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BREAST CANCER METASTATIC POTENTIAL: CORRELATION WITH INCREASED HETEROTYPIC GAP JUNCTIONAL INTERCELLULAR COMMUNICATION BETWEEN BREAST CANCER CELLS AND OSTEOBLASTIC CELLS

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The breast cancer metastasis-suppressor gene *BRMS1* is downregulated in metastatic breast cancer cells. Previous reports have shown restoration of gap junctional intercellular communication (GJIC) in the metastatic human breast carcinoma cell line MDA-MB-435 (435) transfected with *BRMS1* cDNA. Metastasis, to a large extent in most breast cancers, occurs to bone. However, the reason for this preferential metastasis is not known. We explored cell-to-cell communication between 435 carcinoma cells and a human osteoblastic cell line, hFOB1.19, to determine whether carcinoma cells can form gap junctions with bone cells and to explore the role of these heterotypic gap junctions and the *BRMS1* gene in breast cancer metastasis to bone. 435 cells displayed greater cell-to-cell communication with hFOB 1.19 cells than with themselves. Transfection of *BRMS1* into 435 cells increased homotypic gap junctional communication but did not significantly affect heterotypic communication with hFOBs. However, heterotypic communication of *BRMS1* transfectants with hFOB cells was reduced relative to homotypic communication. In contrast, parental 435 cells displayed greater heterotypic communication with hFOBs relative to homotypic communication. Our results suggest that there are differences in the relative homotypic and heterotypic GJIC of metastasis-capable and -suppressed cell lines.

Key words: *adhesion; breast cancer; connexin; cadherin; gap junctional intercellular communication; metastasis*

Gap junctions are intercellular channels that facilitate the movement of small (<1 kDa) ions and signaling molecules between cells.[1] A breakdown in GJIC has been correlated with tumorigenesis.[2] Previous studies suggested that the breakdown in homotypic communication may contribute to breast cancer metastatic potential.[3]

The breast metastasis suppressor gene *BRMS1* suppresses metastasis in human breast carcinomas.[4] Transfection of *BRMS1* cDNA into the metastatic breast cancer cell line MDA-MB-435 (435) reduces their metastatic potential and restores homotypic GJIC, supporting the concept that metastasis-suppressed cells are capable of significant homotypic GJIC as well as the correlation between breakdown in homotypic GJIC and metastasis.[3]

The purpose of the current study was 3-fold: *(i)* to determine whether breast carcinoma cells can form gap junctions with bone cells; *(ii)* to ascertain whether heterotypic GJIC might be relevant to cancer metastasis; and *(iii)* to explore whether *BRMS1* expression might regulate heterotypic GJIC. Since breast carcinomas so commonly colonize bone, we explored heterotypic GJIC (*i.e.,* between 435 cells and hFOB1.19 cells). We examined 435 cells because they metastasize to bone when injected intracardially in mice.[5] *BRMS1*-transfected 435 cells were used to study the effect of metastasis suppression on heterotypic GJIC with hFOB cells since *BRMS1* transfectants are genotypically related to 435 cells. Osteoblasts were used for heterotypic studies because osteoblastic bone lining cells cover all bone surfaces[6] and tumor cells might have to adhere to and migrate through a layer of bone lining cells in addition to a layer of endothelial cells to reach the marrow cavity.[7]

MATERIAL AND METHODS

Cell lines

HTERT-HME1 (HTERT) cells were maintained in specially formulated medium (both cells and medium from Clontech, Lexington, KY).[8] MCF-7 cells were purchased from the ATCC (Rockville, MD)[9] and maintained in EMEM supplemented with 10% FBS (Hyclone, Logan, UT) and 1 mg/ml bovine insulin. 435 cells[10] were maintained in DMEM/F-12 medium (GIBCO Life Technologies, Rockville, MD) supplemented with 5% FBS. 435/BRMS1 cells (clones 3 and 6) are 435 cells transfected with *BRMS1* cDNA[4] and were maintained in the 435 cell medium supplemented with geneticin (500 μg/ml). The hFOB1.19 cell line[11] was maintained in DMEM/F-12 medium supplemented with 10% FBS and 1% penicillin–streptomycin. In previous studies, 435/BRMS1 cells were subcultured by an EDTA method while parental 435 cells were maintained by trypsinization.[12] Therefore,

