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ONCOGENES AND TUMOR GROWTH FACTORS IN BREAST CANCER

A minireview

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Abstract

Five oncogenes have been implied as having a role in human breast tumorigenesis: int-2, c-erbB-2 (HER-2), c-myc, c-Ha-ras and the recessive Rb-1. As far as the function and biochemistry of these oncogenes have been studied, they act at different levels and have totally different functions in the cells. They are normally cellular genes, likely to have important functions in normal cell growth or differentiation. In the tumors their regulation or function is altered, due to a wide class of mutations. The oncogenes may cooperate to result in the malignant cell phenotype. However, different oncogenes are mutated in different tumors, so that the tumors show a variable pattern at the molecular level, underlining the individuality of these tumors already described as differences in histopathology, hormone receptor expression and clinical course. The main importance of the oncogene studies is still to reveal basic pathogenetic mechanisms. Where appropriate it is important to test diagnostic or prognostic significane of the oncogene mutations.

Key words: Breast cancer, oncogenes, int-1, neu, RB-gene, C-myc, transgenic mice, ras.

Human breast cancer defines a heterogenous group of neoplasia, both regarding histopathologic characteristics and, as far as we can judge, etiologic factors. The heterogeneity is also reflected at the molecular and biochemical level.

The extensive epidemiologic data available for breast cancer ranges from showing a strong familial (genetic) risk factor (10-fold higher risk with e.g. sister or mother with bilateral breast cancer) to relatively weak environmental factors (e.g. age at first pregnancy, alcohol, other dietary factors?) (1). The etiology is multifactorial, and varies between different individuals. Irrespective of the contributing pathogenetic factors, it is likely that the tumor cells develop due to genetic changes at the somatic cell level.

The oncogenes and their products have given us new and powerful tools to analyze critical genetic and molecular events leading to tumor cell development and growth. We have an increased knowledge of cellular changes at the DNA level and around the cell membrane. Much less is known about the communication between the membrane and the genes. We are just beginning to see the application of this knowledge to human tumors, including breast cancer.

While clinical stage, number of histopathologically positive lymph nodes, hormone receptor status, tumor grade and other histologic features (lymphatic or vascular invasion) have to be considered reproducible prognostic factors, oncogene recombination and expression belong to a group of parameters still to be evaluated for diagnostic and prognostic importance, together with others like cell kinetic characteristics, cytogenetics and cell surface markers.

Breast cancer has been studied experimentally in animal model systems. The animal breast cancers have been induced by chemicals, viruses or were spontaneous, in animals strains with inbred high susceptibility. These are not models directly applicable to human breast cancer, but they have provided a basis for fruitful discussion of multifactorial pathogenesis, hormone dependent tumor growth and molecular events in breast cancer. After a brief review of current knowledge about oncogenes, I will discuss some of the experimental findings in breast cancer.

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Oncogenes

Around 40 oncogenes have been identified until now. There is no unequivocal definition of oncogenes. They are perturbed normal cellular genes, which have been shown to confer properties to tumor cells or tumor-like cells essential for their behavior as tumor cells. Several of these genes were shown to be evolutionary conserved genes and to control important normal cell physiologic functions related to cell growth and differentiation. When the normal control is disrupted by disregulation of the oncogene, this may contribute to tumor cell development. The disruption is due to 'mutations' in a wide sense (Table 1), either changing the protein coded from the gene (point-mutations) or the regulation of its expression (promoter insertion, amplification, translocation, regulation by virus).

The oncogenes can be grouped according to normal biochemical function or location (Table 2). Several oncogenes are involved in growth factor regulation, either as growth factors, or receptors for growth factors. In several instances it seems that the oncogene is a mutated version of the growth factor receptor that may leave the receptor in a permanently activated state without any ligand/growth factor bound to it. Other oncogenes code for proteins involved in controlling the signal from the cell surface into the cell and to secondary messengers (G-like proteins, membrane bound tyrosine kinases). Yet other oncogenes are active in the cytoplasm or show a nuclear location. The function of nuclear oncogenes is still obscure. One group of proteins have been identified within this group, that act as factors controlling transcription, and can thus modulate activity of a whole set of other genes.

