Oncogene Expression in Mammary Epithelial Cells

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Abstract   Mouse strains which develop tumors at a high incidence with characteristics very similar to human cancers have been derived over the last 8 years. The tumors are caused by defined genetic alterations in the mouse genome. Three areas of research have contributed to the derivation of these mouse strains: (1) Molecular analysis of human tumors has shown that distinct oncogenes and tumor suppressor genes are consistently involved in a high percentage of primary tumors. (2) Regulatory enhancer-promoter sequences have been identified which direct gene expression to specific target cells, preferentially mammary epithelial cells. (3) The introduction of recombinant DNA molecules into fertilized mouse eggs by microinjection and integration of the injected DNA into the genome of injected cells has given rise to mutant mouse strains with unique and defined genetic alterations. Studies with different promoter-oncogene combinations introduced into transgenic mouse strains have led to the following general conclusions: (1) Oncogenes expressed in mammary gland cells predispose transgenic mice to mammary tumors. (2) The oncogenic potential of indivdiual oncogenes in mammary epithelial cells differs. (3) Oncogene expression initially often causes a preneoplastic state affecting growth and differentiation parameters of cells. (4) The expression of different oncogenes synergizes to reduce tumor latency. Synergism can also be observed with physiological growth signals like estrogen or growth hormone. The oncogenes with a role in mammary carcinomas which have been investigated in transgenic mice will be described here. The phenotypic consequences of oncogene expression and the implications for the multistep carcinogenesis model will be discussed.   © 1992 Wiley-Liss, Inc.

Key words: epithelial hyperplasia, breast cancer, oncogene transfer, oncogene mutations, oncogene cooperation

CONSISTENT ONCOGENE ACTIVATIONS IN SPONTANEOUS MOUSE MAMMARY TUMORS AND HUMAN BREAST CANCER

A subset of oncogenes which is consistently involved in the transformation of mammary epithelial cells and the etiology of mammary tumors has been identified. Some of these oncogenes have been functionally evaluated in transgenic mice. Several experimental strategies have been employed in order to identify these oncogenes. The molecular cloning of sequences which are located in the vicinity of tumor specific MMTV proviral integration sites has led to the identification of several cellular gene activated by insertional mutagenesis in mouse tumors (Peters, 1990). These genes include the int-1, int-2, int-3, and int-4 genes (Nusse, 1988). The int-1 and int-2 genes have been tested in transgenic mice. Activated H-ras genes have been detected by genomic transfec-

tion experiments into NIH3T3 cells with DNA from chemically induced mouse mammary carcinomas (Dandekar et al., 1989; Kumar et al., 1990). Oncogenic versions of the H-ras and the N-ras genes have also been tested in transgenic mice.

Hybridisation experiments with known oncogene probes, immunohistochemical analysis, and the detection of the loss of heterozygosity of restriction fragments hybridizing to cellular gene probes have led to the identification of genes involved in human breast cancer. The frequency of alteration and their correlation with clinical parameters have been evaluated (Callahan et al., 1989, 1992). The c-myc and the c-erbB-2 genes were found to be amplified and overexpressed in a fraction of primary human breast cancer cells. These genes have been used to derive transgenic mice. Amplification has been found for the chromosomal region (11q13) comprising the human homologue of the int-2 gene. The gene which is enhanced in its expression as a result of this amplification in human breast tumors has not yet been unambiguously identified. The int-2 gene has been tested in transgenic mice.

The most frequent genetic alterations in human breast cancer are mutations due to interstitial deletions, chromosomes losses, or aberrant mitotic recombinational events. These mutations can be detected by loss of heterozygosity (LOH) of distinct restriction fragments and are thought to affect tumor suppressor genes (Seemayer and Cavenee, 1989; Hollingworth and Lee, 1991). At least eight loci which show frequent LOH have been detected in the genome of human breast cancer cells. These loci were assigned to chromosomes 1p (Bieche et al., 1990), 1q (Merlo et al., 1989), 3p (Ali et al., 1989), 11p (Ali et al., 1987; Theillet et al., 1986), 13q (Callahan et al., unpublished data), and 17p, 17q, and 18q (Cropp et al., 1990) and are discussed by Callahan et al. (1992). An additional frequent LOH in primary breast cancer DNA was found on chromosome 16q (Sato et al., 1990).

