tetraspanin CD151 is likely to play an important role in the development of this disease. Specifically, we found that (a) elevated expression of CD151 regulates proliferation and controls morphogenetic behavior of non-tumorigenic mammary epithelial cells both in vivo and in 3D ECM; (b) removal of CD151 facilitates formation of the internal lumen by cells cultured in 3D ECM and their partial polarization; and (c) CD151 controls behavior of cells in 3D ECM via activation of ERK1/2 and c-Akt. The physiologic relevance of these findings is emphasized by our observation that increased expression of CD151 is associated with development of DCIS, a preinvasive form of breast cancer characterized by proliferation of neoplastic cells filling the lumen of ducts.

It has been previously shown that various biological activities of CD151 in nonhematopoietic cells are dependent on its associated integrin partners (21, 35). Here, we found for the first time that at least one of the CD151-dependent functions in nontumorigenic mammary epithelial cells (i.e., its pro-proliferative activity) does not require a direct contact of the tetraspanin with integrin molecules. Yet, the presence of fully functional CD151 and α6 integrins was not sufficient to allow proliferation of α3β1-negative cells in 3D ECM. This suggests that CD151 functions as a costimulatory molecule. Importantly, our report and an earlier study, which focused on tetraspanin CD81 in T cells (42), have shown that costimulatory activities of tetraspanins are linked to activation of Erk1/2. Thus, there may exist a common mediator of tetraspanin-dependent activation of Erk1/2, which seems to be utilized in the context of signaling pathways activated by various tetraspanin-associated receptors.

In addition to its role in cell proliferation, CD151 (or rather CD151-α3β1 complex) controls cell-cell interactions. Importantly, this became apparent only when cells were cultured in 3D ECM. Although earlier and more recent data have shown that CD151 regulates cell-cell interaction via E-cadherin (15, 43), we found that distribution of E-cadherin or its association with actin cytoskeleton in HB2 cells were not affected in HB2/CD151(-) cells (Fig. 5A and data not shown). Furthermore, we were unable to coprecipitate E-cadherin with either CD151 or α3β1 integrin. Thus, it seems that the effect of CD151 on intercellular adhesion in mammary epithelial cells is likely to be mediated by a noncadherin receptor. Among the possible candidates are EpCAM and one of the claudins, both of which are known to associate with tetraspanins and function as cell-cell adhesion molecules (44).

Our data point to a previously unknown function of CD151 as a potential regulator of epithelial cell polarity. In this regard, it has been reported that CD151 regulates the activity of CDC42 (13, 43), a key player in establishing and maintenance of epithelial cell polarity (45). Therefore, it is possible that CD151 depletion results in refining GTP CDC42 ⇌ GDP CDC42 balance in HB2 cells, thus allowing the establishment...
of polarity in 3D ECM. Furthermore, attenuation of Erk1/2-dependent signaling observed in HB2/CD151(−) cells may also facilitate their polarization (46). It is important to emphasize that despite the prominent effect of CD151 removal on repositioning Golgi, polarization of HB2 cells in 3D Matrigel was incomplete. Indeed, we showed that MUC1 was predominantly delivered to the basal surface irrespective of CD151 expression (Fig. 5B). Furthermore, we found that various tight junction proteins (e.g., occludin, claudins, and ZO-1) were distributed along the entire cell-cell contact membranes in HB2/CD151(−) colonies (not shown). Hence, CD151 deletion restores only certain features of polarized epithelial cells. Nevertheless, it is tempting to speculate that overexpression of CD151 in mammary tumor cells can contribute to changes in cell polarity, one of the early events in the development of DCIS.

The fact that the elevated expression CD151 is specifically associated with high-grade forms of DCIS (which is characterized by a high level of proliferation and inhibition of apoptosis) further supports the physiologic relevance of our findings, linking function of CD151 with proliferation of HB2 cells in 3D ECM. In normal breast, expression of CD151 in the ducts is restricted to the myoepithelial cell layer with luminal cells either being completely negative or weakly positive. Therefore, one may consider that the upregulation of CD151 expression in some forms of DCIS could simply reflect a transition from the luminal to basal/myoepithelial phenotypes (47, 48). However, basal-like DCIS accounts for only 5% to 10% of DCIS, whereas upregulation of CD151 is evident in >70% of ducts with DCIS in our study. This suggests that dysregulated expression of CD151 is not related to differentiation but is more likely to be a driver of the high proliferation seen in this high-grade disease. Although this study was specifically focused on pure DCIS, in some of the selected mixed DCIS cases (i.e., which included an invasive component), we observed that the expression of CD151 was either reduced or lost in the invasive tumor cells (results are not shown). Although the significance of this observation remains to be investigated, these data seem to indicate that the major function of CD151 in DCIS is to stimulate intraductal proliferation of cancerous cells.

In conclusion, we found that CD151 plays a critical role in controlling proliferation of nonmalignant mammary epithelial cells. The upregulation of CD151 in a majority of DCIS, and its significant association with high-grade DCIS, supports the idea that CD151 is involved in driving proliferation of epithelial cells in the early stages of breast cancer. It will be of interest to determine at what point in disease evolution CD151 becomes upregulated; to address this, future studies will need to analyze earlier precursor lesions including atypical ductal hyperplasia and usual type hyperplasia.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank all our colleagues for their generous gifts of the reagents that were used in this study.

Grant Support

Cancer Research UK grant C1322/A7075 (F. Berditchevski).

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Received 12/01/2009; revised 03/09/2010; accepted 03/26/2010; published OnlineFirst 05/25/2010.

References


www.aacrjournals.org Cancer Res; 70(11) June 1, 2010 4707

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Vera Novitskaya, Hanna Romanska, Marwa Dawoud, et al.

Cancer Res 2010;70:4698-4708. Published OnlineFirst May 25, 2010.

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