Comparative Analysis of the Work of Morphofunctional Zones in Normal Epithelium, Fibroadenoma, and Cancer of the Breast

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 8, pp. 201-205, August, 2005
Original article submitted October 6, 2004

Twenty-four rosettes (proliferative epidermal units) in epithelial tissue are united into morphofunctional zones, where cambial cells proliferate with the formation of maternal and daughter cells and these latter ones differentiate under the influence of electrical field generated by 12 maternal cells. The number of cambial cells in morphofunctional zone of a malignant tumor is reduced at least 2-fold. Hence, the number of maternal cells is reduced to 6, which is insufficient for electric field generation and stimulation of daughter cells differentiation. The percentage of cambial cells in a benign tumor decreases 1.5 times. Daughter cells are differentiated in an electric field whose strength is below optimal, but above the threshold value at which cells do not differentiate; hence, differentiation is incomplete.

Key Words: *morphofunctional zones; cambial cell differentiation; fibroadenoma; cancer*

Modern data suggest that impaired structural organization of epithelial tissue can lead to the development of pathological states, *e.g.* cancer [1,2,12]. However, most reports describe an individual structural proliferative unit in normal epithelium, while studies of tumor tissue are scanty [1]. In addition, differentiation of stem cells in the center of this structural unit was considered mainly as a result of the effects of environment ("niche"), hormones, or growth factors [9-11].

We studied cooperative interactions of tissue units and possible differentiation of cambial cell in the system of these units under normal conditions and during tumor growth.

MATERIALS AND METHODS

Tumor tissue from 2 groups of patients (15 patients per group) with breast cancer or fibroadenoma aged 40-60 years was studied. Impressions of cancer and fibroadenoma tissue treated with silver nitrate by the method of Ranvier were examined. Morphometric analysis was carried out on a Video-Test-3.2 image analyzer. Cell area (S) and degree of their elipticity (ED) were estimated and the counts of cell populations was evaluated from these parameters: daughter cells (S= 52.4 μ^2, ED=0.61), early daughter cells (S=61 μ^2, DE= 0.93), "reserve" (S=43.7 μ^2, ED=0.58), "elongated" (S=43.0-47.4 μ^2, ED=0.26-0.42), "oval" cells (S=51.7-89.0 μ^2, ED=0.62-0.78). Two thousand cells were examined in each case. The percentage of different cells in the population was evaluated per 250 cells, which corresponded to their content in one functional zone.

RESULTS

We previously found that cells in the epithelial tissue are grouped in rosettes with 6-7 cells at the periphery and 3-4 in the center [4,5,7]. Rosettes, in turn, are united in morphofunctional zones (about 24 rosettes per zone, a total of 240-250 cells) formed from cambial cells of the same type and presenting a cell base

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0007-4888/05/1402-0231

of the system regulating proliferation and differentiation processes in the epithelium [4,5]. As soon as half of cambial cells starts mitosis, the release of the other half of these cells is inhibited, which leads to division of the zone into two parts or subunits with equal number (12) of cambial cells (Fig. 1, *a*). Division of cambial cells of sububit 1 yields maternal and daughter cells possessing properties of dipole cells and serving as sources of electric field [5]. Initially, maternal cells are in close contact with the daughter cells, and then displace them from the rosette center. This creates a balance between maternal and daughter cells in subunit 1; the cells are still closely contacting, on the one hand, and are already separated, on the other (Fig. 1, *a*, *b*). Daughter cells separated from maternal cells need time for translocation into the rosette centers of another subunit for differentiation, and hence, the predominance of 6 maternal cells over daughter cells is created, but this number of maternal cells is insufficient for generating electric field and differentiation. Only after the number of similarly directed maternal cells increases to 12 at the expense of the other half of these cells, contacting with daughter cells in subunit 1 before, the daughter cells are differentiated (Fig. 1, *c*). Newly formed 12 differentiated daughter cells inhibit further proliferation of these cambial cells, which, in turn, stimulates the proliferation of these cambial cells in subunit 2, which works similarly as subunit 1 [4,5]. Differentiated daughter cells formed during the work of subunits 1 and 2 gradually transform into "oval" cells, which supplement the pool of the so-called "reserve" cells (30%) required for physiological regeneration of the plast [3,6,7]. With increase of the proliferative activity of the epithelium, the reserve cells are transformed into "elongated" cells, which directly participate in mitosis.