Two tumor suppressor genes which correlate with LOH in breast tumor cells have been characterized. They are the RB-1 gene on chromosome 13q and the p53 gene on 17p. Both tumor suppressor genes encode nuclear phosphoproteins involved in the regulation of the cell cycle (DeCaprio et al., 1989; Sturzbecher et al., 1990). These genes can partially revert the transformed phenotype upon introduction of their wild type alleles into cultured tumor cells (Huang et al., 1988; Eliyahuet et al., 1989). p53 mutations have also been found to be transmitted through the germ line in a hereditary cancer syndrome (Malkin et al., 1990). Two additional cellular genes, potential candidates for tumor suppressor genes present at loci identified through LOH are the nm23-H1 gene and the DCC gene. The nm23-H1 gene (Steeg et al., 1988) located on chromosome 17q encodes a nucleoside diphosphate kinase possibly involved in the regulation of G protein activity. This protein is down-regulated in metastatic cells (Liotta et al., 1991). The DCC gene on 18q encodes a protein which is structurally related to neural cell adhesion molecules (Fearon et al., 1990). The absence or decreased expression of this gene could result in the disturbance of cell-cell or cell matrix interactions and thereby contribute to malignancy.

These genes, which display their phenotype as a loss of function, are more difficult to identify and more difficult to test in transgenic mice. Disruption experiments with the genes described above are in their early stages.

TARGETED ONCOGENE EXPRESSION IN MAMMARY TISSUE OF MICE
Experiments With Activated ras Oncogenes

The ras genes, H-, K-, and N-ras, encode guanine nucleotide binding proteins possibly involved in the intracellular signal transduction. Single amino acid mutations distinguish the oncogenically activated versions of the gene from the wild type. These mutations interfere with the normal regulation of ras activity and maintain the protein in a constitutively active state. Activation of the ras oncogenes has not been frequently detected in human breast cancer, but plays a crucial role in rodent mammary tumors induced by chemical carcinogens.

To direct the expression of oncogenes to mammary epithelial cells, two promoter regions have been utilized, the whey acidic protein gene promoter and the mouse mammary tumor virus (MMTV) LTR. The whey acidic protein (Wap) gene encodes a milk protein and its expression is restricted to mammary epithelial cells during the late pregnancy and lactation phases. This regulation is also confered on chimeric transgenes (i.e., the expression of oncogenes under the transcriptional control of the Wap promoter is strictly confined and dependent on the hormonal stimuli governing pregnancy and lactation) (Pittius et al., 1988). The MMTV LTR, in contrast, is not a tissue or stage specific promoter. The endogenous proviral DNA of MMTV LTR regulated transgenes is expressed in mammary and salivary epithelial cells, lung, kidney, seminal vesicles, and in lymphoid cells (Ross et al., 1990). The highest expression occurs in mammary epithelial cells. A third promoter which was shown to direct transgene expression to the mammary in a highly specific fashion, the β-casein promoter, has not yet been exploited for oncogene targeting studies (Greenberg et al., 1991).

The activated H-ras gene was introduced into transgenic mice under the control of the MMTV LTR promoter (Sinn et al., 1987; Tremblay et al., 1989) or the Wap promoter (Andres et al. 1987, 1991). Expression of the transgene and tumor formation in MMTV LTR H-ras mice were found in several organs. In addition to mammary tumors, bilateral hyperplasia of the harderian lacrimal gland, salivary gland carcinomas, lung adenocarcinomas, and splenomegaly were observed. Apparently clonal mammary tu-

mors arose in animals of 4 to 10 months of age and were classified as adenocarcinomas and adenocanthomas. The tumor incidence was higher in female than in male transgenic mice.

The expression of an activated H-ras gene in transgenic mice under the control of the Wap promoter led exclusively to the formation of mammary tumors in female mice (Andres et al. 1987, 1991). A comparison of Wap H-ras strains with different levels of H-ras expression showed that the tumor incidence is a function of the level of oncogene expression. Only 2% of the females of the low expressing strain developed tumors, compared with 72% of the high expressing strain. The Wap promoter utilisation was affected by the transformation of the cells. Whereas the endogenous Wap gene was only expressed in normal mammary tissue of lactating animals, the Wap H-ras transgene escaped from its normal hormonal control and was constitutively expressed in tumor cells. A single line of the Wap H-ras transgenic mice showed an unusual pattern of transgene expression, which was probably due to the site of transgene integration. In this strain the transgene was integrated on the Y chromosome and salivary and/or mammary gland tumors were observed in the male mice. The salivary tumors were adenosquamous carcinomas arising from the serous areas of the submandibular gland with extensive immune cell infiltration. Mammary tumors were adenosquamous carcinomas with multiple foci of squamous metaplasia and adenocarcinomas containing glandular tissue. Microscopic lung metastasis were present in 14% of the animals (Andres et al., 1987; Nielsen et al., 1991).