Abbreviations: BRMS1, breast metastasis suppressor gene; Cx32, connexin 32; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate (DiIC18); E-cadherin, epithelial cadherin; ECL, enhanced chemiluminescence; GJIC, gap junctional intercellular communication; hFOB, human fetal osteoblast; HRP, horseradish peroxidase; MAb, monoclonal antibody; N-cadherin, neural cadherin; OB-cadherin, osteoblast cadherin.

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in initial GJIC experiments, 435/BRMS1 cells were detached by treatment with EDTA. Parallel studies were also performed by detaching cells with trypsin. Our studies demonstrate that while the GJIC properties of the cells changed if they were continually treated with trypsin for 4–5 passages, a single treatment with trypsin did not alter the GJIC properties of the cells (not shown). Thus, in all subsequent experiments, 435/BRMS1 cells were detached using trypsin.

Gap junction function and connexin expression

Cells were grown on tissue culture Petri dishes. Breast epithelial cells were plated at a density of 1×10^6/dish in 3 ml medium, and hFOB cells were plated at 6×10^5/dish in 3 ml medium on day 0. Cells were used for GJIC assays on day 3 at 90–100% confluence. A GJIC protocol was employed based on a modification of the methods described by Goldberg *et al.,*[13] Ko *et al.*[14] and Czyz *et al.*[15] Donor and recipient cell populations were differentially labeled for 30 min with the gap junction–permeable dye calcein AM (3 μM; Molecular Probes, Eugene, OR) and the lipophilic dye DiI (12.5 μM, Molecular Probes) in DMEM/F-12 containing 1% FBS, respectively. After washing, donor cells were trypsinized and added to acceptor cells at a 1:5 donor:acceptor ratio for 2 hr at 37°C. Cocultures were harvested by trypsinization, washed with DMEM/F-12 containing 5% FBS, resuspended in PBS and subjected to FACS analysis. Cells were analyzed for the presence of calcein, DiI or both subsequent to flow-cytometric analysis (Fig. 1, R1). If GJIC occurred, calcein was transferred from donor cells (green) to recipient cells (red). Thus, double-labeled cells represented the communicating cells in this assay (Fig. 1, R4). For studies of heterotypic GJIC (between cancer and hFOB cells), cancer cells were added to hFOB cells to observe the directional transfer of the dye to hFOB cells. Cytometric data were expressed in terms of the coupling index (coupled acceptor cells/total potential acceptor cells) per donor cell, [R4/(R4+R5)]/R2 (Fig. 1), and plotted as means ± SEM of 6 experiments with 5 samples in each experiment. A one-way ANOVA and Student-Newman-Keuls test were used to assess statistical significance. Total RNA isolated from cells was subjected to real-time RT-PCR as described previously for connexin expression.[11] Experiments were performed three times.

Cadherin expression

Membrane-enriched protein was isolated from confluent cell cultures and quantified using the Bradford method.[16] Equal amounts of protein (50 μg/well) were electrophoresed on 5% SDS-polyacrylamide gels under reducing conditions according to Laemmli.[17] Western blotting on nitrocellulose membranes was performed according to Towbin *et al.*[18] at 25 V for 18 hr at 4°C. Immunodetection of cadherins was performed with 1:1,000 dilution of primary mouse anticadherin MAbs specific for E-cadherin (catalogue 13-1700, clone HECD-1; Zymed, San Francisco, CA), N-cadherin (catalogue 33-3900, clone 3B9, Zymed) or OB-cadherin (catalogue 32-1700, clone 5B2H5, Zymed) in 0.02% Tween/PBS, 1% nonfat dry milk for 2 hr at room temperature. This was followed by treatment with HRP-coupled goat antimouse immunoglobulin (catalogue 170-6516; Bio-Rad, Hercules, CA) at a 1:5,000 dilution of IgG in 0.02% Tween/PBS, 1% nonfat dry milk for 1 hr at room temperature. Antibody binding was visualized using ECL reagent (Amersham, Piscataway, NJ). Experiments were performed 5 times.

