Demonstration of Human Breast Carcinoma Cells in Cryosections and Primary Monolayer Cultures of Surgical Biopsies by Neotetrazolium Reductase Cytochemistry

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ABSTRACT

The present study describes a cytochemical approach to demonstrate human breast carcinoma cells in cryosections and in primary monolayer cultures from surgical biopsies. The material consisted of biopsies from 52 carcinomas and 29 benign lesions. Cryosections and cultures were incubated to demonstrate NADPH-neotetrazolium reductase in an atmosphere of 99.5% oxygen. Incubation time and section thickness were adjusted to accomplish the same level of reaction in cells of cryosections and corresponding cultures. Positive reaction was thus confined to epithelial elements and to the wall of some smaller blood vessels. More than one-half of the carcinoma cells showed moderate to strong reaction in cryosections from 29 of 52 carcinomas whereas no reaction was seen in ductules of normal appearance adjacent to these carcinoma cells. Positive reaction was seen in epithelial cell islets in primary cultures of 16 of the 40 carcinomas cultured.

In cryosections from fibroadenomas and fibrocystic disease specimens only apocrine metaplasia consistently showed positive reactions compared with less than 10% of other ductular profiles in a given cryosection. This pattern of reaction was reflected in the derived primary cultures in which positive reaction was found in epithelial cell islets in only one of 19 biopsies cultured.

The presence of human milk fat globule membrane antigen was used to demonstrate the epithelial nature of the cell islets seen in cultures of biopsies from both benign lesions and carcinomas. NADPH-neotetrazolium reductase positive islets from carcinoma biopsies were frequently aneuploid whereas most negative islets from carcinoma biopsies were diploid as were all islets from benign tissues.

INTRODUCTION

The conditions for cultivation of normal human breast epithelial cells from surgical biopsies are gradually becoming better defined (1–3). This is in contrast to results obtained so far with cultures of breast carcinoma cells. Thus, although epithelial cells from breast carcinomas are sometimes present in culture, we and others have questioned whether they actually represent the carcinoma cell populations of the tumor (4–7). Many solid carcinomas contain both hyper- and metaplastic epithelial elements as well as remnants of the original uninvolved mammary gland. These may also form epithelial monolayer islets in culture. Since a distinction between cultured carcinoma cells and other epithelial cell islets based on either traditional morphological criteria or on recently discovered tumor associated antigens is still open to question (7), alternative approaches are required.

It has often been suggested that malignant epithelial cells exhibit a higher level of activity of the pentose-shunt enzyme than do normal cells (8–11), and this activity is preserved when cells are explanted to primary culture (4). Although it is generally accepted that specific tumor markers do not currently exist (12), a clearcut difference has recently been documented cytochemically in the G6PDH reaction between normal and malignant epithelial cells from human lung, stomach, colon, and breast (13–15). By incubation in pure oxygen the reaction for G6PDH can be completely suppressed in normal epithelial cells of lung, stomach, colon, and breast whereas it is retained in the corresponding carcinomas (13–15). The studies on G6PDH activity have led to the suggestion that endogenous intermediate electron carriers (flavoproteins of NADPH-NR reductase) play a significant part in the cytochemical demonstration of G6PDH in pure oxygen (8, 15, 16). Such electron carriers may be identical to the particulate (structural bound) fractions of NADPH cytochrome c (cytochrome P-450) (EC 1.6.4.2) reductases (17), and/or they may be important for many biosynthetic steps required for cellular growth (18). If NADPH-NR reductase cytochemistry, which is very easy to perform, could be applied routinely to both cryosections and primary monolayer cultures from human primary breast carcinoma in order to demonstrate at least some of the carcinoma cell populations, it would represent a very simple approach compared to G6PDH-, DNA-, and immunocytochemistry.

The present study therefore is addressed to a direct demonstration of the particular fractions of NADPH-NR reductase during incubation in an atmosphere of pure oxygen of human breast epithelial cells in cryosections and in culture. For both cryosections and corresponding primary monolayer cultures of surgical biopsies, we show that provided tightly controlled conditions and section thickness are observed a marked NADPH-NR reductase reaction is preserved in the majority of carcinoma epithelial cells from approximately one-half of the carcinomas during oxygen incubation whereas the majority of benign biopsies are negative.