The activated N-ras oncogene under the MMTV LTR control was also tested as a transgene (Mangues et al., 1990). Expression of the transgene in harderian, mammary, and salivary glands was observed and tumors resulted in these tissues. In addition, male sterility was observed in these mice. Thus, two members of the ras gene family have a similar effect on mammary gland transformation.

The observations made with the transgenic mouse strains described above were corroborated by experiments in which a different mode of gene transfer was used. Infection with recombinant retroviruses or transfection of primary epithelial cells and reconstitution of the mammary epithelium by reimplantation of the cells has been used to gain information on the potential of individual oncogenes. Poorly differenti-

ated, invasive mammary tumors were observed in animals after infection of the mammary epithelial cells with a recombinant Harvey murine sarcoma virus, directing the expression of the H-ras oncogene, and reimplantation of the infected cells into gland-cleared mammary fat pads (Strange et al., 1989). In these experiments the number of infected cells seemed to influence tumor formation. Dysplasic, non-invasive mammary outgrowth was observed when the primary epithelial cells were infected with a replication-deficient H-ras expressing virus. The inclusion of a helper virus, promoting viral spread and thus yielding a higher percentage of infected cells, resulted in tumor formation. Mammary hyperplasia was observed when the activated H-ras gene was transfected into normal mouse mammary epithelial cells and the transfected cells were transplanted into mice (Miyamoto et al., 1990). The hyperplasias caused by the transfected cells were preneoplastic (i.e., they were not immortal in vivo). Cell lines could be established only with very low frequency from cells infected with an H-ras expressing retrovirus (Redmond et al., 1988). Similar observations were made when the SV 40 large tumor (T) antigen or the v-H-ras oncogene were introduced into primary luminal epithelial cells cultured from human milk. The cells were not immortalized in vitro, senesced, and passed through a crisis period with only a low frequency. Few tumorigenic cell lines emerged (Bartek et al., 1991).

The transfer of the activated H-ras oncogene into established, clonal mammary epithelial cell lines provided further insights into its influence on growth and differentiation properties. The HC11 cell line retains characteristics of normal mammary epithelial differentiation and expresses milk protein genes upon treatment with the lactogenic hormones prolactin and glucocorticoids. Activated H-ras suppressed the ability of the cells to respond to the lactogenic hormones and caused inhibition of β-casein gene transcription. It also led to tumor formation of transplanted cells in nude mice (Hynes et al., 1990). The level of p21-ras expression directly influenced the phenotype of a second established cell line, NOG8 cells. The morphology, cloning efficiency in soft agar and tumorigenicity of the epithelial cells was a function of the extent of H-ras gene expression (Redmone et al., 1988).

Several conclusions can be drawn from these experiments. The expression of the activated ras

oncogene promotes proliferation and causes hyperplasias, but is not sufficient for tumor formation of primary mammary epithelial cells. In contrast, ras oncogene expression can confer tumorigenicity onto established cell lines. In addition, its expression suppresses lactogenic hormone action in cultured cells and in transgenic animals.

Experiments With the c-myc Oncogene

The myc genes, c-myc, N-myc, and L-myc, encode nuclear proteins which display DNA binding activity. They are rapidly and transiently induced upon growth factor stimulation of cells and may function as modulators of gene expression in response to proliferative signals. It is possible that they regulate the promoter activity of cellular genes important for cell division. The integration of the c-myc gene into the genome of transforming retroviruses, proviral promoter insertions adjacent to the myc gene, the location of the gene at chromosomal translocation junctions, and the amplification of the c-myc locus in a number of different human tumors have drawn attention to its role as an oncogene. Deregulated expression seems to be the major mode of its activation.