For cadherin immunofluorescence, cells were grown on glass coverslips, fixed in cold methanol and permeabilized with 0.05% Triton X-100. Cells were incubated with antibodies that recognize human E-cadherin, N-cadherin or OB-cadherin. Binding of primary antibodies was visualized by epifluorescence microscopy after incubation with FITC-labeled goat antimouse secondary antibodies (catalogue 115-095-062, Jackson Immunoresearch, West Grove, PA). Experiments were performed three times.

RESULTS

"Normal" breast HTERT cells displayed greater homotypic GJIC than all other cells examined. HTERT cells displayed greater homotypic GJIC than poorly or nonmetastatic MCF-7 cells, which in turn displayed greater homotypic GJIC than highly metastatic 435 cells (Fig. 2). HTERT cells displayed heterotypic GJIC with hFOBs to a degree similar to that of MCF-7 cells with hFOBs (Fig. 2). However, heterotypic GJIC between HTERT and hFOB cells and between MCF-7 and hFOB cells was significantly greater than that between 435 and hFOB cells (Fig. 2). In HTERT cells, homotypic GJIC was significantly greater than heterotypic GJIC with hFOBs (Fig. 2, Table I). However, in the metastatic breast cancer cell lines MCF-7 and 435, homotypic GJIC was significantly lower than heterotypic GJIC of either cell line with hFOB cells (Fig. 2, Table I).

Both 435/BRMS1.3 and 435/BRMS1.6 displayed significantly greater homotypic GJIC than the highly metastatic 435 cells (Fig. 3). Heterotypic GJIC between individual breast cancer cells and hFOBs was similar for all cell lines examined (Fig. 3). Metastasis-

