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Association between Cell Membrane Potential and Breast Cancer

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Abstract
Cell membrane potentials were measured in breast tissue and in breast epithelial cells to explore the relation between cell membrane potentials, oncogenesis and electrical potentials previously measured on the surface of the breast. The mean membrane potential in breast biopsy tissue from 9 women with infiltrating ductal carcinoma was significantly depolarized, compared with values measured in tissue from 8 women with benign breast disease. Depolarization was also observed in transformed breast epithelial cells, compared with normal breast cells; the transformed cells were particularly sensitive to the action of K^+ channel blockers. The results were consistent with previous observations of electropositivity of skin sites over malignant tumors of the breast.

Introduction

Electrical potentials measured on the surface of the skin have been correlated with the presence of cancer [1, 2]. In patients with either cancerous or benign lesions of the face, when the potential was measured at the lesion and at a contralateral control position, the lesion was significantly more positive only in the cancer group [2]. Similarly, sites of basal-cell carcinomas were significantly electropositive compared with control sites in normal tissue, but noncancerous lesions yielded no potential difference between the lesion and control sites [1]. Elevated skin potentials were also associated with cancerous lesions beneath the skin: in women with a palpable breast mass, the skin site above the tumor was

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significantly electropositive only when the mass was a cancer, as determined by a subsequent biopsy [3, 4]. Detection of cancer within the female genital tract by electrical potential measurements was reported [5], but the result was not corroborated in a later study [6].

Electrical potentials originate in tissue as a result of ionic concentration gradients [7]. The electrical differences observed in patients with skin and breast cancer could therefore have arisen from changes in the ionic composition of various tissue compartments occasioned by the cancer: If so, a correlation would be expected between the electrical potential on the surface and the membrane potential of local cells because the potential difference across a semipermeable membrane depends on the concentrations of diffusible ions in accordance with the Goldman equation [8]. We measured the cell membrane potential in both breast tissue and breast epithelial cells to explore the possible relation between membrane potentials, oncogenesis and previously reported electrical potentials measured on the surface of the skin [3, 4].

Methods

Cell membrane potential (V_m) was measured in breast tissue obtained during open surgical biopsies. After approval by the Louisiana State University Medical Center IRB, breast tissue was obtained from 17 patients selected on the basis of clinical suspicion regarding the ultimate diagnosis, in an attempt to produce cancer and benign groups of about equal size. A 5-mm cube of tissue was obtained by a pathologist from the portion of the biopsy specimen not needed for clinical diagnosis, and placed in bath solution (defined below). V_m measurements were made using standard methods [9, 10] modified as follows. Wedge-shaped slices, about 1 mm along the thickest edge, were cut and fixed to the bottom of Petri dishes using stainless-steel wires. The tissue slices were equilibrated in the bath solution at 25 °C for 10 min before measurement, which was accomplished by advancing a microelectrode through the tissue at a constant rate of 0.6 μm/s using a motorized micromanipulator. The criteria for an acceptable measurement were: (1) an abrupt change in potential on entry into the cell; (2) a stable potential for ≥ 5 s and (3) return of the potential to baseline after passage of the microelectrode out of the cell. The mean of a minimum of 20 cells was used to characterize the V_m of the tissue; if the requisite number was not obtained within 3 h of the biopsy, the data were discarded. Following the measurements, the tissue was fixed in formalin and prepared for histological examination.