MATERIALS AND METHODS

Biopsy Specimens. The carcinoma specimens consisted of 52 biopsies removed from patients undergoing mastectomy due to primary breast cancer. The noncarcinomatous specimens consisted of biopsies from 29 patients with either FA or FD undergoing tumorectomy on the suspicion of breast cancer. All specimens received were divided into two pieces, one for cryosections and one for tissue culture.

Cryosections. The portion for cryosection was frozen in isopentane cooled at −70°C in an alcohol-solid carbon dioxide mixture within 5–10 min of receipt. The tissue was stored mounted and sectioned in a motor driven cryostat to ensure uniformity of section thickness as described in a previous study (15). The thickness setting of the cryostat was 6.5 μm; thicker sections caused formation of reaction product in ductular epithelial elements and thinner sections provided a lower G6PDH enzymatic activity than normal cells (8–11). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4 The abbreviations used are: G6PDH, glucose 6-phosphate dehydrogenase; CDM1, CDM2, chemically defined medium 1 or 2; MFGM-A, human milk fat globule membrane antigen; FA, fibroadenoma; FD, fibrocystic disease; NT, neotetrazolium; TDLU, terminal duct lobular unit.

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reaction in malignant cells than that seen in corresponding cultures. Sections were cut at two different levels in each block, picked up on clean slides, and surrounded by a Perspex ring inside which the incubation medium was placed.

The incubation medium consisted of 4.49 mM chloroform-purified neotetrazolium chloride (Serva)-1.19 mM NADPH (Sigma Chemical Co.)-100 mM β-glycerophosphate (Sigma) in 0.05 mM phosphate buffer, pH 7.20. The medium was saturated for 5 min at 37°C with 99.5% oxygen before use. The reaction was performed at 37°C for 15 min in an atmosphere saturated with 99.5% oxygen. Specificity of the enzyme reaction was ensured by incubation of control sections in medium without NADPH addition. At the end of the incubation period the slides were transferred to distilled water at 0–4°C for 5 min and thereafter postfixed 1 h in phosphate buffered 4% formaldehyde, pH 7.2, at 0–4°C; hematoxylin stained, dried, and mounted in glycerol-gelatine. The intensity of reaction in the cells was evaluated semiquantitatively as strong if clearly visible with a ×10 objective in combination with a ×12.5 eyepiece and moderate if only visible with a ×25 objective. In addition, the relative proportion of cellular profiles in a given section with positive reaction was estimated. The histological classification of the component parts of the benign lesions was performed according to WHO recommendations (19). No attempts were made to morphologically subdivide the malignant lesions; no cytological malignancy or nuclear atypia classifications could be applied to the cells in the cryosections.

Cultures. Primary short-term cultures were established from 40 of the carcinomas and from 19 of the benign lesions (FD and FA specimens). The portion of the fresh biopsy for cultivation was immediately transferred to growth medium (CDM2, see below). After a short storage (1–3 h) at 0–4°C the biopsy was gently dissected into small pieces, enzymatically digested by collagenase (C 0130 from Sigma), 900 IU/ml for 24 h at 37°C on a rotary shaker (70 rpm) in CDM2 plus soybean trypsin inhibitor (T9003 from Sigma), 0.1 µg/ml. The cell suspension was then dispensed into 4 T25 flasks (Nunc, Roskilde, Denmark) for further cultivation in various growth media.

Short-term primary cultures were established in 4 different growth media: (a) CDM1; this medium was a modification of a medium for growth of the established human breast cancer cell line MCF-7 (20), (b) HBCA; this medium was a modification of the recently introduced medium for growth of various malignant cell lines (21), (c) CDM2; this medium was a modification of the recently introduced medium for cultivation of human breast epithelial cells (3), and (d) CDM2 supplemented with 20% fetal calf serum (4, 5).

The culture flasks were either uncoated or coated with collagen I (Vitrogen; Flow Laboratories) (21) or collagen IV (Sigma) (4, 5). All cultures were maintained for 10–14 days in a humidified atmosphere of 5% CO2, 20% O2, and 75% N2. The medium was changed every 2–3 days and always the day before any cytotoxic analysis. All media were supplemented with penicillin, 250 IU/ml, and streptomycin, 25 µg/ml.

