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New Developments in the Molecular Biology of Breast Cancer

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INTRODUCTION

SEVERAL genes and their protein products have been identified over the last few years which may play a role in the development and progression of breast cancer. These genes, termed oncogenes, have often been initially identified by their presence in transforming retroviruses which cause cancer in animals. Work by Varmus and Bishop and their colleagues subsequently demonstrated that these are derived by recombination with normal genes present in the host organism's nuclear DNA. These normal genes have therefore been called proto-oncogenes. When human tissues and human tumours are examined differences either in the structure or expression of such genes are often observed. Other experiments in which artificial transfer of oncogenes into immortalized rodent cells confers a dominant malignant phenotype have confirmed their transforming potential.

The identity of the proteins encoded by some of these genes have now been established. Initial clues to their functions were provided by their subcellular location. Some genes encoded cell surface proteins while others were present in the cytoplasm or nucleus.

Oncogene research and work in the area of cell growth control became overlapping with the discovery that certain genes such as those encoding the EGF receptor, M-CSF receptor and platelet derived growth factor had been acquired by transforming viruses.

These discoveries provided confirmation of the hypothesis that alterations in growth control may contribute to the tumour phenotype. Cancer after all is a problem only because tumour cells grow more rapidly than they die.

Other oncogenes specified proteins located in the cell nucleus. Examination of their structure and various laboratory experiments have now shown that some of these (possibly all) are transcription factors whose function is to regulate the rate of expression into mRNA of sets of apparently normal genes. Work by Weinberg *et al.* has demonstrated that whereas single dominantly acting oncogenes can transform immortalized fibroblasts, more than one oncogene was needed to transform normal senescing cells [1].

The discovery of dominantly acting oncogenes was a breakthrough in the understanding of the transformation process. We are, however, at the beginning of another advance which is the discovery of anti-oncogenes or tumour suppressor genes [2]. These genes become involved in transformation by being deleted or damaged, producing a 'loss of function mutation'. It is hypothesized that such changes may allow cells to enter the cell cycle and begin to divide.

A picture is thus emerging that alterations to genes controlling cell growth rate together with changes in genes involved in transcriptional regulation and possibly those affecting processes controlling the cell cycle are necessary to fully transform indicator cells. Such a series of changes at the level of DNA fit well with the mathematical models of transformation which suggest an accumulation of events leading to full transformation.

NEW ONCOGENES IN BREAST CANCER

Two new players have recently joined the subset of oncogenes which are altered in human breast cancer. One is a putative growth factor receptor which may provide a dominantly acting aberrant stimulation for cells to grow and the other encodes a nuclear protein which may be an additional member of the small but growing family of anti-oncogenes.

1. *c-erbB*-3

The discovery of the c-erbB-3 gene has recently been reported by Kraus *et al.* [3]. The archetype of growth factor receptors with protein tyrosine kinase activity has been the epidermal growth factor receptor. This gene, which is also known as c-erbB-1, can become a transforming oncogene when it is overexpressed in NIH 3T3 cells in the presence of its ligand, presumably by providing a strong growth stimulation to already immortalized and cycling cells. ECF receptors are overexpressed in some human tumours at variable levels reaching perhaps 10–20 times that found in normal cells. This is often as a consequence of gene amplification as in some head and neck, brain and gynaecological tumours, but seems to be predominantly by increased transcription (or message stabilization) in human breast cancer cells. Occasional examples of gene amplification have been reported, however, which lead to very high levels of protein expression [4]. In 1985 the c-erbB-2 gene was independently discovered by the groups of Ullrich and Schlessinger [5] (who called it HER2) and by Yamamoto [6]. This gene is related in structure to the EGF receptor and is probably also a growth factor receptor for an as yet unidentified ligand. The c-erbB-2 gene is amplified in about 20% of breast, stomach and ovarian cancers and occasionally in some other tumour types, leading to elevated levels of protein expression [7]. In a few cases, however, high expression appears to occur without amplification. Again artificial overexpression will transform NIH 3T3 cells in culture.