Cell membrane potential was also measured in MCF 10A cells (ATCC, Rockville, Md., USA), a non-transformed cell line ('normal') from a patient with fibrocystic breast disease, MCF 7 cells (ATCC), an estrogen-receptor-positive (ER+) cell line from a patient with breast adenocarcinoma, and MDA 435L2 cells, an estrogen-receptor-negative (ER–) cell line from a patient with breast ductal carcinoma (kindly provided by Janet Price, MD Anderson, Houston, Tex., USA). MCF 10A cells were cultured in a 1:1 mixture of Hamm's F12 medium and Dulbecco's modified Eagle's medium (MEM) containing cholera enterotoxin (100 ng/ml), insulin (10 μg/ml), hydrocortisol (0.5 μg/ml), EGF (20 ng/ml), 95%, and fetal bovine serum, 5%. MCF 7 cells were cultured in MEM, insulin (10 μg/ml), 90%, and fetal bovine serum, 10%. MDA 435L2 cells were cultured in MEM 95%, and fetal bovine serum, 5%. The cells were seeded at 10^5 cells/flask; the MCF 7 and MDA 435 cells reached confluence in 6–8 days, and the MCF 10A cells in about 14 days. For measurements of V_m, cells from monolayer cultures were trypsinized, resuspended twice in culture medium, and then allowed to settle for 4 h (10^5 cells/cm^2). Immediately prior to measurement, the medium was replaced with a bath solution having the following composition (in m*M*): NaCl, 145; KCl 5.4; $CaCl_2$, 1.0; HEPES, 5.0; glucose, 5.0; pH, 7.4. Membrane potentials were determined in isolated cells. The impalement technique consisted of placing the microelectrode tip at an angle of 30–45°, with enough pressure exerted to cause a small dimple on the membrane surface; a slight jog of a micromanipulator moved the electrode tip into the cell. The criteria used to characterize a successful impalement were: (1) an abrupt change in potential on entry into the cell; (2) a stable potential (± 3–4 mV) for 20 s and (3) return of the potential to baseline (± 4 mV) after withdrawal of the microelectrode. The role of K^+ and Na^+ channels in mediating V_m was evaluated by adding tetraethylammonium (TEA), 4-aminopyridine (4-AP) or tetrodotoxin (TTX) to the bath solution. About 10^6 cells from

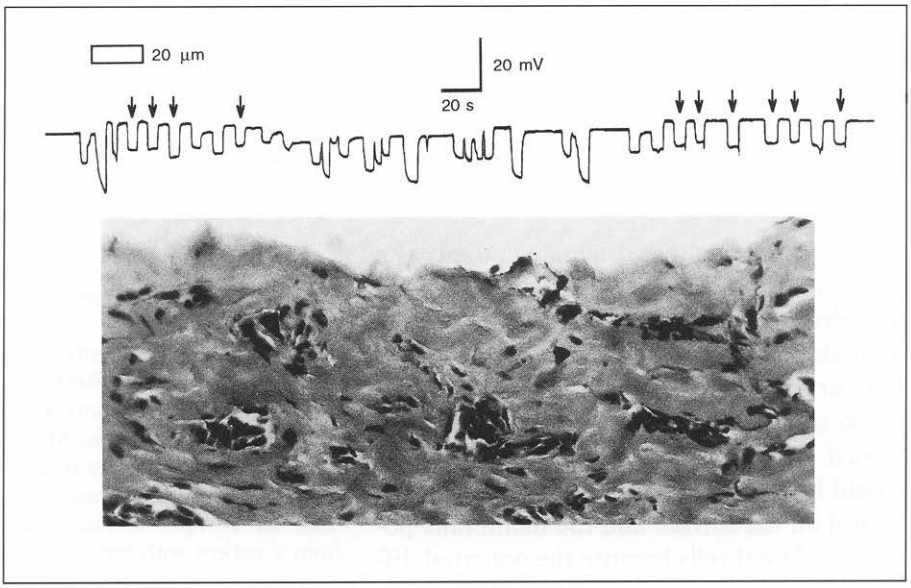

Fig. 1. Membrane potentials in breast cells from a patient with intraductal carcinoma (table 1, No. 7). The arrows indicate the measurements from individual cells that met the acceptance criteria. The microelectrode was advanced at 0.6 µm/s and traversed the illustrated pockets of tumor cells and fibrous matrix present in the tissue (the path of the micropipette was along the upper margin). The distance scale applied to both illustrations.

each of the cell lines were implanted in the breast of female nude mice, and after 1–2 months, V_m was measured in the resulting solid tumor using the tissue measurement method described above.

Micropipettes were pulled from filament capillaries and filled with 3 *M* KCl (electrode resistances, 100–200 MΩ). A silver-chloride electrode in the micropipette was connected to an electrometer (Keithley, model 603, Keithley Instruments, Cleveland, Ohio, USA), and the membrane potentials were recorded on a strip-chart recorder, with reference to a silver-chloride electrode in the bath solution. The data were analyzed using the unpaired t test at a significance level of $p < 0.05$, after testing for normality using the χ^2 test.