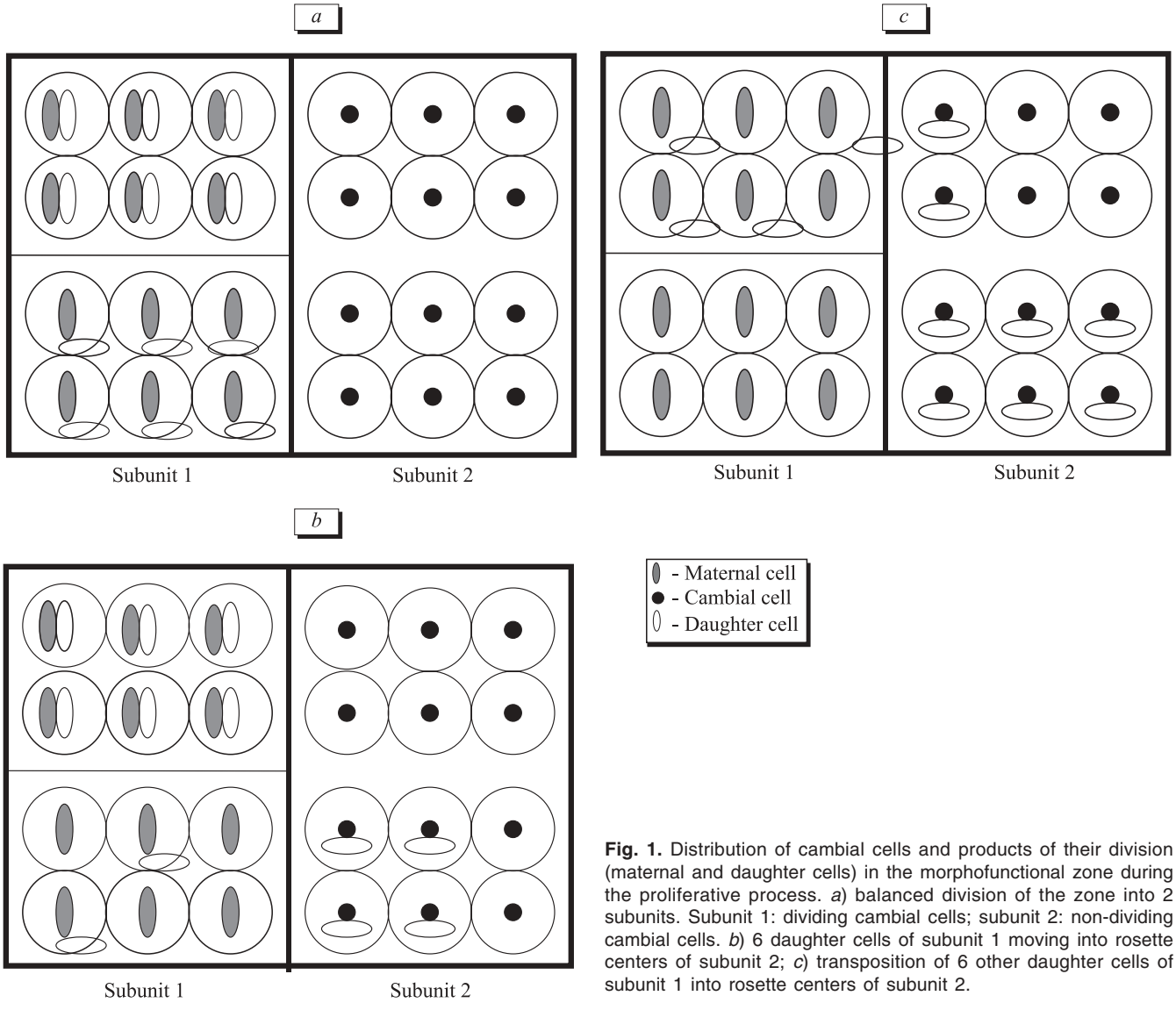

Fig. 1. Distribution of cambial cells and products of their division (maternal and daughter cells) in the morphofunctional zone during the proliferative process. *a*) balanced division of the zone into 2 subunits. Subunit 1: dividing cambial cells; subunit 2: non-dividing cambial cells. *b*) 6 daughter cells of subunit 1 moving into rosette centers of subunit 2; *c*) transposition of 6 other daughter cells of subunit 1 into rosette centers of subunit 2.

During the work of subunit 1, 15% oval cells are formed, supplementing the pool of reserve cells only by half, and thus inhibiting their proliferation, which determines attenuation of reserve cell proliferation. Due to functioning of subunit 2 the number of oval cells increases to 30%, this completely restoring the reserve cell depot [4,7]. Hence, subunit 1 works during active proliferation of reserve cells, while subunit 2 during its attenuation.