For NADPH-NT reductase cytochemistry, cultures were rinsed twice in phosphate buffered saline, dried for 15 min at room temperature, and incubated under the same conditions as described for the cryosections. The evaluation of reaction level followed the same criteria as for cryosections and only cells lying strictly in monolayer with a clear temperature in 5 mM Tris with 1 mM EDTA, pH 7.4, containing Hoechst 33258 (2 µg/ml), washed in distilled water for 15 min at room temperature, and mounted with coverslips using 0.16 M sodium phosphate with 0.04 M sodium citrate, pH 7.4 (22). The microscope fluorometer consisted of a Leitz Orthoplan microscope stand (Leitz GmbH, Wetzlar, Federal Republic of Germany) equipped with a Ploemopak for epi-illumination fluorescence exitation and combined with a Leitz MPV II photometer system (see also Ref. 5). A total of 100 NADPH-NT reductase positive epithelial cells, 100 NADPH-NT reductase negative epithelial cells, and 100 fibroblast-like cells were measured from each culture. The peak from these latter cells was taken to represent the diploid cell population (2C). Aneuploidy was stated if the leftmost peak of the epithelial cells was higher than 2.5C and not in the tetraploid a rea (3.8–4.2C).

RESULTS

NADPH-NT Reductase in Cryosections

The NADPH-NT reductase reaction causes formation of reaction product deposited as fine blue granules and larger brownish-blue crystals. Moreover, a pink nongranular staining due to monoformazans may also occur. Only the formation of crystals and granules was considered a positive reaction (Fig. 1). The reaction for NADPH-NT reductase is substrate specific since no reaction is seen without NADPH in the medium. Stromal and inflammatory cells were NADPH-NT reductase negative whereas weak reaction was sometimes seen in the walls of smaller blood vessels.

Epithelial Cells of FA and FD. The majority of epithelial components in FA and FD specimens showed no reaction. In particular, no reaction was seen in normal TDLU as revealed in those sections of FA and FD containing both terminal ducts and lobules. The most consistent positive reaction was recorded in apocrine metaplasia (Table 1). On the average, in those cryosections with any reaction at all, NADPH-NT reductase positive ductular profiles constituted less than 10% of the total amount of profiles.

Epithelial Cells of Ductules within Carcinomas. Except for those affected by apocrine metaplasia, no reaction was recorded in ductular profiles (including normal TDLU) adjacent to carcinoma cells present in 24 of 52 of the carcinoma biopsies (Fig. 1; Table 1).

Carcinoma Cells. As seen in Table 2, 55% of the carcinomas showed moderate to strong NADPH-NT reductase reaction in more than one-half of the carcinoma cells within a given section (Fig. 1; Table 2). In biopsies containing both invasive and intraductal carcinoma (8 of 52) the intraductal elements in general showed the strongest reaction.

NADPH-NT Reductase in Short-term Primary Cultures

Identical results were obtained with respect to NADPH-NT reductase activity irrespective of the matrix and the medium used. Like the stromal cells in cryosections, fibroblast-like cells characterized by their spindle-shaped form, multilayered appearance, superimposed oblong nuclei, and lack of MFGM-A showed no NADPH-NT reductase reaction in culture (Fig. 2b). However, primary cultures derived from carcinomas rich in NADPH-NT reductase positive vascular structures contained clusters of fibroblast-like cells with weak NADPH-NT reductase reaction.

Epithelial Cell Islets in Cultures from FD and FA Specimens. Only one of 19 biopsies gave rise to predominantly NADPH-NT reductase positive epithelial cell islets in the cultures. Cultures derived from FA and FD specimens containing ductular profiles with positive reaction in the cryosections (<10% of the profiles; Table 1) were totally negative. After 2–3 wk of cultivation and also after subcultivation a weak NADPH-NT reduc-
Fig. 1. Cryosections (6.5-μm thickness) with (a, b, and c) and without (d) nuclear staining from three different human breast carcinoma biopsies. Sections were incubated for NADPH-NT reductase in an atmosphere of pure oxygen. As seen in a, b, and c the carcinoma cells (CA) are positive for NADPH-NT reductase whereas ductules of normal appearance (DU) exhibit no reaction. The ductules of normal appearance are composed of a distinct inner layer of lining epithelial cells (LEP) and an outer layer of myoepithelial cells (MEP). The absence of reaction in normal ductules is clearly seen in the two consecutive sections c and d. × 500; bar, 50 μm.
Table 1  Reaction patterns of NADPH-NT reductase in benign epithelial components as present in cryosections of 29 benign lesions and 24 carcinomas