The frequent involvement of the c-myc gene in lymphoid malignancies has been the reason for the development of many transgenic mice in which the c-myc expression was directed towards hematopoietic cells (Adams and Cory, 1991). The phenotypic observations were similar to the ones made in mice in which the c-myc expression was directed to other cell types. They included a benign, polyclonal, hyperproliferative phase followed by the emergence of stochastically appearing clonal tumors. The MMTV LTR and the Wap gene promoter have been used to direct c-myc expression to mammary gland cells. MMTV LTR myc mice developed mammary adenocarcinomas during their second or third pregnancy (Stewart et al., 1984), although deregulated myc expression was observed earlier and did not interfere with cell proliferation or normal development. Tumors were not restricted to female animals and were eventually observed in testicular cells, B and T lymphocytes, and mast cells (Leder et al., 1986). The transgenic mice expressing the myc gene under the control of the Wap promoter showed high expression of myc during their lactation periods (Schoenenberger et al., 1988). c-myc expression impaired the development of the glandular epithelium and milk

protein synthesis was decreased. Epithelial cell proliferation, however, was partially uncoupled from the hormonal status of the animal and continued during lactation and post-lactationally (Andres et al., 1988). Tumors occurred in mammary tissue several months after the onset of Wap-myc expression (i.e., after the first lactation) and the adenocarcinoma incidence reached about 80%. The expression of the milk protein genes was also affected in these tumors, but differently from the tumors elicited through an activated H-ras transgene. The expression of the milk protein genes, the endogenous Wap gene, and the β-casein gene became independent of lactogenic hormone stimulation and persisted even in transplanted nude mouse tumors (Schoenenberger et al., 1988).

Hyperplastic growth of the mammary epithelium as a consequence of v-myc oncogene expression has also been shown in retrovirally infected cells. Primary mammary cells transplanted into cleared mammary fat pads formed a hyperplastic pattern of ducts (Edwards et al., 1988). A crucial difference in the consequences of H-ras and c-myc expression was also confirmed in cell culture. Whereas H-ras expression suppressed the lactogenic hormone response of mammary epithelial cells, c-myc expression did not inhibit the production of milk proteins upon hormonal stimulation (Ball et al., 1988).

Experiments With the int-1, int-2, and TGF-α Genes

The int genes (int-1, int-2, int-3, and int-4) were discovered when the integration sites for the mouse mammary tumor virus (MMTV) proviral DNA were analysed (Nusse, 1988; Peters, 1990). These genes are found adjacent to the proviral integration sites and their expression is activated through the insertion of the promoter-enhancer region present in the MMTV LTR. Although they share similar names, these genes are unrelated and involved in transformation through different modes of action.

The int-1 gene encodes a protein which appears to be associated with the cell surface or the extracellular matrix and may have the properties of a growth factor for adjacent cells. It has a distinct expression pattern during mouse embryogenesis and its product is found in the developing nervous system between days 9 and 15. Gene disruption experiments have confirmed that it has an essential role in normal mouse brain development. Expression has also been

found in the testis of mature males. Its *Droso-phila* homologue (wnt-1) has been identified as the segment polarity gene wingless, a protein which functions in the pattern formation of the developing *Drosophila* embryo. The proviral insertions found in MMTV induced mammary tumors occur outside of the coding region of int-1, either upstream in the opposite transcriptional orientation or downstream in the same orientation. This configuration has been mimicked in transgenic mice in which a transgene consisting of the MMTV LTR inserted upstream of the int-1 gene in the opposite orientation was introduced (Tsukamoto et al., 1988). The alveolar and ductal mammary epithelium in male and female transgenic mice was hyperplastic. Adenocarcinomas were frequently observed in female and less frequently in male mice. Int-1 expression seems to promote cellular proliferation and thus initiation of carcinogenesis.

The int-2 gene encodes a protein which is related to the basic fibroblast growth factor. It is normally not expressed in the mammary gland or other adult mouse tissues, but expression has been detected in developing mouse embryos between days 7 and 9. In transgenic mice int-2 expression under the control of the MMTV LTR resulted in a pronounced enlargement of the mammary gland, indicating epithelial hyperplasia. A truncated version of the MMTV LTR directed the expression of the int-2 transgene to the prostate of male mice and caused a benign prostatic hyperplasia, similar to the benign prostatic hypertrophy commonly observed in humans. Int-2 seems to function as a potent epithelial growth factor in the mammary gland as well as in the prostate (Muller et al., 1990).