The histostructures of benign tumors and cancer represent the same systems of cell groups of the epidermal-proliferative unit, functions or rosettes typical of normal epithelium, and hence, such tumors emerge as a result of certain restructuring of normal tissue functions [2,8].

Mammary fibroadenoma develops from a solitary morphofunctional zone of normal epithelium with 1.5-fold lower number of cambial cells. This is seen from a 1.5-fold decrease in the number of daughter cells formed during division of cambial cells in the tumor. This zone works similarly as the normal zone, that is, both subunits function; but the decrease in the number of cambial cells to 16 in the zone leads to the formation of a pathological process in it. Eight daughter cells formed during the work of subunit 1 will be differentiated in the electric field generated by only 8 maternal cells, the energy of this field being below optimal (created by 12 cells), but higher than the threshold level (generated by 6 cells), when there is no differentiation; hence, differentiation will be in-

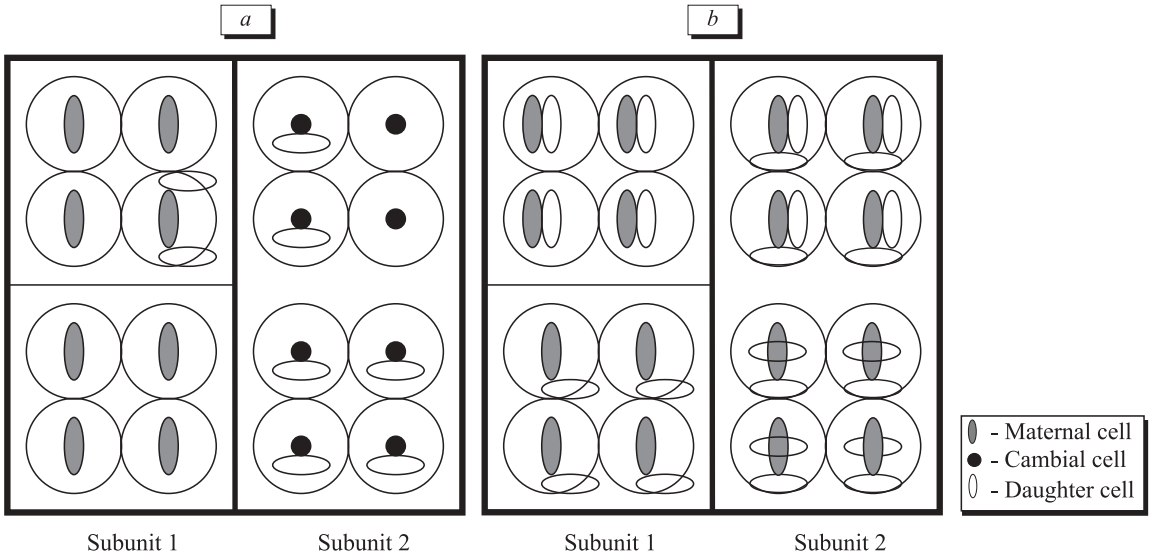

Fig. 2. Distribution of cambial, maternal, and daughter cells in the morphofunctional zone during development of fibroadenoma. *a*) differentiation of 8 daughter cells of subunit 1; *b*) repeated mitosis of cambial cells of subunit 1.