None of the epithelial components was found to be present in all of the 29 benign biopsies. Normal lobules and terminal ducts were visible in the sections can be compared to the TDLU in the classification of Wellings (29). Apocrine cysts showed the strongest reaction recorded among epithelial elements. The reaction was visible using a ×10 objective and a ×12.5 eyepiece. Reaction in the other epithelial components was only visible using a ×25 objective. Within individual sections NADPH-NT reductase positive epithelial profiles never constituted more than 10% of the total amount of profiles.

<table>
<thead>
<tr>
<th>Epithelial components classified into histological types according to the WHO recommendations</th>
<th>Number of biopsies containing an epithelial component with positive reaction relative to the total no. of biopsies showing that particular component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign biopsies (N = 29)</td>
<td>Carcinomas (N = 24)</td>
</tr>
<tr>
<td>Uninvolved ducts</td>
<td>2/13</td>
</tr>
<tr>
<td>Normal lobules</td>
<td>0/9</td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>2/15</td>
</tr>
<tr>
<td>Adenosis</td>
<td>0/4</td>
</tr>
<tr>
<td>Hyperplastic cysts</td>
<td>0/1</td>
</tr>
<tr>
<td>Atrophic cysts</td>
<td>0/3</td>
</tr>
<tr>
<td>Apocrine cysts</td>
<td>8/8</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>0/4</td>
</tr>
<tr>
<td>Fibroadenomatous hyperplasia</td>
<td>2/3</td>
</tr>
</tbody>
</table>

Table 2  Reaction patterns of NADPH-NT reductase in carcinoma cells in cryosections of 52 human breast carcinomas

<table>
<thead>
<tr>
<th>% carcinoma cells with positive reaction</th>
<th>Intensity of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;80</td>
<td>2+/&lt;sup&gt;a&lt;/sup&gt; 1+/&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50–80</td>
<td>14/52 9/52</td>
</tr>
<tr>
<td>50–80</td>
<td>2/52 4/52</td>
</tr>
</tbody>
</table>

*<sup>a</sup> 2+, reaction easily visible when using a ×10 objective and a ×12.5 eyepiece.  
*<sup>b</sup> 1+, weaker reaction visible only when using a ×25 objective and a ×12.5 eyepiece.

ductase reaction appeared in islets from benign lesions. The weak reaction appeared as brownish-red crystals only.

Epithelial Cell Islets in Cultures from Carcinomas. In contrast to cultures from benign biopsies, NADPH-NT reductase positive cell islets appeared in 39% of the short-term cultures derived from carcinomas (Fig. 2). The demonstration that NADPH-NT reductase in cryosections was mainly confined to carcinoma cells suggests that the NADPH-NT reductase positive cell islets seen in primary cultures from such biopsies actually represented at least some of the carcinoma cells of the tumor of origin. This notion was further supported by the following observations: (a) NADPH-NT reductase positive cell islets appeared exclusively in cultures derived from those carcinomas characterized by the presence of a large number of carcinoma epithelial cells with NADPH-NT reductase positive reaction (compare Figs. 1 and 2); (b) if the biopsy of origin contained both NADPH-NT reductase positive carcinoma cells and NADPH-NT reductase negative carcinoma cells and/or ductular cells, this pattern was reflected in the corresponding cultures by the presence of both NADPH-NT reductase positive and negative cell islets (Figs. 1 and 2c); (c) the level of NADPH-NT reductase reaction in the NADPH-NT reductase negative cell islets, as evaluated semiquantitatively, often corresponded to that of the carcinoma cells in the original biopsy; and (d) NADPH-NT reductase positive cell islets were sometimes aneuploid whereas the NADPH-NT reductase negative cell islets of FA and FD specimens and of most carcinoma biopsies were diploid (Fig. 3; Table 3). The NADPH-NT reductase reaction of aneuploid cells was a very stable feature in culture showing no decrease within at least 4 wk of cultivation.