Results

Results of a typical measurement of membrane potentials in breast tissue from a patient with intraductal carcinoma are shown in figure 1, along with a corresponding histological view. Groups of values from individual cells were observed as the micropipette traversed the tissue, which contained nests of anaplastic pleomorphic cells with frequent mitoses. The other cancer tissues presented a similar histological appearance, but varied in the number of sheets or nests of tumor cells and in the extent of necrosis. Patients with benign breast disease characteristically yielded irregularly spaced individual values of V_m. The results from 9 patients with infiltrating ductal carcinoma and 8 patients with benign breast disease are shown in table 1 in relation to pertinent patient characteristics. V_m was significantly depolarized in patients diagnosed with infiltrating ductal carcinoma, compared with patients who had benign breast disease (grand mean, table 1). The asso-

Table 1. Mean membrane potential (n ≥ 20) of breast tissue cells from patients with malignant or benign lesions

Patient	Age	Diagnosis	Receptor status		Membrane potential, mV
			ER	PR	
1	46, W	IDC	–	+	−10.5
2	34, B	IDC	+	+	−14.5
3	53, B	IDC	–	–	−17.9
4	44, W	IDC	–	–	−13.5
5	68, B	IDC	+	+	−14.4
6	46, W	IDC	–	–	−13.0
7	26, W	IDC	–	–	−10.6
8	55,W	IDC	–	–	−12.8
9	47, B	IDC	–	–	−12.1
Grand mean ± SD					−13.3* ± 2.2
10	37, W	fibroadenoma			−15.7
11	39, B	fibroadenoma			−20.0
12	28, W	fibroadenoma			−16.6
13	41, B	fibrosis			−11.0
14	54, W	fibrosis			−16.3
15	54, B	fibrosis			−19.5
16	20, B	fibroadenoma			−14.7
17	31, W	fibrosis			−15.6
Grand mean ± SD					−16.2 ± 2.8

IDC = Infiltrating ductal carcinoma; ER = estrogen; PR = progesterone. W, B = white and black female, respectively. Receptor status – and + corresponds to less than and greater than 15 fmol/mg cytosol protein, respectively. * $p < 0.05$.

ciation between lower V_m and cancer did not depend on the age of the patient (fig. 2).

The distributions of the measurements of V_m in isolated normal and transformed breast epithelial cells are shown in figure 3, and the results are summarized in table 2; both transformed cell lines were signifciantly depolarized, compared with the normal cells. The K^+ channel blockers significantly reduced V_m in all three cell lines, but the effect was more pronounced in the transformed cells, particularly following the addition of 4-AP (fig. 4). Addition of 10 μM TTX (a Na^+ channel blocker) had no effect on V_m in any cell line. A total of 9 mice were implanted with the epithelial

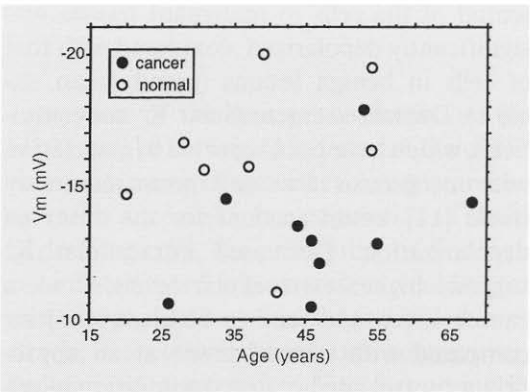

Fig. 2. Relationship between patient age and the mean V_m of cells from surgically obtained breast tissue (data from table 1).

