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Progress in Molecular Biology of Breast Cancer

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INTRODUCTION

CANCER IS caused by damage to certain genes, leading to alterations in their expression or an altered structure of their encoded proteins. The genes concerned can be roughly divided into two groups. Gain-of-function mutations of proto-oncogenes result in an abnormal and excessive growth stimulus (such mutated, 'activated' proto-oncogenes are designated oncogenes); loss-of-function mutations affecting both alleles of tumour-suppressor genes, which normally exert a negative effect on cellular proliferation, likewise result in or contribute to excessive growth. In some instances, mutation of one allele only of a tumour suppressor gene already has a significant effect, such as in the case of a 'dominant negative' mutation, where the protein derived from the mutated gene interferes with the action of the wild type protein derived from the other, intact allele.

The exact molecular biological pathways of growth signal transduction and the role of the large number (well over one hundred) of proto-oncogenes and tumour-suppressor genes in these pathways is only partially known; however, it has become clear that there is a limited number of major signal-transduction pathways, and that the products of various proto-oncogenes and tumour-suppressor genes may act within a single signalling pathway; some tumour-suppressor gene products inhibit the expression of a proto-oncogene, or the activity of its protein [1].

It has become apparent that in individual maligant tumours, more than one oncogene or tumour-suppressor gene is usually mutated; the dysregulated and excessive growth which is characteristic of tumours is the net result of the simultaneously perturbed action of a number of genes, rather than of one gene only. Even in a rare familial childhood cancer such as familial retinoblastoma, where the pattern of inheritance and age distribution would suggest the involvement of only one gene (namely, the RB-1 gene on the long arm of chromosome 13), the frequent presence of cytogenetic lesions at specific different sites in the genome point to the involvement of additional genes [2]. Perhaps the most detailed data on the simultaneous involvement of several genes during tumorigenesis are based on studies of adenomas and adenocarcinomas of the colon [3]; however, it is clear that also in malignant tumours of other organs, including the carcinomas of the breast, accumulation of a number of mutations in oncogenes and tumour-suppressor genes is basic to their pathogenesis.

In this short review, we shall discuss some recent insights in the molecular basis of breast carcinoma tumourigenesis and progression, with special emphasis on those data presented and discussed at the EORTC 5th Breast Cancer Working Conference in Leuven, 3–6 September 1991.

ONCOGENES

In about 15–20% of invasive primary breast carcinomas, an amplification of the c-*erb*B-2 (*neu*, HER/2) gene has been reported [4, 5], and this prevalence figure was confirmed in several series presented at the Leuven meeting [6, 7]. In the large majority of cases, the amplification is associated with a marked increase in expression of the gene; indeed, the increase in expression may exceed the level of amplification by an order of magnitude [8].

The c-*erb*B-2 gene was originally detected in rat neuroblastoma (hence its alternative designation *neu*), where it is activated by a point mutation in the transmembrane region. Such an activating mutation has not been found in human breast carcinomas [9].

Breast carcinomas harbouring an amplification of the c-*erb*B-2 gene are usually of the ductal type, and often exhibit large tumour cells and an extensive intraductal component, with a comedo (centrally necrotising) pattern. Indeed, in purely intraductal (*in situ*) carcinoma of the breast with a large cell type, c-*erb*B-2 overexpression is found in a much higher percentage of cases, namely, about 70% [5] and when intraductal carcinoma extends to the nipple, resulting in mammary Paget's disease, c-*erb*B-2 overexpression is even more common [10, 11]. The impression that c-*erb*B-2 amplification appears to vanish in some tumours when they progress from an *in situ* lesion (70% amplification) to an invasive cancer (15–20% amplification) is certainly spurious: the likely explanation is that in a subset of breast carcinomas, the c-*erb*B-2 amplification occurs as an early event and leads to the development of an extensive *in situ* component; these tumours are detected relatively often at this early stage, before invasion has occurred. Other breast carcinomas, which arise through the perturbed action of other genes, less commonly have such an extended *in situ* phase, and therefore 'dilute' the numbers of invasive cancers with a c-*erb*B-2 amplification detected at this later, invasive stage.