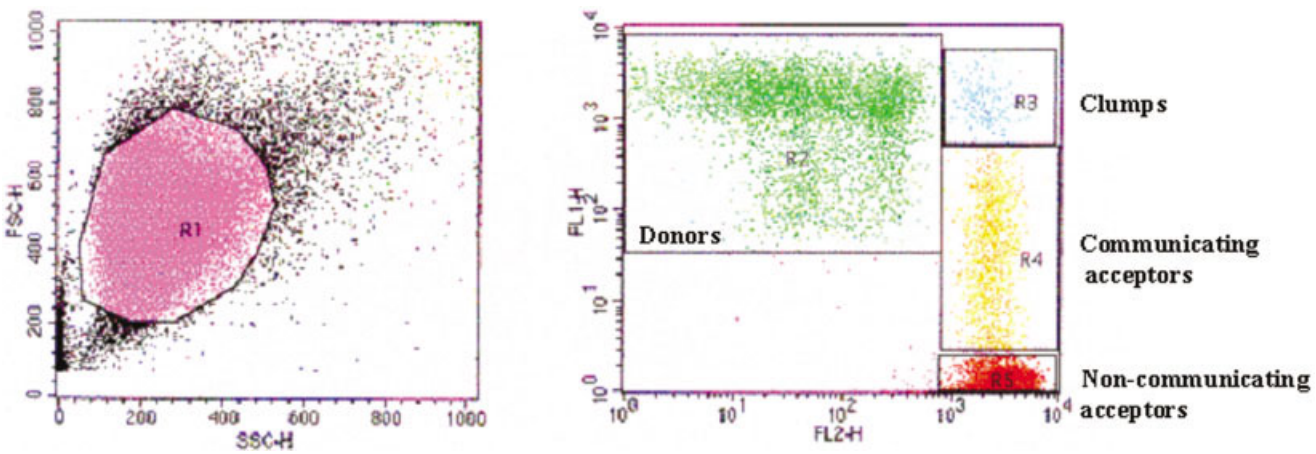

Figure 1 – Flow cytometry to measure GJIC. Cells in region 1 (R1) were quantified for the presence of calcein, DiI or both. Double-labeled cells (R4) are the communicating cells in this assay. This figure represents homotypic GJIC in HTERT cells. Donor cells (1×10^4) and acceptor cells (5×10^4) were used. The total number of events measured is 50,000. The total number of counted events (R1) is 47,230 (100%). The number of events in R2 (donors) is 9,935 (21.04%), that in R4 is 12,149 (25.72%) and that in R5 is 20,235 (42.84%). Thus, the coupling index in this case is 37.5.

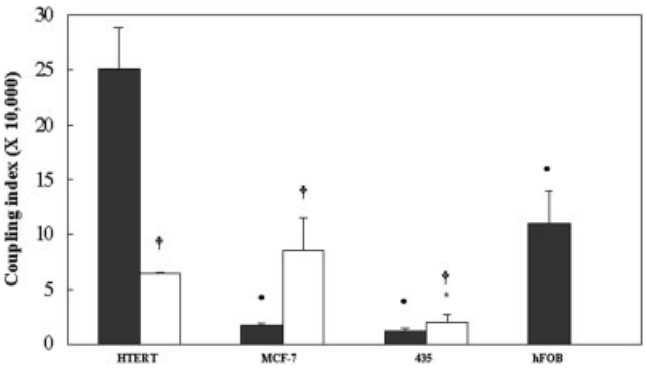

FIGURE 2 – Homotypic and heterotypic GJIC in breast epithelial cells of differing metastatic potential. GJIC studies were performed as described in the text. In the homotypic studies (black bars), donor cells were added to cells of the same type. In the heterotypic studies (white bars), breast cancer cells were used as the donor cells and hFOB cells were used as the acceptor cells. Results are means ± SD of 5 experiments done in quintuplicate. •Significantly different from homotypic GJIC in HTERT cells ($p < 0.001$). *Significantly different from heterotypic GJIC in HTERT cells ($p < 0.001$). †Significantly different from homotypic GJIC values for the same epithelial cell line ($p < 0.001$).

TABLE I – COMPARISON OF HOMOTYPIC AND HETEROTYPIC GJIC WITH hFOB CELLS IN BREAST CANCER CELLS OF DIFFERING METASTATIC ABILITY

Cell line	Homotypic GJIC	Heterotypic GJIC with hFOB cells	Relative GJIC within a cell line (heterotypic/ homotypic)
HTERT	25.144 ± 3.719	6.296 ± 2.211	0.3*
MCF-7	1.607 ± 0.352	8.500 ± 3.002	5.3*
435	1.181 ± 0.198	2.029 ± 0.699	1.7*
hFOB	11.049 ± 2.983		

*$p < 0.001$.

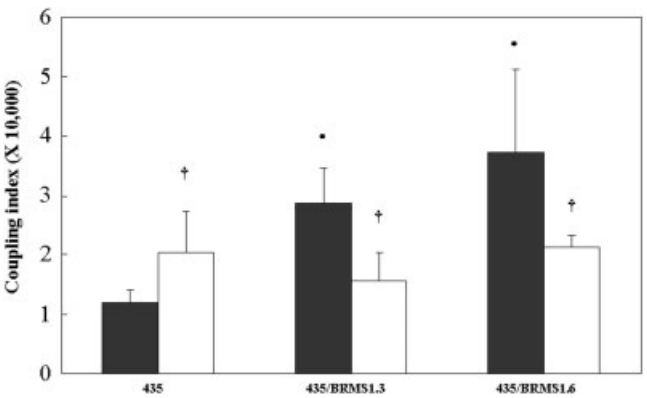

FIGURE 3 – Effect of *BRMS1* transfection on homotypic and heterotypic GJIC in the 435 cancer cell line. GJIC studies were performed as described in the text. In the homotypic studies (black bars), donor cells were added to cells of the same type. In the heterotypic studies (white bars), breast cancer cells were used as the donor cells and hFOB cells were used as the acceptor cells. Results are mean ± SD values of 5 experiments done in quintuplicate. •Significantly different from homotypic GJIC in 435 cells ($p < 0.001$). †Significantly different from homotypic GJIC for the same cell line ($p < 0.001$).