The transforming growth factor-α (TGF-α) gene encodes a growth factor related to epidermal growth factor (EGF) and a growth factor encoded by vaccinia virus. It is secreted by a number of transformed cells and H-ras transformation of cultured mammary epithlial cells induces this secretion (Hynes et al., 1990). EGF and TGF-α bind to the EGF receptor, which in a permanent state can assume oncogenic properties. The secretion of TGF-α by tumor cells suggests a potential role in transformation, as an autocrine factor and transgenic mice have been derived to test the oncogenic potential of an ectopically expressed TGF-α gene. TGF-α expression directed by the metallothionein promoter caused epithelial hyperplasia in several organs, including the mammary gland. Initially

it did not cause major alterations in tissue architecture, but later in postlactational mammary glands induced secretory mammary adenocarcinomas (Sandgren et al., 1990). Similar observations were made when the TGF-α gene was introduced into transgenic mice under the control of the MMTV LTR (Matsui et al., 1990). Hyperplasia of alveoli and terminal ducts in virgin female and pregnant mice were seen, as were lobular and cystic hyperplasias, adenomas, and adenocarcinomas in mammary tissue. The transforming potential of the TGF-α gene could also be confirmed in cell line experiments. HC11 mammary epithelial cells were able to grow as nude mouse tumors after transfection with a TGF-α gene (Hynes et al., 1990).

Experiments With the c-erbB-2 and ret Oncogenes

The largest group of oncogenes is constituted by the protein kinases which phosphorylate tyrosine residues on their substrates. One subgroup of these tyrosine specific protein kinases is represented by the growth factor receptors with an extracellular ligand binding domain, a single transmembrane domain, and an intracellular tyrosine kinase domain. The prototype of this receptor gene family is the EGF receptor. Two oncogenes with characteristics of growth factor receptors, the c-erbB-2 and the ret genes, have been tested in transgenic mice and shown to be able to cause mammary tumors. Both oncogenes were introduced into the germ line as MMTV LTR constructs.

The c-erbB-2 encodes a 185 kDa transmembrane protein which is structurally related to the EGF receptor. An amino acid substitution has been discovered in an oncogenically activated rat homologue of the human c-erbB-2 gene. This mutation induces receptor oligomerisation, causes ligand independent tyrosine kinase activation, and confers a strong transforming activity onto the receptor. The c-erbB-2 receptor is not mutated in human tumors and its transforming activity is most likely based on overexpression. High expression of the normal c-erbB-2 gene also induces autophosphorylation and enhanced tyrosine kinase activity. The activated rat neu gene under the control of the MMTV LTR was employed in the first transgenic mouse experiments with this oncogene (Muller et al., 1988). In one transgenic mouse line, uniform malignant transformation of the mammary epithelium was observed. In another

transgenic line non-uniform expression of the c-neu oncogene was found. This resulted in the stochastic appearance of mammary tumors. The expression of c-neu was restricted to the transformed cells which were surrounded by non-expressing normal cells. The authors concluded that the expression of the activated c-neu oncogene is sufficient to induce malignant transformation of mammary tissue. Expression of the same gene in the parotis or epididymis led to benign, bilateral epithelial hypertrophy and hyperplasia, but did not progress to full malignant transformation. It was concluded that the tissue context is a major determinant of the malignant potential of individual oncogenes. In a second study with a MMTV LTR neu transgene slightly different results were obtained (Bouchard et al., 1989). Epithelial hyperplasias of the epididymis, seminal vesicles, and salivary glands were observed and dysplasia of harderian glands were found. In addition, independent multiple mammary tumors arose stochastically in females between 5 and 10 months of age. High transgene expression in normal tissue preceded the mammary tumors. These results suggest that the c-neu oncogene expression has an important role in transformation but that its expression is not a sufficient requirement. A third study was conducted in which the human c-erbB-2 gene was introduced into transgenic mice, also under the control of the MMTV LTR and under the control of an immunoglobulin enhancer-SV40 early gene promoter (Ig/Tp) chimera (Suda et al., 1990). In this study the normal allele of the gene and an activated version of the gene were compared. The normal c-erbB-2 under MMTV LTR control induced adenocarcinomas and B lymphomas with a late onset. Ig/Tp regulation of the normal c-erbB-2 also induced pre-B cell lymphomas within 6 to 10 months. Very rapid tumor induction was observed when the activated c-erbB-2 allele was introduced under the Ig/Tp control. Lymphomas were induced neonatally in highly expressing lines. Even in the early onset tumors, immunoglobulin heavy chain gene rearrangement indicated that the tumors arose clonally.

The ret oncogene was originally detected in transfection experiments with genomic DNA from human lymphoma cells. It is activated by DNA rearrangement of a receptor tyrosine kinase gene with another cellular gene (rpf). It has not been implicated in mammary tumor formation. Nevertheless, introduction of an MMTV LTR ret gene into transgenic mice gave rise to stochastically appearing mammary and salivary gland adenocarcinomas, as well as hyperplastic and dysplastic lesions of the harderian glands and male reproductive tracts. High ret expression and high phosphotyrosine containing protein levels were associated with the tumors (Iwamoto et al., 1990).

