also a posttranscriptional control or even a control of activation of the TGF-β1 latent form into the biologically active form.

As TGF-β1 synthesis may be regulated by estrogen or antiestrogen through ER and because in postmenopausal women (independent on the con-

centration of plasma estradiol) there are mechanisms which enable the main-

tenance of higher levels of estradiol concentrations in tumor tissue (7) (as an adaptation of the tumor cell enabling a higher degree of proliferation) then it is logical to assume that this increased estradiol concentration induces an increased TGF-β1 synthesis. However, as it is known that TGF-β1, due to its multifunction, in later phases of tumor progression, behaves as an invasive marker, this may potentiate the tendency of tumor tissue towards autonomy.

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KEYWORDS: Breast Neoplasms; Tumor Markers, Biological

ABSTRACT

It is shown that steroid hormone receptors by themselves are not sufficiently strong prognostic factors in management of breast cancer. For that reason, simultaneous consideration of different biomarkers seems to be more appropriate for clinical use, i.e. selections of patients with high/inter-

mediate/low risk of disease outcome. However, the amount of tumor mater-

ial available from breast carcinoma can preclude determination of estrogen-

regulated biomarkers together with estrogen receptor and progesterone recep-

tor. The aim of this study was to assess the possibility of estrogen recep-

tor and progesterone receptor determination by a single-point instead of fi-

ve-point biochemical method. Our results demonstrated that the correla-

tion between measurements of estrogen and progesterone receptor contents obtained by the five-point and single-point assay in the total population was very high. Consequently, we could use the single-point assay instead of five-

point assay for estrogen receptor and progesterone receptor determination, thus making possible determination of other molecular biomarkers from the same breast carcinoma.

BREAST CANCERGENESIS

Over 85% of the spontaneous mammary cancers that occur in women originate in the luminal mammary epithelial cells (LMECs). Mammary cancers are classified (1,2) according to their requirement for proliferation as being either hormone-dependent tumors or hormone-independent tumors. Whether a cancer is a hormone-dependent tumor or a hormone independent tumor is ultimately based on the response to hormone therapy of metastatic disease. Hormone dependent tumors require the presence of hormones for their proliferation, whereas hormone independent tumors do not. Why are hormones required and how do they regulate the genesis of mammary cancers of heterogeneously phenotypes, including hormone dependent tumors and hormone independent tumors?

It is well established that hormones from several endocrine glands act as key regulators for LMEC proliferation (3,4). Besides hormones, it seems that

Address correspondence to:
Milan Markojevic, Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Yugoslavia. E-mail: marcojevic@ionc.ac.yu

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growth factors are an additional class of mammary regulators. Studies during last two decades show that growth factors, most of which are produced locally in the mammary gland, have a profound influence on the growth of LMECs. Figure 1 schematically represents our current concepts of the roles of hormones in mammary carcinogenesis, based on the literature.

**Figure 1. Model explaining the role of hormones in mammary carcinogenesis in humans**

Normal mammary glands contain many subcompartments, each with its own specific mitogenic microenvironment. Hormones and/or locally produced growth factors create microenvironments that are responsible for the proliferation of different luminal epithelial subcompartments. The concept suggests that there are two populations of LMECs to begin with: directly ovarian hormone responsive (DOHR) cells with functional estrogen receptors (estrogen receptor+), and indirectly ovarian hormone responsive (IOHR) cells lacking functional estrogen receptors (estrogen receptor-). When exposed to hormones from ovary and pituitary gland, directly ovarian hormone responsive cells are stimulated to proliferate, as well as to synthesize and secrete local mammmogenic growth factors. Much evidence exists that hormones can induce growth-factor expression in different compartments of mammary cell (5). Indirectly ovarian hormone responsive cells, on the other hand, are unresponsive to hormones. They proliferate only in response to the hormone-induced stimulation and secretion of growth factors that are produced locally by directly ovarian hormone responsive or other cell types. Thus, the indirectly ovarian hormone responsive cells are indirectly dependent on hormones for their development.

Following chemical carcinogen exposure, adduct formation will occur in one or more of these expressed genes. Upon further cell division, fixation of the mutation will occur within specific genes giving rise to initiated cells. Further promotion will result in a tumor with a specific phenotype. Particular subcompartments of directly ovarian hormone responsive cells are thought to give rise to hormone dependent tumors of specific phenotypes or genotypes, depending on hormonal milieu around the time of carcinogen exposure. These hormone dependent tumors may undergo further progression, giving rise to diverse phenotypes that may include hormone independent tumors. In a similar fashion, specific indirectly ovarian hormone responsive subcompartments, responding to particular mitogenic growth factors, available at the time of carcinogen exposure, will give rise to hormone independent tumors of specific phenotypes or genotypes. These also may undergo further progression to hormone independent tumors of heterogeneous phenotypes. This scenario explains why all of the tumors, hormone dependent or hormone independent tumors, depend initially on hormones. It also explains how normal LMECs can give rise to tumors with such variety of phenotypes and genotypes. Hence, from biological point of view, estrogen and progesterone hormones, as well as growth factors within their corresponding receptors, express pronounced effect on breast carcinoma cell growth (proliferation, differentiation and apoptosis), malignant cell invasion, metastatic activity and autonomy.

