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Review paper

BIOMARKERS IN BREAST CANCER

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Summary: Breast cancer is a disease characterized by abnormal growth and development of normal breast tissue. When the growth and development of a normal cell no longer obey biological control mechanisms, tumour cells begin to appear. An understanding of normal biological processes relating to cell growth, such as the cell cycle, angiogenesis, and apoptosis, and of other abnormal processes, such as neo-plastic transformation, tumour progression, and metastasis, would aid not only in identifying tumour markers, but also in determining the best uses of these tumour markers for diagnosis and treatment of patients with cancer. Biomarkers are improving our understanding of the biology and management of breast cancer.

Key words: biomarkers, breast cancer, biological processes

Introduction

Biological properties of breast cancers were described when Sir George Beatson, the Chief Cancer Surgeon in Glasgow, proposed in 1896 (1) that the human breast was regulated by »secretions from the ovary«. He carried out oophorectomy on a number of premenopausal women with aggressive breast cancer and quickly noted that some of the patients did not appear to benefit from the oophorectomy. In the succeeding 70 years, breast cancer surgeons and physicians became increasingly aware that ablative surgery (oophorectomy for premenopausal women and adrenalectomy plus hypophysectomy for post-menopausal women) was of considerable benefit to some breast cancer patients but of no value to others. The major question facing these clinicians was how to select the patients for whom surgery would be beneficial, whilst saving great trauma to those for whom surgery would be of no avail. As more is known about the biology of different types of breast cancer and as different approaches are proposed for prevention, it is up to the scientists to develop markers that will allow for each patient to be managed as an individual (2). In the last decade, the knowledge of biomarkers' bases and the clinical use of breast cancer markers have been evolving greatly and new data are continuously emerging. We grouped them into *tissue* or *blood markers* according to the principal site where they are performing evaluation for clinical use. Tissue markers include different categories such as intracellular membrane receptors, oncogenes, and tumor suppressor genes, nuclear antigens and growth factors, while circulating markers include the wide category of tumour-associated antigenes (TAA) and others (2).

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Tissue markers in clinical management of breast cancer patients

Estrogen and progesteron receptors

The first measures to provide markers of breast cancer biology were established during the 1950's and 60's when endocrinologists tried to discriminate hormone sensitive disease from hormone independent disease by analyzing changes in steroid metabolism either in the urine or plasma of the patient (3). None of these approaches were very successful. At the same time, evidence accumulated that estrogen action was mediated through an intracellular receptor (4). In the 1970's, ligand binding assays for these receptors were developed (5) and evidence began to pile up that sensitivity to endocrine therapy was confined to those individuals whose tumours contained functional estrogen receptors, though it took some time before possible cut-off levels were proposed for clinical decision making.

Early results suggested that about 50% of estrogen receptor (ER) positive patients experienced at least six months of objective response to endocrine therapy (6). However, it was also noted that 10% of ER-tumours also responded to endocrine therapy. This raised two obvious questions. Firstly, why did only 50% of the ER+ patients respond to endocrine therapy, and, secondly, why did 10% of apparently ER– tumours respond? It was recognized that tumour cells often express abnormal or mutant proteins, and subsequent work has shown that breast cancer cells contain a number of different mutated (usually splice variants in which one exone of the gene is deleted at mRNA level) proteins. The logical step was to develop assays for the »functional« receptor to show that the tumour really did respond to estrogen. Unbound estrogen receptor is found in the nucleus of target cells, but is a soluble protein, and so is recovered in the soluble fraction after tissue homogenization. After binding steroid, the activated steroid-receptor complex becomes bound to DNA (specific sites usually upstream of estrogen-regulated genes). As a result of estrogen action, various new proteins are synthesized and cells enter S-phase and cell division. »Functional« receptor can, therefore, be defined as:

Table I Main tissue molecular markers of breast cancer and their clinical outcome according to the Tumour Marker Utility Grading System (TMUGS) (2)

Marker	Structure	Function	Utility scale of clinical outcome			
			Gene amplification and/or mutation	Expression	Relapse (prognostic)	Response to therapy (predictive)
ER	protein	nuclear receptor	no	increased	++	++
ER-alpha	phosphoprotein	nuclear receptor	no	increased	+/–	+/–
ER-beta	protein	nuclear receptor	no	increased	+/–	+/–
PR	protein	nuclear receptor	no	increased	++	++
PR-A	protein	nuclear receptor	no	increased	+/–	NA
PR-B	protein	nuclear receptor	no	increased	+/–	NA
c-erbB-2	proto-oncogene	encodes for HER/2*neu*	yes	–	+	+
HER2/*neu*	glycoprotein	membrane receptor	–	increased	+	+
p53 gene	tumour suppressor gene	encodes for wild type p53	yes	–	+/-	+/–
wild type p53	nuclear phosphoprotein	transcription factor	yes	increased	+/-	+/–
Ki67/MIB-1	nuclear protein	proliferation-associated nuclear antigen	–	increased	+	+
VEGF 121,145, 165[1], 189, 206 (5 isoforms)	protein	growth factor	–	increased	+/-	+/–