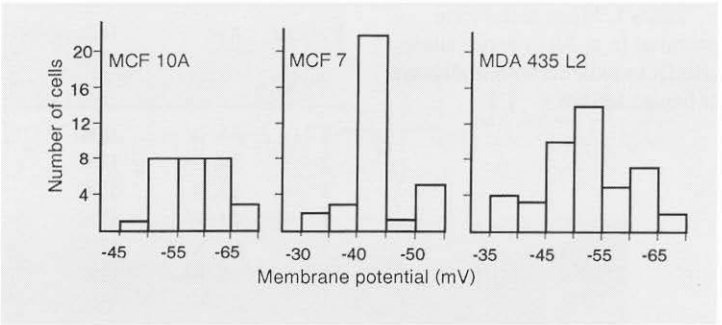

Fig. 3. Frequency histogram of the membrane potential of normal (MCF 10A) and transformed (MCF 7, MDA 435 L2) breast epithelial cells.

cells (3 mice/cell line), and V_m measurements were obtained on 1–2 mice/cell line; the resulting range of V_m was 16–18 mV.

Discussion

The breast tissue specimens contained a variety of cell types, and the measurement method did not permit a determination of the cell type corresponding to each value of V_m. It was therefore not possible to characterize the membrane potential of the malignant cells of epithelial origin in relation to normal epithelial cells. But, when measured under standardized conditions, the mean membrane potential of the cells in malignant lesions was significantly depolarized, compared with that of cells in benign lesions (grand mean, table 1). Decreased intracellular K^+ concentrations, which have been reported in association with oncogenesis in rat and mouse mammary tissue [11], could account for the observed depolarization. Decreased intracellular K^+ suggests higher extracellular levels; if so, a cancer site would tend to be electropositive compared with normal tissue at an appropriate control site because the addition of relatively few ions can easily produce electrical potentials [12] comparable in magnitude to those measured on the skin surface in cancer patients [3, 4]. Decreased intracellular K^+ could occur as a result of altered kinetics of K^+ channels, and the effects of K^+ channel blockers on the membrane potential of normal and transformed breast epithelial cells (fig. 4) is evidence of a change in K^+ channel conductance in association with expression of the oncogenic phenotype. The membrane potential in transformed cells, but not in normal cells, was determined almost completely by 4-AP-sensitive channels, indicating either a change in membrane permeability to 4-AP (thereby permitting increased blocking from the inside of the cell) or an alteration in K^+ channel expression. Increased intracellular Na^+ can occur in association with oncogenesis in mammary tissue [11] and would also tend to depolarize the cell; however, the absence of TTX-sensitive channels in the epithelial cells suggests that Na^+ channel conductance is not the basis of the depolarization of the tissue cells (table 1).

The mean membrane potentials of the transformed cells were depolarized compared with normal cells (table 2). Use of micropipettes for measurement of membrane potential can result in errors due to loss of cytoplasmic components following cell impalement; such errors are usually reflected by skewing in the membrane potential frequency histogram in the direction of low V_m [13]. The measured

membrane potentials, however, were normally distributed for all three cell lines (fig. 3), suggesting that the impalement process itself did not introduce significant errors. Thus, the epithelial-cell measurements indicated a true association between depolarization and expression of the oncogenic phenotype, in agreement with the results found in the tissue measurements. Too few mice were implanted with the epithelial cells to permit a determination of whether the V_m relationships observed in vitro were preserved when the measurements were repeated in the corresponding solid tumors (n = 9 implanted, N = 6 survived, n = 5 tissues available for measurement), but the mouse breast tissue V_ms were significantly less than those measured in the cell line from which the solid tumors were derived, indicating that gap junctions and matrix interactions profoundly affected V_m.

An association between membrane potential and cancer has been observed in many previous studies using normal and transformed cell lines, in situ measurements, and excised tissues measured in a standard bath solution (table 3). Such data were one reason that prompted Cone [14] to suggest that V_m might control the mitotic cycle. The general idea was that a decreased V_m initiated progression through the cycle; oncogenesis was explained on the basis of an assumption that, following mitosis, the cell was prevented from assuming its normal electronegative resting membrane potential and thus forced to reenter the cycle. It was subsequently established that V_m indeed changed during the cell cycle [15–17]. From an initially negative resting level, V_m became progressively electropositive beginning in G_1, reached a peak in late G_1 or early in the S-phase, and decreased following mitosis to assume its relatively electronegative value. But since V_m is decreased during a substantial portion of the cycle, the frequently observed association between V_m and