Table 1

Oncogene activation and dysregulation in tumors

Increased expression/disregulation
Controlled by viral promoter-enhancer
Controlled by integrated viral enhancer
Chromosome translocation: oncogene under influence of non-autologous promoter/enhancer
Amplification
Changed product with new function
Point-mutation

Table 2

Groups of oncogenes

Characteristics	Member/example
Growth factor	c-sis
Growth factor receptor	c-erbB/EGF-receptor/c-neu
G-protein	c-Ha-ras
Tyrosine kinase	c-src/EGF-receptor
Nuclear protein	c-myc
Transcription factor	c-jun

In recent years suppressor oncogenes, or simply suppressor genes have been identified. Much less is known about their mode of action. In the case of the retinoblastoma the oncogene coded protein, Rb, has been identified, and it interacts with some other oncogene coded proteins. It has been suggested that it blocks or modifies their function (2).

Suppressor genes have to be inactivated before the tumor cell can arise. This can occur at the genetic level by mutations. In tumors where suppressor genes have been implicated, they are the result of allelic expression. In dominant homozygous or heterozygous situations the suppressor activity is expressed, while in homozygous recessive situations the suppressor activity is lost and tumor can develop. Important examples are retinoblastoma and Wilms' kidney tumor in children (3).

Oncogenes in breast cancer

A number of oncogenes have been implicated playing a role in breast cancer, either since they have been found in a mutated form in the cancer cells (truncation, amplification), or because they were originally found adjacent to the mouse mammary tumor virus (MMTV) integration sites in mouse breast cancer, and could possibly be under the control of the MMTV controlling promoter-enhancer element. These genes are the int-1 and int-2 genes, named after virus *int*egration. The oncogenes of relevance are listed in Table 3.

Int-1 and int-2. These, the most studied genes of a 'pseudofamily' of oncogenes, derive their name from the mode of detection. They are localized in the vicinity of *int*egrated MMTV in rodents. These genes are frequently activated as a result of their localization close to the strong MMTV-promoter-enhancer (the long terminal repeat, LTR). I deliberately call this a pseudofamily as there is no indication that they are or have to be functionally related. After the discovery of these genes in mice, it has been shown that homologues can be amplified or activated by

Table 3

Cellular oncogenes and putative oncogenes associated with mammary cancers

Gene	Species	Mode of activation
c-erbB-2	Human	Amplification
int-1	Mouse	MMTV-insertion mutagenesis
int-2	Mouse	MMTV insertion mutagenesis
	Man	Amplification
c-myc	Human	Amplification
	Mouse (transgenic)	Heterologous promoter
c-Ha-ras1	Mouse/rat	Point mutation (codon 12 or 61) in chemically induced tumors
N-ras	Human	Amplified
Suppressor gene (RB)	Human	Deletion/inactivation

other means in human breast cancer. Int-1 is likely to code for a secreted protein, and thus is a candidate to be a growth factor. In this case the conservation throughout evolution has provided a surprise. The int-1 homologue in the Drosophila flies is involved in the development of the fruitfly wings and legs (4). Int-2 is coding for a fibroblast growth factor (FGF) homologue. There are more members of this family discovered in the mouse system, but int-1 and int-2 are those known to be affected in some human breast cancers int-2 has been shown to be amplified in some human breast cancer (5).

c-Ha-ras. The normal counterpart of this oncogene is a GTP-binding or G-protein homologue, involved in signal transduction from membrane receptors to activation of intracellular second messengers. By point mutations in two particular regions, at aminoacids 12–13 or 59–61, the proteins seem to acquire a more active form that does not release bound, activating GTP. Such point mutations have been observed in chemically induced mouse mammary tumors, specifically at position 61 in dimethylbenzanthracene-induced tumors and in spontaneous tumors (6), and in codon 12 after N-nitroso-N-methylurea induction (7). Another member of the 'ras-family', N-ras has been found amplified in one of the human breast cancer cell lines (8).

c-erbB-2/HER-2/neu. This oncogene is closely related to the human EGF-receptor, but the ligand is unknown. Neu is the rodent homologue of the human c-erbB-2 (or HER-2, from human EGF-receptor 2). The natural ligand is not known, but it has been shown that if the intracellular part of c-erbB-2 is linked to the extracellular domain of the EGF-receptor, the hybrid receptor acts as an EGF-receptor (9). This strongly supports the suggestion from the molecular homology to the EGF-receptor, that this is a membrane bound growth factor receptor. Amplification of this gene has been detected in up to 20% of stage I and 40% of stage II breast cancer patients, suggesting a role in progression of the disease (10–12). Amplification is predominantly seen in estrogen receptor negative tumors. Amplification usually correlates well with higher levels of protein expression.