Following the initial report by Slamon and colleagues [4] on an association of c-*erb*B-2 overexpression and poor prognosis in breast cancer, there has been intense debate on this matter. Most groups now report an association with decreased overall and disease-free survival [12–14]; specifically, early recurrence is associated with c-*erb*B-2 amplification and overexpression. However, after long follow-up periods, the differences in survival figures tend to diminish somewhat and indeed, not all investigators agree about the prognostic significance of c-*erb*B-2 amplification. In a recent report where no correlation with disease-free or overall survival was found [15], the prevalence of this amplification was 33%, i.e. significantly higher than the figures found by many other investigators. It seems possible that tumours with a low level of c-*erb*B-2 amplification, resulting in only a small increase in gene copy number and level of expression, detectable only by highly sensitive techniques, may dilute out the difference in behaviour which emerges when tumours with a high copy number and a marked overexpression of c-*erb*B-2 are studied. Also, differences in adjuvant treatments may influence the results [15].

Several groups reported an inverse correlation between c-*erb*B-2 overexpression and steroid receptor positivity [7], and in a series reported by Berns and colleagues [16], response to hormonal therapy was inversely correlated with c-*erb*B-2 amplification.

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The c-*erb*B-2 (*neu*) oncogene encodes a transmembrane protein with a high degree of homology to the epidermal growth factor receptor (EGF-R). The c-*erb*B-2 ligand remains to be identified, but it seems plausible that, like the EGF-R, the c-*erb*B-2 protein constitutes a growth factor receptor. At the Breast Cancer Working Conference, Mareel [17] presented data of *in vitro* cell motility experiments which suggested an alternative function, namely, that of a receptor promoting cellular invasion. Although further work will be needed before this matter becomes clear, it should be realised that the two hypotheses are not mutually exclusive: it is not excluded that the c-*erb*B-2 protein has more than one physiological function, or that abnormally increased expression results in an additional effect which is absent when it is expressed at physiological levels.

Amplification of c-*erb*B-1, the epidermal growth factor receptor (EGF-R) gene, is seen in only a few per cent of breast carcinomas; however, an increase in levels of expression of this gene, as evidenced by increased positivity in *in situ* hybridisation and immunocytochemical assays, is seen in about a third to more than half of all breast cancers, and is correlated with an increased growth fraction, as evidenced by increased Ki-67 positivity or S-phase fraction, and inversely with oestrogen receptor positivity in a number of series reported at the Leuven meeting [18]. Clinically, an association with early progression and a poor prognosis was found by several groups [7, 19]. Injection of anti- EGF-R antibodies resulting in inhibition of growth of xenotransplants of human breast carcinoma with a high EGF-R content, was reported by Schnürch and colleagues [20].

Amplification of c-*myc* was found in 16% of a large series of breast tumours investigated by the group of Klijn, and was a strong unfavourable prognostic indicator [6].

Rarely, K-*ras* and N-*ras* amplifications have been identified [21]. It is of interest that in contrast to so many adenocarcinomas of other organs, such as the lung and colon, activating point mutations in *ras* genes are very rare in breast cancer.

The 11q13 region, which includes the *int*-2 gene, has recently attracted much interest. The *int*-2 gene, which is activated by retroviral insertion in the majority of MMTV-induced mammary tumours in some mouse strains, is amplified in about 15–20% of human breast cancers, and is correlated with ER-positivity and with the presence of lymph node metastases [22]. However, since the *int*-2 gene is not expressed in human breast tissue or in breast carcinomas, no biological effect can be expected from the amplification of the *int*-2 gene. It was therefore hypothesised that the 11q13 amplicon (the segment of DNA amplified) on which *int*-2 is located contains another gene which is overexpressed and which may thus confer a biological advantage. The amplified 11q13 region is large, and includes the *sea* and *hst* oncogenes, which are also not expressed in these tumours, as well as the bcl-1 locus. Recently, Schuuring and colleagues [22] succeeded in identifying two more genes within the 11q13 amplicon, which did exhibit increased expression: these genes are: PRAD-1, which encodes a G1 cyclin recently named cyclin D1 [23] and which was recently identified as the gene activated by translocation in a parathyroid adenoma [24], and a hitherto unknown gene, which was designated *EMS*-1 [22]. Further studies of these two genes are under way, in order to assess whether they are related to the postulated selective advantage conferred by the 11q13 amplification.