**ESTROGEN - INDUCED BIOMARKERS**

Current consensus recommends routine clinical use of only estrogen (ER) and progesterone (PR) receptors as molecular biomarkers for patients with newly diagnosed breast cancer. Estrogen and progesterone receptors are estrogen-regulated proteins (6). Estrogen receptor is ligand-dependent transcription factor that regulates proliferation and differentiation of breast carcinoma cells, as well as apoptotic activity (7). Progesterone receptor is an end product of estrogen action (8). Knowledge of steroid hormone receptor status, i.e. knowledge of their quantitative values, has significantly improved the clinician’s ability to select appropriate patients for endocrine treatment within primary (10) and metastatic breast cancer (9). It is shown that steroid hormones by themselves are not sufficiently strong prognostic factors (11) and it has been suggested that estrogen receptor, as well as progestereone receptor, may be best used in combination with classical tumor-host and tumor prognostic factor parameters.

In an effort to explain non-homogeneity in response to therapy, as well as non- homogeneity in natural course of disease, and perhaps to improve the subgrouping of breast cancer patients, tumor-host-related (12, 13) and tumor-related (12, 14) steroid hormone receptor contents have been assessed. In spite of significant association between steroid hormone receptor expression and clinicopathologic features, there was no biologically significant association due to a wide range of individual estrogen receptor and progesterone receptor values. Accordingly, the knowledge of steroid hormone receptor status is necessary in all clinicopathologic-related primary operable breast carcinoma subgroups.

In addition to endocrine growth control of breast cancer cells, there is currently an increasing interest in the role of the autocrine/paracrine growth factors in modulation of tumor growth. Particularly, interest has been focused on a cross-talk between steroid hormone receptor and c-erbB-2 and receptor for epidermal growth factor (EGFR) pathways (15, 16). Studies of new biomarkers form an extensive part of the oncology literature. These studies may offer insight into the molecular pathogenesis of the disease and also may help in medical decision making.

The purpose of investigation of estrogen regulated pS2 protein is to answer the question whether its expression may be a marker of functional heterogeneity in relation to steroid hormone receptor status (17). In context of that it is necessary to assess more precisely the cut-off value of pS2 that best predicts hormone responsiveness (18).

One of the most paradoxical biomarkers in breast cancer is cathepsin D. A number of studies have implicated cathepsin D in promoting tumor growth as a proteolytic enzyme (19). Another study reports that the expression of cathepsin D is regulated by estrogen in breast carcinomas with the functional integrity of the estrogen response pathway (20). Accordingly, the positive association between cathepsin D and steroid hormone receptor status is puzzling, confounding that they should provide opposite prognostic as well as predictive information (21).

In the search of new potentially useful prognostic and predictive biomarkers, the expression of the p53 suppressor gene protein is of particular interest. It is known that it plays crucial role in regulating cell proliferation and apoptosis (22, 23). It is important to point out that p53 mutation and loss of estrogen receptor expression is late event in breast cancer progression. Hence, the p53 suppressor gene products may be important breast cancer progression-related biomarkers.

Generally in each of the above reported studies the authorities in breast cancer investigation concluded that either the strength of marker was insuffi-
cient to justify a change in treatment based on the results, or that insufficient data were available to reliably estimate either the prognostic or predictive strength. That is why simultaneous consideration of different biomarkers seems to be more appropriate for clinical use, i.e. selections of patients with high, intermediate, and low risk of disease outcome. Thus, simultaneous knowledge of the above mentioned biomarkers may help in medical decision making. However, the amount of tumor material available from breast carcinoma can preclude determination of estrogen-regulated biomarkers together with estrogen receptor and progesterone receptor.

VALIDITY OF ONE-POINT BY CHEMICAL ASSAY FOR ER AND PR DETERMINATION

The aim of our study was to assess the possibility of estrogen receptor and progesterone receptor determination by a single-point instead of five-point biochemical method and to enable determination of the other molecular biomarkers from the same breast carcinoma cytosolic fraction. Results of comparison of five-point and single-point assays for estrogen receptor (A) and progesterone receptor (B) contents are shown in Figure 2. There were 49 and 36 samples analyzed for estrogen and progesterone receptor contents, respectively. Measurements were done on the same breast carcinoma cytosol. Our results demonstrated that single-point assay was equivalent to five-point assay in relation to the content of both estrogen and progesterone receptor. The correlation between measurements of estrogen and progesterone receptor contents obtained by five-point assay and single-point assay in the total population was very high (r = 0.996 and r = 0.990, respectively). Consequently, we could use single-point assay instead of five-point assay for estrogen receptor and progesterone receptor determination, thus making possible determination of the other molecular biomarkers from the same breast carcinoma cytosol fraction.

![Figure 2. Measurement of estrogen receptors (A) and progesterone receptors (B) by five-point assay (Rb) and single-point assay (R1). Linear regression analysis between receptor contents obtained by five-point assay and single-point assay in the total population yielded: (A) y = 1.009x (r=0.996, p < 0.05). (B) y = 0.974x (r = 0.99, p < 0.05)](image-url)

At the same time, the validity of intralaboratory quality control of single-point biochemical assay was performed. We analyzed distributions over time of estrogen and progesterone receptor status in qualitative and quantitative manner. No variations were observed over time in estrogen and progesterone receptor phenotypes, as well as in estrogen and progesterone receptor quantitative values (data not shown).

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