See text for details. Prognostic factors are those predicting relapse independent of future treatment effects; predictor factors are those predicting response or resistance to a specific therapy. Brief explanation of the used utility scale: 0 = marker should not be ordered for that clinical use; NA = data are not available; +/- = data are suggestive for a contribution but they are preliminary, thus the marker is still considered highly investigational; + = sufficient data are available to demonstrate a contribution; however, the marker is still considered investigational; ++ = marker supplies information not otherwise available for other measures and it should be considered standard practice in selected situations (203).
[1]VEGF 165 is the predominant isoform.

(a) receptor which is present in both the soluble and nuclear fractions of tissue homogenates (7),

(b) receptor which induces synthesis of known estrogen-sensitive proteins (Progesterone Receptor, Cathepsin D, pS2 etc.),

(c) receptor which is present in cells that undergo increased DNA synthesis after exposure to estrogen.

Application of any one of these tests of functionality results in the same conclusions: 70–75% of functional ER+ tumours will respond to endocrine therapy (8, 9); 5–10% of ER-tumours still respond to therapy; some small sub-groups of tumours (e.g. ER–/PR+) still have a 40–50% chance of response to endocrine therapy.

Studies of prognosis showed that patients with ER+ tumours had a significantly longer overall survival than did those with ER– tumours (10). Much of this benefit appeared to be simply that the patients with ER+ tumours were getting benefit from their therapy, whereas the same was probably not true for many of the patients with ER– disease. Therefore, ER status alone was not very useful as a prognostic index, although its predictive value for response to endocrine therapy was considered very useful. Many studies of the use of adjuvant endocrine therapy were initiated and an international group was established to study the overall follow-up. This overview has now reported on a minimum of 10 years follow-up (11). The main conclusions, as far as estrogen receptor is concerned, are:

(a) the proportion of patients responding to endocrine therapy is directly related to the concentration of ER present in the tumour,

(b) the length of time of response in an individual patient is proportional to the concentration of ER in the tumour,

(c) treatment of completely ER– tumours with endocrine therapies is a waste of time and money.

The overall conclusions from this are that measurements of ER need to be quantitative and, correspondingly, an external quality assurance (QA) scheme is needed to ensure that all values of ER are internationally equivalent (such a scheme was developed by the EORTC Receptor and Biomarker Study Group – see ref. 12).

While the foregoing studies were in progress, monoclonal antibodies against the ER became available. This led to immunocytochemical determinations of ER in fine needle aspirates (by tagging the antibody with a fluorescent probe, it was possible to make this into a semi-quantitative assay, and the values obtained in FNAs were surprisingly similar to those obtained by ligand binding assay on a biopsy of tumour removed at subsequent surgery) and to immunohistochemical (IHC) determination of ER in paraffin-embedded material. The use of IHC normally does require prior epitope retrieval, but good protocols are now available (13) and an agreed approach to semi-quantitative assessment has been developed (13, 14). Evidence has rapidly accumulated that an external QA scheme is equally essential for the IHC method and one active scheme, described by Rhodes (15).

It was initially thought that all breast tumours would begin as ER+ and gradually revert to ER–. However, research has shown that most ER+ tumours remain ER+ until the patient dies, and, similarly, ER– tumours remain negative throughout their history (16). Thus, patients with endocrine sensitive tumours will often respond to second-line endocrine therapy by a mechanism that implies an intact estrogen receptor system, once they relapsed on the initial (usually tamoxifen) therapy – hence the success of second-line aromatase inhibitors etc. The use of endocrine therapy against ER– tumours is, similarly, of little value, though it is important to confirm the absence of functional ER by measuring a product of ER action such as the progesterone receptor (PR). Patients with ER– disease may need entirely separate analysis in terms of both therapy and epidemiology, since the carcinogenic agents that initiate the disease and, certainly, the tumour promoters will be different from those in patients with ER+ disease. A very small number of ER– patients may benefit from tamoxifen through its ability to activate release of TGF from the surrounding breast stroma. There is also good evidence that tamoxifen can reduce myocardial infarctions and plasma cholesterol, as well as stabilize bone calcium content. These benefits of long-term tamoxifen have to be balanced against the agonist effect that tamoxifen has on the reproductive tract. The overview has shown that there are more deaths from endometrial cancer in the tamoxifen arm than in the non-tamoxifen arm, but, in relation to the total number of women–years exposure and the benefit experienced in relation to their breast cancers, the endometrial problem is one to keep in mind, rather than a reason to reduce the use of tamoxifen. It does, however, raise the importance of developing third generation SERMs (selective estrogen receptor modulators) that will reverse the unwanted menopausal symptoms without being agonistic to reproductive tissues.