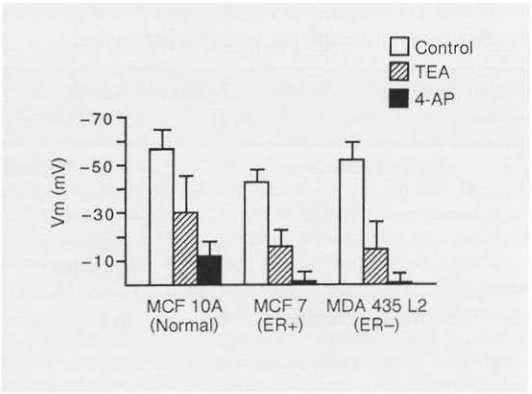

Fig. 4. Effect of K^+ channel blockers on the V_m of normal and transformed breast epithelial cells. Following impalement, the indicated blocker was added to the bath solution to a final concentration of 1 m*M*. n = 5 (n = 10 for controls).

Table 2. Membrane potential in normal and transformed breast epithelial cells (means ± SD)

Cell type	Cells	V_m, mV
MCF 10A (normal)	28	−58.1 ± 5.8
MCF 7 (ER+)	33	−42.1 ± 5.3*
MDA 435 L2 (ER–)	45	−51.8 ± 8*

* $p < 0.05$.

expression of the oncogenic phenotype (table 3) may be explained in terms of the difference in duration of the cell cycle between normal and cancer cells. In a population of rapidly dividing cells, the probability of finding a cell in late G_1 or S is greater than would be the case in a population of cells that divided more slowly. Since cells in late G_1 or S are relatively depolarized, the average V_m in cancer cells would be expected to be less negative, compared with normal cells. Further, V_m was apparently not related to age (fig. 2) or recep-

Table 3. Comparison measurements of membrane potential in normal and cancer cells. Measurements were made using micropipettes, except where noted

Reference	Method	Animal	Normal cell	Comparison cell	Normal	Cancer
18	confluent monolayer	rat	kidney epithelial cells	chemically transformed fibroblasts	−61.9 ± 8.89	−30.7* ± 7.36
		quail	embryonic fibroblasts	chemically transformed fibroblasts	−40.1 ± 8.89	−20.7* ± 5.96
19	confluent monolayer	rat	kidney cells	virally transformed kidney cells[b]	13.5% decrease in V_m in association with change to the transformed phenotype[c]	
20	subconfluent monolayer ($< 10^5$ cells/cm^2)	mouse	3T3 fibroblasts	virally transformed 3T3 cells	−12.3 ± 0.9	−22.7 ± 1.6
This study	subconfluent monolayer (10^5 cells/cm^2)	human	normal breast epithelium	transformed breast epithelium	−58.1 ± 5.8	−42.1 ± 5.3 (ER+) −51.8 ± 8 (ER−)
13	in situ	rat	hepatocytes	hepatoma	−37.1 ± 4.3	−19.8 ± 7.1
		mouse	corneal fibroblasts	fibrosarcoma	−42.5 ± 5.4	−14.3 ± 5.4
21	in situ	rat	muscle cells	rhabdomyosarcoma	−88.7 ± 5.8	−15.5 ± 4.4
9	excised tissue	rat	thyroid cells	thyroid cancers	−47.2 ± 3.3	−21.7 to 31.7[a]
		hamster	thyroid cells	thyroid cancer	−38.5 ± 3.0	−22.6 ± 2.4
22	excised tissue	human	cervical cells	cervical carcinoma	−33	−22
23	excised tissue	human	glial cells	gliomas	−69 ± 10	−32 to −70[a]
This study	excised tissue	human	breast cells	intraductal carcinoma	−16.2 ± 2.8	−13.3 ± 2.2
24	excised tissue	human	gastric mucosa	gastric carcinoma	−24 to −35	−19 to −28

[a] Various cancer types.
[b] Temperature-sensitive mutant.
[c] Voltage-sensitive dye.
* $p < 0.05$.

tor status (table 1), as might have been expected if membrane potential changes directly mediated oncogenesis. We conclude, therefore, that the observed association between V_m and expression of the oncogenic phenotype (table 3) might be a consequence of the increase in mitotic rate.

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