suppressed 435/BRMS1 cells displayed greater homotypic GJIC than heterotypic GJIC with hFOBs (Fig. 3, Table II). Thus, in this regard, 435/BRMS1 behave in a way similar to "normal" breast HTERT cells. Cells pretreated with the gap junctional uncoupler

TABLE II – COMPARISON OF HOMOTYPIC AND HETEROTYPIC GJIC WITH hFOB CELLS IN 435 CELLS TRANSFECTED WITH *BRMS1* cDNA

Cell line	Homotypic GJIC	Heterotypic GJIC with hFOB cells	Relative GJIC within a cell line (heterotypic/ homotypic)
435	1.181 ± 0.198	2.029 ± 0.699	1.7*
435/BRMS1.3	2.857 ± 0.572	1.577 ± 0.450	0.5*
435/BRMS1.6	3.722 ± 1.397	2.117 ± 0.220	0.6*

*$p < 0.001$.

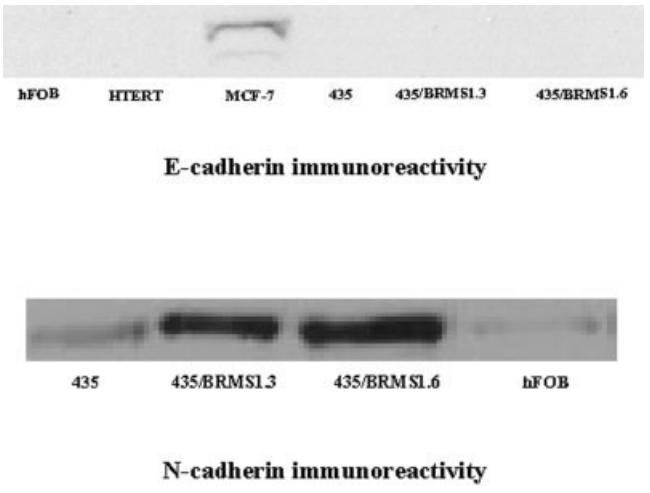

FIGURE 4 – Western blot analysis of E-cadherin and N-cadherin protein levels in parental and *BRMS1*-transfected 435 cells. Results are representative of 4–5 experiments.

AGA (75 μM) for 30 min at 37°C displayed a decrease in dye transfer, confirming that dye transfer was *via* GJIC (not shown).

We were unable to detect E-cadherin in any of the cell lines examined except MCF-7 (Fig. 4). OB-cadherin was detected only in hFOB cells (not shown). Since N-cadherin is elevated in metastatic breast cancer cells,[19] we examined its expression in 435 and 435/BRMS1 cells. We were unable to detect N-cadherin in either HTERT or MCF-7 cells (not shown). N-cadherin expression was detected in hFOB cells, to a greater degree in 435 cells and to an even greater degree in 435/BRMS1.3 and 435/BRMS1.6 cells (Fig. 4). Immunocytochemistry data confirmed the Western blot analysis (not shown).

Nontumorigenic and nonmetastatic HTERT cells expressed relatively high levels of Cx43 but did not express Cx32. Tumorigenic and poorly metastatic or nonmetastatic MCF-7 cells expressed very low levels of Cx43 and did not express any Cx32 (Fig. 5). The tumorigenic and highly metastatic 435 cells, however, did not express any Cx43 but did express high levels of Cx32. The *BRMS1* transfectant (clone 3) had higher levels of Cx43 than did 435 cells and no Cx32. hFOB and normal liver cells were used as positive controls for Cx43 and Cx32 RT-PCRs, respectively.