C-myc. This much studied nuclear oncogene is amplified or overexpressed in 22–32% of human breast cancer (10, 13, 14). When introduced into mice as a transgene under the control of a strong-promoter-enhancer, c-myc is overexpressed and these animals get mammary cancers (15, 16). This is, however, the end of consensus, as the effect of c-myc studied in in vitro growing breast cancer derived cell lines is conflicting regarding its contribution to a (more) malignant phenotype (17, 18).

Experimental model systems

The mouse mammary tumor virus (MMTV) can induce tumors in mice after long latency periods of 4 to 9 months. The pathogenesis is complex and this is an excellent model for multistep pathogenesis (19). MMTV provirus carried in the germ-line genome of the mice, exogenous MMTV fed by the mother's milk, immunologic factors presumably controlling level of MMTV replication in the animals, and hormonal influence on the potential tumor cells are steps identified in the tumorigenic process. As mentioned at the molecular level it has been shown that MMTV frequently integrates close to oncogenes, in particular int-1 and int-2. The virus is usually oriented in opposite direction to the oncogene, and the oncogene exons are never interrupted by the virus integration. It is most likely that the virus activates the oncogene by its strong enhancer in the LTR region, which can act over a wide distance of the genome, since integration has been detected up to 12 000 DNA bases away from the int-genes (4). In this context it is also worth noting that the MMTV LTR also contain sequences that specifically interacts with glucocorticoid- and progesteron-receptor nuclear complexes. This interaction results in activation of the LTR-enhancer, and of course can serve as a basis for the hormonal influence on the progression of these tumors.

In the mouse several steps can be identified preceding the tumor (19): plaques and hyperplastic alveolar nodules (HAN). The HANs are hyperplasias of the lobuloalveolar epithelium, and are maintained by constitutive levels of lactogenic hormone. The nodules can be further transformed to malignant tumors, and sometimes hyperplastic nodules and carcinomas in situ have been observed in the same tumor. Multiple evidence support that the HANs are precursors of cancer. The plaque is another alternative pathway. They appear during pregnancy, and disappear between pregnancies. They can be considered as small, hormone-dependent tumors. By transplantations of these pretumorous lesions in mice models to study details of the multistep tumorigenesis have been developed.

The transgenic mice. Most of the mentioned oncogenes relevant for breast cancer have been introduced into the germ line of mouse strains by the transgene technique. The oncogenes have been put under control of heterologous promoters such as the MMTV LTR itself. Under these conditions int-1, c-erbB-2/neu, c-myc and v-Ha-ras were expressed in breast tissue and with some variation induced mammary adenocarcinomas (20–23). Frequencies were often low and latency periods long, suggesting that none of the genes were sufficient for mammary tumor induction, but that secondary (genetic) events were taking place to result in tumor growth. The oncogene disregulation introduced by the transgene predisposed for these secondary events, correlating to the preneoplastic hyperplasia introduced by the transoncogenes. The method promises to deepen the understanding of the pathogenic process.

Suppressor genes

Suppressor genes, or recessive oncogenes, are cellular genes with an as yet unknown function. Both alleles of

such a gene have to be inactivated by mutations or lost by deletions in tumors where the genes play a role (3). In hereditary retinoblastoma one allele is lost in the germ line, and the change is thus present in all somatic cells, while the second allele is lost/inactivated in the pretumorous cells. This gene codes for the only protein identified from such a suppressor gene. It binds to other oncogenic proteins, particularly some virus-coded transforming proteins, and may inactivate them, when the protein is expressed. Suppressor genes can be searched for by identifying gene losses at the chromosomal level or at the molecular level in tumors. By such methodology the Rb-locus has been shown to be lost in a number of mammary carcinomas (24, 25).

Conclusions

The studies of the oncogenes have introduced totally new concepts and theories to tumor biology. Their main merit is at presently for studies of basic understanding of tumorigenesis. It is, however, of great interest that a limited number of oncogenes have been shown perturbed in breast cancer, different in different tumors, and new experimental models with transgenic mice suggest that they can really be involved in breast tumorigenesis. Intense work is in progress both to reveal their function in the cell biochemistry and in tumorigenesis. In parallell, it is of great interest to pursue studies on their diagnostic and prognostic significance.

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