TUMOUR SUPPRESSOR GENES

Allele loss of the 17p13.3 region is seen in about 60% of informative cases of breast carcinoma [25, 26]. Mutations of the p53 gene, which is located in this region, were detected in 8 out of 60 breast carcinomas, all of which showed the allele loss of the 17p region mentioned [27]. As a result, there is much interest in the role of p53 in the pathogenesis of breast cancer. An exciting finding in this respect was that of the group of Friend *et al.* [28], who reported germ line point mutations in the p53 gene in affected family members of the Li-Fraumeni syndrome, a familial syndrome characterised by a high incidence of a wide variety of malignant tumours, including carcinomas of the breast.

One of the biological effects of the normal (wild type) p53 protein is growth suppression. Some mutations in the p53 gene result in a protein which interacts with wild-type p53, resulting in a functionally defective complex. Mutations in the p53 gene have now been found at high frequency in a very large and varied spectrum of human malignancies; it is likely that further data concerning the highly complex function of wild-type p53 and the various mutated forms will contribute substantially to the understanding of human breast cancer.

Inactivating mutations in the retinoblastoma (RB-1) gene and loss of heterozygosity of polymorphic markers in the direct vicinity of the gene on chromosome 13q14 have also been reported in breast cancer. Non-random allele loss of loci on chromosomes 1, 3 and 11 has also been found, and point to the presence of further putative tumour-suppressor genes.

STEROID RECEPTORS

Much clinical interest has focused on the expression of steroid receptors, and their relation to survival [29] and clinical response to endocrine treatment. ER positivity as assessed by ligand-binding or immunocytochemistry shows a positive but far from perfect correlation with responsiveness to tamoxifen treatment [30]. Also, dextran-coated charcoal and immunocytochemical methods, which assess hormone binding and the presence of an immunoreactive epitope of the receptor, respectively yield discrepant results in about 15% of cases [31]. Intriguingly, a comparison of biopsies before and after tamoxifen treatment revealed a significant reduction of ER levels but not of PR, which is under transcriptional control of the ER [32].

Recently, mutations have been described in the oestrogen receptor, and some of these lead to loss of hormone or DNA binding capacity. The biological effects of these different mutations might theoretically be totally opposite, i.e. a constitutive activation or a total loss of function. The now emerging data on these mutations provide a beginning of a molecular basis for the observation that the currently used steroid receptor assays do not allow a complete assessment of steroid receptor function and tumour responsiveness to endocrine treatment [30]. Expression of pS2, which is controlled at the transcriptional level by the ER protein, has been used as an indicator of ER activity and predictor of clinical response to endocrine treatment, with a modest degree of success [33].

CONCLUSION

At present, the accumulated data on mutated oncogenes, tumour suppressor genes and steroid receptor genes in human breast cancer appear as parts of a still very incomplete jigsaw-puzzle. In some instances, distinct patterns start to emerge from correlations between genetic lesions and some pathological and clinical data (e.g. c-*erb*B-2 amplification and overexpression together with steroid receptor negativity, a large cell type, an extensive *in situ* component of the tumour, and an increased chance of early recurrence) which may foreshadow a rational molecular basis for future classification of these tumours. How-

ever, much more knowledge is needed, both at the basal level concerning the molecular physiology and pathophysiology of the relevant genes, including an elucidation of their complex interactions, as well as at the clinical level, before these recent data will provide a basis for tumour typing and prediction of clinical behaviour, as well as for a better understanding of their pathogenesis.

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