DISCUSSION

Our results show that at least 50% of human breast carcinoma cells in cryosections from 55% of surgical biopsies and most cell islets in primary cultures from 39% of the same biopsies are characterized by a moderate to high NADPH-NT reductase reaction. In contrast, reaction is absent in ductular epithelial cells not affected by apocrine change.

The cytochemical NADPH-NT reductase assay is generally considered to reflect the activity of NADPH:quinone acceptor oxidoreductase (EC 1.6.99.2) and the particulate fraction of NADPH:cytochrome c (cytochrome P-450) reductase (EC 1.6.2.4) (17, 23). Since NADPH:quinone acceptor oxidoreductase can be demonstrated only in cryosections by prefixation with formaldehyde vapor and by addition of vitamin K to the incubation medium (24), we can exclude this enzyme as contributing significantly to the present assay. The NADPH:cytochrome c (cytochrome P-450) reductase is also known to be a part of the microsomal cytochrome P-450 respiratory chain which participates in the bioactivation and detoxification of endogenous and exogenous substances (25).

It is important to stress that NADPH-NT reductase as such is not a marker of cancer. Thus, the lack of NADPH-NT reductase reaction in nonmalignant cells is exclusively obtained by using a restricted section thickness and monolayers of cells without any superimposition, a restricted incubation time, and incubation in oxygen. Moreover, even under these conditions some reaction is present in vascular elements and in apocrine metaplasia. It remains uncertain as to why these structures show reaction. However, since the NADPH-NT reductase under influence of oxygen may reflect the presence of enzymes involved in reductive biosynthetic processes as well as in microsomal respiration (18), it is probable that at least one of these enzyme complexes is expressed to a high degree in some vascular elements (presumably neovascularization) and in apocrine metaplasia. It should be considered that many of the oxidoreductases detectable by enzyme cytochemistry on cryosections of breast tissue exhibit an elevated level of activity in apocrine metaplasia (26). Thus, if the cytochemical enzyme levels reflect potential in situ activity, the metabolism of carbohydrate, fat, and protein is certainly elevated in these cells (26).

It would be of interest if any correlation between precancerous lesions of the breast and the NADPH-NT reductase reaction could be established. Thus, recently much attention has been directed towards TDLU as the site of origin of most pathological mammary lesions including carcinomas (27). However, as also reported in other studies (27, 28), it was very difficult in the present study to identify TDLU by the simple examination of cryosections when the architecture of the lobule was altered by unfolding and proliferation (pathological TDLU). This problem can be circumvented only by the method introduced to examine the tissue subgrossly followed by isolation and histological analysis of the particular ductular segment of interest (27–29). Moreover, identification of epithelial atypia, which is necessary to grade the TDLU, requires paraffin sections. This is incompatible with the present enzyme cytochemical approach. With regard to lobules of normal appearance cut in the plane of the entering terminal duct (normal TDLU) these could more easily be identified, and no reaction was found in any part of this unit nor in larger interlobular ductules of normal appearance.

The present study does not deal with any relationships between the NADPH-NT reductase reaction in carcinoma cells and for instance, histological classification, steroid receptor
Fig. 2. Primary monolayer cultures of different human breast carcinoma biopsies incubated for NADPH-NT reductase in an atmosphere of pure oxygen. In the lower right-hand corner of c is seen some epithelial cells without reaction for NADPH-NT reductase. Arrows, mitotic figures. FIB, fibroblasts; EXP, explant; CA, carcinoma cells. a and b, × 500; c, × 520; bars, 50 μm.