DISCUSSION

Our results support the hypothesis that alteration of GJIC imparts a metastatic phenotype to normal cells. The telomerase-immortalized, nontumorigenic and nonmetastatic HTERT cell line used here exhibits a high degree of homotypic GJIC. Homotypic GJIC is reduced to a great degree in both tumorigenic and poorly metastatic or nonmetastatic MCF-7 cells and in tumorigenic and highly metastatic 435 cells. However, the degree of homotypic GJIC in MCF-7 and 435 cells is similar, suggesting that a decrease of GJIC plays a greater role in tumorigenesis than it does in metastasis. This might, however, be a cell line–related effect, and

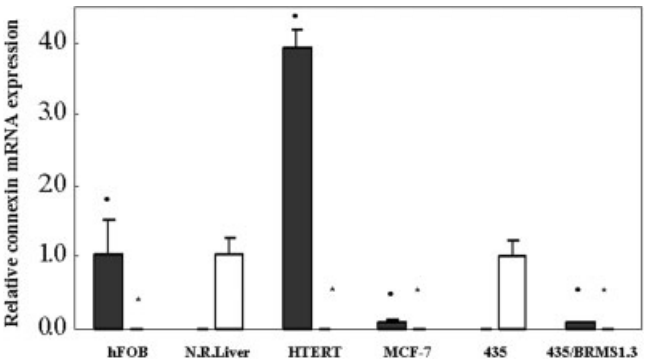

FIGURE 5 – Quantitative real time RT-PCR analysis for Cx43 and Cx32 mRNA. hFOB is the positive control, and the ratio of Cx43 mRNA to 18S mRNA (black bar) in hFOB cells was set as 1. Normal liver tissue is the positive control, and the ratio of Cx32 mRNA to 18S mRNA (white bar) in normal liver tissue was set as 1. All other cell line values are depicted in comparison to hFOB mRNA levels for Cx43 and in comparison to liver mRNA levels for Cx32. Results are representative of 3–4 experiments. ●Significantly different from Cx43 mRNA levels in 435 cells ($p < 0.001$). *Significantly different from Cx32 mRNA levels in 435 cells ($p < 0.001$).

drawing a conclusion on this basis might be misleading. Thus, using metastasis-capable and -suppressed variants of the same cell line would give a better idea of the role of gap junctions in cancer cell metastasis.

To address this issue, we examined GJIC in 435/BRMS1 cells. 435 cells transfected with the *BRMS1* gene are less metastatic *in vivo* than 435 cells.[4] *BRMS1*-transfected cells exhibit greater homotypic GJIC in comparison to the parent 435 cell line.[3] 435/BRMS1 cells also display increased heterotypic communication with normal breast epithelial cells, underlining the fact that transfection of the *BRMS1* gene into cancer cells brings them to a more normal phenotype regarding gap junction expression.[3] Both 435/BRMS1.3 and 435/BRMS1.6 cells demonstrated increased homotypic GJIC relative to 435 cells. These results suggest that a decrease in homotypic GJIC, which could be due to a change in the connexin expression profile of the cells, correlates with tumorigenicity and metastasis in breast epithelial cells.

A more important objective of our study was to determine whether breast cancer cells could communicate with osteoblasts and whether this communication could be correlated to the metastatic potential of breast cancer cells. We demonstrate that breast cancer cells are capable of forming functional gap junctions with hFOB cells. However, the degree of heterotypic GJIC between breast cancer cell lines and hFOB cells did not correlate with metastatic potential. For instance, poorly metastatic or nonmetastatic MCF-7 cells displayed greater heterotypic GJIC with hFOBs than did the highly metastatic 435 cells. Even among cells with similar genetic background, *i.e.,* 435 and 435/BRMS1, heterotypic GJIC did not correlate with metastasis. It should be emphasized that we examined heterotypic communication with only one type of bone cell. However, the possibility remains that heterotypic GJIC between breast cancer cells and other cells in the bone microenvironment, *e.g.,* endothelial cells, osteocytes and stromal cells, or within other environments of metastases, *e.g.,* lung or liver, may distinguish metastasis-competent from –incompetent cells.