An additional effect of tamoxifen is to reduce the incidence of contralateral breast cancers. For this reason, there have been several trials established to use tamoxifen for the »prevention« of breast cancer in high-risk women. All such trials have been designed in the light of our knowledge about the agonist effects of tamoxifen on the reproductive tract. These studies have been reviewed by Craig Jordan (17) and show that where benefit was observed, it was confined to those patients at risk of developing ER+ breast cancers, i.e. there was no reduction in the incidence of ER– tumours.

Most recent clinical data on ER determination have been obtained using commercial kits that

cannot distinguish between ER-alpha and ER-beta isoforms.

Nevertheless, it has also been reported that, unlike in normal breast tissue where ER-beta predominates, in most breast tumors ER-alpha is expressed either alone or in combination with ER-beta (18). Therefore, it is assumed that most available clinical data mainly reflect the ER-alpha function.

While ER determination has been standard practice for a few years in the decision-making process of selected situations, ER-alpha and ER-beta must still be considered highly investigational markers (*Table I*).

Progesterone receptors are ligand-dependent members of the nuclear receptors family of transcription factors and allow progesterone to exert its effects. Two PR isoforms, A and B, as alternate initiations of translation from the same mRNA or transcribed from two promoters on a single gene (19), exist in progesterone target tissue. In the breast, equal expression of the two progesterone receptors is necessary for normal development and differentiation, while PR-A to PR-B ratio is extensively misregulated in breast cancer. Recent results showed that expression levels of PR-A were higher than PR-B in breast cancer tissue. PR-B correlated with the absence of HER-2*neu* indicating good prognosis, while excess PR-A correlated with poorly differentiated phenotype and higher tumour grade (20).

PR as ER determination is standard practice in selected situations, while PR-A and PR-B must still be considered highly investigational markers (*Table I*).

HER2/neu

HER2 is a protooncogene that encodes the human epidermal growth factor with tyrosine kinase activity. HER2 is a member of the erbB family of receptors that plays a major role in promoting breast cancer cell proliferation and malignant growth. The EGFR family is comprised of four homologous receptors: erbB1 (HER1), erbB2 (HER2/*neu*), erbB3 (HER3) and erbB4 (HER4). However, HER2 is known to be the preferred co-receptor for the EGFR, HER3 and HER4. It transmits signals into the cell nucleus, thus regulating normal cell growth, division and differentiation (21). HER2 overexpression, usually caused by HER2 gene amplification, results in oncogenic transformation, and is regarded as HER2 positive (+) status in approximately 30% of breast cancer patients (22). Routinely used laboratory methods for estimating HER2 status are: immunohistochemistry (IHC), fluorescent *in situ* hybridisation (FISH), polymerase chain reaction (PCR), etc (24). Women whose breast cancers are HER2 ++/+++ by IHC and/or FISH positive have more aggressive disease, shortened disease free survival (DFS) and overall survival (OS), and altered response to conventional anticancer agents (23). HER2 receptor thus provides an extracellular target for novel and specific anticancer treatment–monoclonal antibodies. Trastuzumab (Herceptin) is the first targeted therapy for HER2 positive breast cancer. It is evident that anti-HER2 therapy induces objective tumour remission and survival benefit. Anti-HER2 therapy may represent the most innovative breast cancer treatment developed in the last 20 years (24).

p53, gene and protein

The human p53 tumour suppressor gene has been mapped to chromosome 17p53.

Many studies showed that alterations in p53 are associated with poor prognosis. In fact, mutated p53 was found in a higher percentage of patients with inflammatory breast cancer and p53 overexpression was associated with a worse outcome in high risk primary breast cancer patients (25).

KI-67/MIB-1

Ki 67 is a non-histone nuclear protein that is closely linked to the cell cycle. Increased percentages of Ki-67 positive cells have been described as an independent negative prognostic factor for relapse and overall breast cancer survival in some (26), but not all studies (27).

Vascular endothelial growth factor (VEGF)

The molecular mechanisms of the increase in VEGF mRNA and VEGF protein production are not yet understood, although insulin, insulin-like growth factor-1, corticotropin, thyrotropin and steroid hormones have been reported to affect VEGF mRNA production. VEGF protein, also referred to as the vascular permeability factor (VPF), is the most commonly studied vascular growth factor, specific mitogene and survival factor for endothelial cells, and, key promoter of angiogenesis. VEGF has been reported to be an independent unfavourable prognostic factor for relapse and survival in a few studies (28).