status, or other diagnostic and prognostic parameters. The scope of the present study was to assess the potential usefulness of a cytochemical method to correlate the demonstration of carcinoma cells in cryosections and in primary cultures derived from solid carcinomas. Since these often contain ductular components corresponding to those found in FA and FD in the
Fig. 3. Primary culture from a breast carcinoma specimen incubated for NADPH-NT reductase in an atmosphere of pure oxygen. Three epithelial cell islets (E₁, E₂, and E₃) are shown at low magnification (a) surrounded by a confluent layer of mostly superimposed fibroblasts (FIB). E₁ is positive for NADPH-NT reductase whereas E₂ and E₃ are negative. Moreover, E₁ exhibits a bimodal aneuploid DNA pattern (b) whereas the fibroblasts and the NADPH-NT reductase negative epithelial cell islets (E₂ and E₃) are diploid (c and d). The NADPH-NT reductase reaction product is strictly confined to the perinuclear region (e) not interfering with the DNA measurements of the nuclei (N). When stromal cells (FIB) become confluent and superimposed they may exhibit some reaction for NADPH-NT reductase as well (f). In contrast, the epithelial cells in monolayer (E₂) as identified by expression of epithelial membrane antigen (g) remain without reaction. a, x 55, bar, 500 μm; b, x 500 μm; f, x 200, bar, 100 μm; g, x 500, bar, 50 μm.
vicinity of the cancerous cells, FA and FD specimens were included in the study. The problem of distinction between carcinomatous and noncarcinomatous epithelial cells in culture exclusively exist within cultures of primary carcinomas and metastatic lesions of the skin and other epithelial tissues; therefore cultures from lymph node metastases and pleural effusions were not included in the study. Some studies in the field of immunocytochemistry on tumor associated antigens classify for various reasons a particular tumor specimen as “positive” if just any tumor cell in a section shows reaction (30). This type of classification is too flexible in the present comparison of biopsy sections with short-term cultures. We found NADPH-NT reductase positive cell islets in primary cultures from carcinomas of breast of the NADPH-NT reductase positive and negative cell islets. A culture was only designated diploid if all the epithelial cell islets present were diploid. The presence of two C values indicates a clearly bimodal DNA distribution within one epithelial cell type. A culture was only designated as diploid if all the epithelial cell islets present were diploid.

Table 3 DNA distribution in NADPH-NT reductase positive and negative epithelial cell islets in primary cultures from carcinomas

<table>
<thead>
<tr>
<th>Biopsy code</th>
<th>NADPH-NT reductase positive</th>
<th>NADPH-NT reductase negative</th>
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<tbody>
<tr>
<td>3770</td>
<td>Nondiploid (4.6C, 8C)</td>
<td>Diploid</td>
</tr>
<tr>
<td>3778</td>
<td>Nondiploid (2.8C)</td>
<td>Not present</td>
</tr>
<tr>
<td>3779</td>
<td>Not present</td>
<td>Diploid</td>
</tr>
<tr>
<td>3785</td>
<td>Not present</td>
<td>Nondiploid (3.5C)</td>
</tr>
<tr>
<td>3790</td>
<td>Not present</td>
<td>Diploid</td>
</tr>
<tr>
<td>3793</td>
<td>Nondiploid (3.0-3.5C)</td>
<td>Diploid</td>
</tr>
<tr>
<td>3798</td>
<td>Not present</td>
<td>Nondiploid (3.5C)</td>
</tr>
<tr>
<td>3805</td>
<td>Diploid</td>
<td>Diploid</td>
</tr>
<tr>
<td>3808</td>
<td>Not present</td>
<td>Diploid, tetraploid</td>
</tr>
<tr>
<td>3809</td>
<td>Nondiploid (6.7C)</td>
<td>Not present</td>
</tr>
</tbody>
</table>

any increase in number of nucleoli per nucleus showing a DNA content exceeding the model G2 DNA content of the fibroblasts. Accordingly, at least in short-term cultures aneuploidy can be considered as an indicator of the presence of carcinoma cells from the human mammary gland. It is, however, very time-consuming to localize and estimate the proportion of such aneuploid cell islets without use of the NADPH-NT reductase reaction to localize specific epithelial islets for DNA measurements. It has recently been reported that human breast epithelial cells from carcinomas in a monolayer culture system were consistently diploid (35). However, in the present study some of the NADPH-NT positive epithelial cell islets were aneuploid. The reason for this discrepancy remains uncertain.

In conclusion, the present NADPH-NT reductase technique is a simple cytochemical method for routinely demonstrating carcinoma derived epithelial cell islets in primary monolayer cultures based on the reaction patterns of the tumor of origin. Our approach may thus serve as an efficient tool in, for instance, evaluating the preservation of phenotypic traits of cultured breast carcinoma cells.5

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