Several reports have suggested that overexpression of either EGF receptors or c-erbB-2 are indicators of short relapse free interval and survival in breast cancer patients. Harris and colleagues also showed that combinations of both were apparently additive in predicting the course of the disease [8]. Interestingly, this result has now been confirmed experimentally in that combinations of moderate levels of expression of EGF receptors and c-erbB-2 which are not individually capable of cell transformation can produce fully malignant cells [9].

The c-erbB-3 gene is a new member of the EGF receptor/c-erbB-2 gene family. It is somewhat less related in structure to EGFR and c-erbB-2 than they are to each other suggesting that

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it may have evolved prior to the former two's divergence. Particularly important is the observation that the c-erbB-3 gene is overexpressed up to 100-fold at the mRNA level in six out of 17 human breast cancer cell lines apparently without gene amplification [3]. No doubt we will soon know whether the c-erbB-3 protein is concomitantly overexpressed and whether artificial overexpression is transforming. It will be interesting to explore, on the basis of these results, whether c-erbB-3 is ever overexpressed in primary human breast cancers. It seems likely that it will be. Finally, it may be that this gene product may also have some prognostic significance. If we learn from experience with the c-erbB-2 gene, large (greater than 200 cases), carefully performed studies employing multivariate analyses will be necessary to determine this reliably.

2. *p53*

The second gene with an apparently emerging role in breast cancer is p53. The gene was discovered in 1979 by Lane and Crawford as a protein associated with the T antigen of simian virus 40 [10]. The p53 gene was cloned in 1982 and was shown in initial experiments to be a dominantly acting transforming gene in NIH3T3 cells. Other work demonstrated that this gene would co-operate with a mutant ras gene in transforming normal cells. Subsequently, it was shown that the original gene used in these experiments contained a mutation which activated it. The work was then reinterpreted and the picture emerged that truly normal p53 can in fact act as a tumour suppressor gene [11]. In summary, normal p53 seems to be involved in providing a break to cell cycling. Inactivation of the p53 gene 'renders the normal cell predisposed to become transformed' [11]. The mutant p53, however, is transforming in a 'trans-dominant' fashion since it apparently complexes normal p53 expressed by the wild type allele together with the heat shock protein hsc70 thereby stabilizing the normal protein but sequestering it in an inactive form. Thus, three modes of transforming action may be envisaged. Loss of one normal allele may by a reduction in p53 protein levels give cells a growth advantage. Loss of both gene copies would remove all restraints imposed by p53. Finally, expression of a mutant p53 would produce effectively a 'loss of function mutation' by inactivating any wild type protein present (of course the normal copy could be deleted as well). Studies by Vogelstein and colleagues have shown that allelic deletions to the short arm of chromosome 17 occur in up to 75% of colorectal carcinomas. The region deleted contained the p53 gene which has been mapped to chromosome 17p 13.1 in humans. The remaining normal allele in the two cases studied in detail had suffered mutations in single codons [12].

A recent paper by Thompson *et al.* [13] has examined the expression of p53 mRNA and the loss of genetic material from the short arm of chromosome 17 in breast cancer biopsies. The VNTR probe employed was informative in 69% of the 76 tumour/lymphocyte DNA pairs examined but was several megabases telomeric to the p53 gene. However, polymorphic probes for the p53 gene itself were not available. The level of expression of p53 was determined by Northern analysis.

The results presented showed that approximately one-third of cases contained negligible p53 mRNA, one-third normal levels and one-third elevated levels when compared to normal breast extracts. In addition, loss of heterozygosity had occurred in 58% of the informative cases. Loss of one gene copy was positively correlated with high levels of mRNA expression (and absence of oestrogen receptors). This would, in the model described above, require also that the remaining copy be mutated as has been observed in colorectal tumours. The authors state that they are now searching for mutations in these cases. One might have expected loss of both gene copies in those cases with little or no p53 mRNA, however more subtle mutations not visible as gene deletions might account for this instead. These intriguing observations will stimulate further examination of clinical samples, particularly for the presence of mutations.

The number of genes involved or implicated in the aetiology of breast cancer is rapidly increasing. It is an exciting time in this field and hopefully greater understanding of the molecular basis of the disease will generate improvements in diagnosis, prognosis and therapy for breast cancer patients.

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