While we found no correlation between heterotypic GJIC and metastatic potential, we did demonstrate that an increase in heterotypic GJIC relative to homotypic GJIC in an individual cell line correlated with metastatic potential. Thus, metastatic 435 and MCF-7 cells display greater heterotypic GJIC with hFOBs relative to homotypic GJIC, whereas nonmetastatic 435/BRMS1 and HTERT cells display less heterotypic GJIC with hFOBs than homotypic GJIC. Therefore, the degree of heterotypic GJIC with hFOB cells relative to homotypic GJIC rather than the absolute degree of homotypic or heterotypic GJIC correlates with breast cancer cell metastatic potential.

Real time RT-PCR data demonstrated that HTERT cells have a Cx43-positive and Cx32-negative profile while 435 cells have a Cx43-negative and Cx32-positive profile. Transfection of *BRMS1* into the 435 cancer cell line reverses the profile of the cells to a Cx43-positive and Cx32-negative one.[3] hFOB cells have a Cx43-positive and Cx32-negative profile. This may explain the greater heterotypic communication between HTERT cells and hFOBs compared to 435 cells and hFOBs since the former have a more compatible connexin profile with the hFOBs than the latter. In *BRMS1* transfectants, Cx43 levels were only marginally higher than those observed in parental cells. This may explain the equivalent heterotypic GJIC values of parental and *BRMS1*-transfected 435 cells with osteoblasts.

Since functional gap junction channel formation also requires appropriate cell–cell adhesion and cadherins are adhesion molecules that are involved in the formation of functional gap junctions,[20] we examined the cadherin profile of 435 and 435/BRMS1 cells. The reason for studying the cadherin profile of these cell lines was to determine if the differences observed in the homotypic GJIC of the 435 and 435/BRMS1 cells were a consequence of differential cell adhesiveness and cadherin expression.

A large number of cadherins are present in cells, but we focused on E-cadherin, N-cadherin and OB-cadherin. We focused on E-cadherin because it is present in cells of epithelial origin. E-cadherin is absent or downregulated in most invasive breast and other epithelial carcinomas[21] and replaced by aberrant expression of other cadherins.[22] We focused on N-cadherin because it has been shown previously that 435 cells, despite being of epithelial origin, express high levels of N-cadherin.[19] N-cadherin is involved in increased motility, invasion and metastasis of breast cancer cells.[19,23] Normally, N-cadherin is expressed by cells of mesenchymal origin, including osteoblasts. Osteoblasts also express OB-cadherin,[24] so we also measured OB-cadherin in these cells.

E-cadherin was not detected in any cells except MCF-7, in which we detected E-cadherin by both Western blotting and immunocytochemistry, as reported earlier.[25] The absence of E-cadherin in HTERT cells was unexpected since they are presumably very similar to "normal" epithelial cells. However, it is possible that, since HTERT cells are telomerase-immortalized, this might alter certain characteristics in these cells despite their being normal in other respects. E-cadherin expression was lost in 435 cells and not restored in 435/BRMS1 cells, suggesting that the increase in homotypic GJIC of these cells relative to 435 cells was not dependent on the E-cadherin profile. Furthermore, expression of OB-cadherin in osteoblastic cells but not in breast cancer cells suggests that alterations in OB-cadherin expression do not explain differences in relative homotypic and heterotypic GJIC.

Surprisingly, we found that N-cadherin expression was greater in 435/BRMS1 cells (both clones 3 and 6) than in either 435 or hFOB cells. This suggests that alterations in N-cadherin expression do not explain the increased heterotypic GJIC between 435 and hFOB cells relative to the homotypic GJIC among 435 cells. Taken together, our results suggest that alteration in cell–cell adhesion molecules other than E-, OB- or N-cadherin contribute to increased heterotypic relative to homotypic GJIC in 435 cells. However, increased N-cadherin expression in 435/BRMS1 cells may at least partially explain why these cells display increased homotypic GJIC relative to 435 cells.