Circulating tumour markers

Many authors reported on the usefulness of tumour associated antigens (TAAs) in breast cancer patients to post-operatively detect relapses and monitor response to treatment (29). Among them, only CA15.3 are considered clinically relevant. (29) (*Table II*). However, the last updated guidelines of the American Society of Clinical Oncology (ASCO) (30) do not recommend the use of any circulating tumour marker post-operatively as they are not considered sufficiently accurate to be used routinely. In the last decade, suitable criteria for the use of circulating TAAs have been defined. Consequently, many authors reported the high accuracy of CEA, CA15.3, TPA association in the post-operative follow-up of breast cancer patients

Table II Main circulating markers of breast cancer and their clinical outcome, according to the Tumour Marker Utility Grading System (TMUGS)

Marker	Structure	Function	Prognostic or predictive value – clinical outcome				
			Relapse (P)			Monitor course (M)	
			Sensitivity % (range)	Specificity % (range)	Utility scale	[1]Low, high correspondence	Utility scale
TAAS							
CEA	glycoprotein	cell-cell interaction	7–50%	88–100%	+	low to high	+/–
CA15.3	glycoprotein	mucins	37–67%	92–100%	+	high	+
TPA	protein	cytokeratines	51–86%	48–72%	0	high	0
MCA	glycoprotein	mucins	43–84%	43–89%	0	high	0
CA549	glycoprotein	mucins	50–70%	79–98%	0	low to high	0
Others							
ECD HER2/*neu*	protein	antigen	31–45%	100%	+/-	unclear	+/–
p53 antibodies	immunoglobulins	antibodies	0–46%	>81% – >99%	+/-	unclear	+/–
Nucleophosmin antibodies	immunoglobulins	antibodies	NA	NA	+	NA	+/–
Cytokeratin-positive cells	protein	cytokeratines	NA	NA	+[2a]	NA	+/–[2b]
ICAM-1	glycoprotein	adhesion molecule	NA	NA	+/-[2a]	NA	+/–[2b]
VCAM-1	glycoprotein	adhesion molecule	NA	NA	+/-[2a]	NA	+/–[2b]
E-selectin	glycoprotein	adhesion molecule	NA	NA	+/-[2a]	NA	+/–[2b]

See text for details. For explanation of utility scale, see *Table I*. For TAAs, ECD HER2/*neu*, p53 and nucleophosmin antibodies, prognostic value derives from a rising level predicting an impending relapse; for predictive value see text. P = primary cancer; M = metastatic cancer; ECD = extracellular domain. [1]Referred to progression of disease, or response to therapy (complete or partial), or no change: high > 60%. Although they are investigational (+) or highly investigational (+/–), data are available as prognostic factor (2^a) or predictor factor (2^b). Data are not available (NA).

and identified clinically important benefits from their use (31). While their preoperative levels did not show relevant prognostic or predictive value, their clinical use in the yearly detection and monitoring of relapses is widely documented. None other circulating marker, according to the *Table II,* should be considered as standard practice.

Conclusion

Many efforts are ongoing to define either tissue or circulating biomarkers that independently, or in addition to the conventional pathological findings (T and N), better select patient management. In the adjuvant setting, c-erbB2/HER2*neu* positive tumours with concomitant high value of Ki67/MIB-1 define a subgroup of lymph-node negative patients with higher risk of relapse. Cytokeratin-positive cells in the peripheral blood or high KI67/MIB-1 values proved to be independent negative prognostic factors. Cytotoxic chemotherapy is an important therapy for breast cancer patients, but it is limited by toxicity, non-specificity and the inevitable development of resistance. The above-mentioned new markers can help in the decision-making process on the use of antracyclines and/or taxanes in place of CMF (2). However, an effective therapy has to target cellular pathways involved in growth regulation. The term »targeted therapy« refers to a known therapeutic target that is important in the biology of the cancer cell and

indicates a specific agent that acts by modifying the expression or activity of the target in the growth and progression of cancer. According to this approach, only patients with the likelihood of benefit are treated, so hopefully the therapeutic index will be improved. Tissue biomolecular markers, aside from being prognostic and predictor factors, undoubtedly play a central role in targeted therapies that are among the most promising directions of clinical research.

BIOMARKERI U KARCINOMU DOJKE

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Kratak sadržaj: Rak dojke je bolest abnormalnog rasta i razvoja do tada normalnog tkiva dojke. Tumorska ćelija nastaje kada ćelija više ne prepoznaje biološke kontrolne mehanizme. Za bolje razumevanje biologije tumora, ćelijskog ciklusa, angiogeneze, apoptoze, neoplastične transformacije, fenomena progresije i metastaziranja veoma su značajni biomarkeri kao prognostički i prediktivni faktori. Biomarkeri raka dojke imaju posebnu vrednost u proceni agresivnosti bolesti i ranoj detekciji progresije.

Ključne reči: biomarkeri, rak dojke, biološki procesi

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