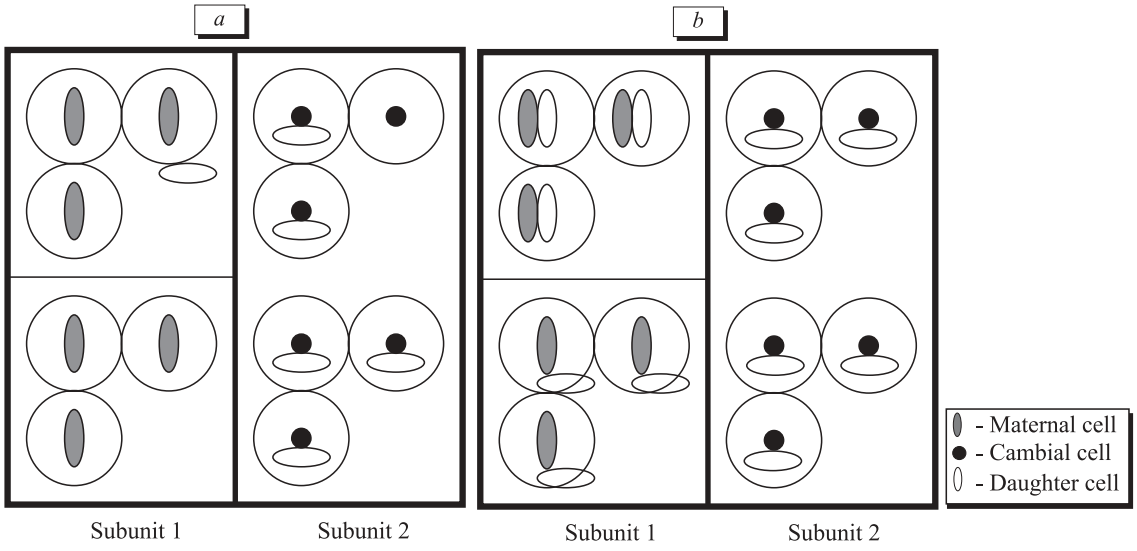

Fig. 3. Distribution of cambial, maternal, and daughter cells in the morphofunctional zone during development of cancer. *a*) 6 undifferentiated daughter cells of subunit 1; *b*) repeatedly dividing cambial cells of subunit 1.

complete and abnormal (Fig. 2, *a*). As a result of decreased number of cambial cells, a strictly constant share of daughter cells (12 cells per subunit) is maintained in this morphofunctional zone (similarly as in normal epithelium); the daughter cells are transformed into other epithelial cells and provide the work of the epithelium [3,5,7]. Eight formed daughter cells will not inhibit proliferation of subunit 1 cambial cells, and these letter will again divide (Fig. 2, *b*). Activation of cambial cells during this period can be seen from 1.5-1.6-fold increased number of earlier forms of daughter cells in comparison with the normal epithelium. Eight more daughter cells form during second division of subunit 1 cambial cells. As soon as the sum of daughter cells resultant from the first and second mitosis reaches 12, subunit 2 starts its work (the formation of 12 daughter cells is a signal to proliferation of its cambial cells). Hence, subunit 1 cambial cells are still proliferating, while subunit 2 started functioning (Fig. 2, *b*). Hence, in contrast to normal epithelium, where only one subunit works during each period of the proliferative process, two subunits start functioning simultaneously in a benign tumor. A similar situation emerges in each of these subunits during the next proliferative process: subunits again divide into 2, subunit 1 dividing first and subunit 2 after it, that is, the normal order of their function is preserved. Hence, 4, then 6, 8..., 24 subunits form from two subunits of the initial zone; these new subunits will be located radially from the initial zone. The maximum number of zones does not exceed 24, because division of the primary zone does not involve the last indivisible structure in the zone: one rosette developing from one daughter cell. Disturbed work of the morphofunctional zone manifests in the appearance of specific spatial organization of a pathological focus. Studies of nodular hyperplasia and benign tumors of the thyroid and mammary glands showed that foci of non-tumor growth were characterized by radial symmetry with 4, 6, 8, 12, and 24 radial sector zones forming these foci [8], which is in line with our data.

Malignant tumor develops from a single morphofunctional zone of normal epithelium, where the number of cambial cells (maternal and daughter) decreased at least 2-fold. Only 6 daughter cells form during the function of subunit 1 (corresponding to the active proliferation period of "reserve" cells) *vs.* 12 in normal (Fig. 3, *a*). However, these cells do not differentiate, because the number of maternal cells is reduced to 6, a critical level at which no electric field is generated and hence, differentiation is not triggered. In addition, 6 daughter cells formed during the work of subunit 1 (instead of the needed 12 cells) will not inhibit the proliferation of cambial cells, and these letter will again divide (Fig. 3, *b*). Hence, subunit 1 cambial cells proliferate not once (as in normal epithelium), but 2 times running. As a result, 15% "oval" cells, resultant from transformation of daughter cells, are forming longer than normally. Therefore, oval cells will not block proliferation of reserve cells for a longer time, which leads to increase of the tumor cell bulk. This is confirmed by a reduction to 8-10% (*vs.* normal 16%) in the number of "elongated" cells (characterizing the degree of the reserve cell proliferation), which start active mitosis. The work of subunit 2 is in general similar to that of subunit 1. The difference is as follows: subunit 2 cambial cells function under conditions of inhibited release of the "reserve" cells for proliferation, as 15% oval cells, resultant from the work of subunit 1, suppressed them.

Hence, the increase in the tumor bulk is mainly due to the work of subunit 1. This manifests in a specific symmetry of cancer foci (concentric spherical), when solitary tumor tissue structures are located at concentric orbits [8].

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