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REFERENCES

1. MacDonald C. Gap junctions and cell–cell communication. Essays Biochem 1985;21:86–118.
2. Yamasaki H, Mesnil M, Omori Y, Mironov N, Krutovskikh V. Intercellular communication and carcinogenesis. Mutat Res 1995;333: 181–8.
3. Saunders MM, Seraj MJ, Li Z, Zhou Z, Winter CR, Welch DR, Donahue HJ. Breast cancer metastatic potential correlates with a breakdown in homospecific and heterospecific gap junctional intercellular communication. Cancer Res 2001;61:1765–7.
4. Seraj MJ, Samant RS, Verderame MF, Welch DR. Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. Cancer Res 2000;60:2764–9.
5. Harms JE, Welch DR, Manni A. MDA-MB-435 human breast carcinoma metastasis to bone: effects of alpha-difluoromethylornithine on local recurrence and pulmonary metastasis from MDA-MB-435 breast cancer xenografts in nude mice. Clin Exp Metastasis 2003;20:327–34.
6. Chole RA, Tinling SP. Bone lining cells of the mammalian cochlea. Hear Res 1994;75:233–43.
7. Donahue HJ, Saunders MM, Li Z, Mastro AM, Gay CV, Welch DR. A potential role for gap junctions in breast cancer metastasis to bone. J Musculoskeletal Neuronal Interactions 2003;3:156–61.
8. Vaziri H, Benchimol S. Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr Biol 1998;8:279–82.
9. Soule HD, Vazguez J, Long A, Albert S, Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 1973;51:1409–16.
10. Price JE, Polyzos A, Zhang RD, Daniels LM. Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. Cancer Res 1990;50:717–21.
11. Donahue HJ, Li Z, Zhou Z, Yellowley CE. Differentiation of human fetal osteoblastic cells and gap junctional intercellular communication. Am J Physiol Cell Physiol 2000;278:C315–22.
12. Samant RS, Seraj MJ, Saunders MM, Sakamaki TS, Shevde LA, Harms JF, Leonard TO, Goldberg SF, Budgeon L, Meehan WJ, Winter CR, Christensen ND, et al. Analysis of mechanisms underlying BRMS1 suppression of metastasis. Clin Exp Metastasis 2000;18: 683–93.
13. Goldberg GS, Bechberger JF, Naus CC. A pre-loading method of evaluating gap junctional communication by fluorescent dye transfer [erratum in Biotechniques 1995;19:212]. Biotechniques 1995;18:490–7.
14. Ko K, Arora P, Lee W, McCulloch C. Biochemical and functional characterization of intercellular adhesion and gap junctions in fibroblasts. Am J Physiol Cell Physiol 2000;279:C147–57.
15. Czyz J, Irmer U, Schulz G, Mindermann A, Hulser DF. Gap-junctional coupling measured by flow cytometry. Exp Cell Res 2000;255: 40–6.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
18. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.
19. Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J Cell Biol 1999;147:631–44.
20. Trosko JE, Ruch RJ. Cell–cell communication in carcinogenesis. Front Biosci 1998 ;3:D208–36.
21. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 1998;392:190–3.
22. Moll R, Mitze M, Frixen UH, Birchmeier W. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 1993;143:1731–42.
23. Hazan RB, Phillips GR, Qiao RF, Norton L, Aaronson SA. Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis. J Cell Biol 2000;148:779–90.
24. Kawaguchi J, Kii I, Sugiyama Y, Takeshita S, Kudo A. The transition of cadherin expression in osteoblast differentiation from mesenchymal cells: consistent expression of cadherin-11 in osteoblast lineage. J Bone Miner Res 2001;16:260–9.
25. Mauro L, Bartucci M, Morelli C, Ando S, Surmacz E. IGF-I receptor–induced cell–cell adhesion of MCF-7 breast cancer cells requires the expression of junction protein ZO-1. J Biol Chem 2001;276:39892–7.