COOPERATING EVENTS IN MAMMARY TUMOR FORMATION

The recurring theme in the description of oncogene expression in primary mammary epithelial cells is hyperplasia and a preneoplastic state. Oncogenes with diverse modes of action exhibit similar phenotypes (i.e., they promote mitogenesis but their expression does not suffice for full malignant transformation). The current model of multistep carcinogenesis is based on distinct steps—tumor initiation, promotion, and progression. It is assumed that the accumulation of mutations in oncogenes and tumor suppressor genes accounts for the rate limiting steps in this sequential process. This might not be the only mechanism by which tumor formation can be promoted. A number of experiments in cultured cells and transgenic mice indicate that the level of oncogene expression also has a profound influence on the tumorigenic phenotype. Cultured mammary epithelial cells show dose dependent transformation parameters when transfected with the H-ras oncogene (Redmond et al., 1988) and the tumor latency is drastically shortened when the c-erbB-2 oncogene is expressed at high levels in transgenic mice (Suda et al., 1990). The prevailing concept, however, remains oncogene cooperativity. It has been developed in experiments with cultured cells and has been tested in some cases in transgenic mice (Weinberg et al., 1989; Adams and Cory, 1991).

The most straightforward approach to evaluate the consequences of the simultaneous expression of two oncogenes and possible oncogene cooperativity in transgenic mice has been to mate separate strains, each transgenic with a different oncogene. When MMTV LTR v-Ha-ras and MMTV LTR c-myc mice were crossed, the offspring expressing both oncogenes exhibited accelerated tumor formation (Sinn et al., 1987). The tumors still arose stochastically and were apparently monoclonal in origin. Coexpression of the H-ras and c-myc oncogenes under the control of the Wap promoter synergistically affected differentiation and resulted in a high num-

ber of neoplastic foci. Tumors were only observed after a latency of 3 to 4 months (Andres et al., 1988). These experiments suggested that although the H-ras and c-myc oncogenes cooperated and reduced tumor latency, they are not sufficient for full transformation and additional somatic events were required.

It has been suggested that the environment in which the oncogene expressing cells exist exerts a restraining influence on their growth properties (i.e., normal surrounding cells inhibit the growth of initiated, for example, oncogenic ras expressing cells) (Weinberg et al., 1989). The transgenic mouse experiments allow a test of this suggestion. Instead of isolated islands of oncogene expressing cells surrounded by wild type cells, as they might be encountered in spontaneously mutated somatic cells in the normal course of tumorigenesis, transgenic mice contain large numbers of oncogene expressing cells in hyperplasias. The interaction with neighboring cells might be affected and the tumor latency is usually shortened, but cooperating events still seem to be required. The situation is even more accentuated in retroviral transfer experiments. Introduction of the v-H-ras and the v-myc oncogenes into primary mouse epithelial cells and transplantation of such doubly altered cells into cleared mammary fat pads is accompanied by an intensive disruption of the cellular tissue context. Nevertheless, such cells lead to tumors only after six to eight weeks and progression through a hyperplastic intermediate state (Bradbury et al., 1991).

The progression from ras expressing initiated, premalignant cells to tumor cells is not restricted to the action of a cooperating oncogene, but can also be affected by physiological growth signals. This has been described in ras expressing skin cells, where the activation of protein kinase C by TPA leads to papilloma formation. Mammary epithelial cells with activated ras oncogenes can be proded into tumorigenicity by such diverse signals as growth hormone or estrogen. MMTV LTR H-ras mice with low p21 expression and a low tumor frequency of 2% could be converted into a high incidence strain of 76% by crossing with a line which ubiquitously expresses high levels of the human growth hormone gene under the control of the hydroxymethylglutaryl coenzyme A reductase promoter. High levels of growth hormone expression caused precocious mammary gland development and potentiated the tumorigenic potential of the ras oncogene (Bchini et al., 1991; Andres et al.,

1991). The activation of the ras oncogene also precedes the onset of neoplasias in chemically induced mammary tumors. Rats exposed at birth to the carcinogen nitrosomethylurea develop mammary tumors with a high incidence at least 2 months after the carcinogen treatment. Molecular analysis revealed the presence of activated H-ras and K-ras oncogenes 2 weeks after the treatment, but these oncogenes remained latent until the mammary epithelial cells were exposed to estrogen. Estrogen induced mammary development cooperated with the activated ras oncogenes in the full malignant transformation (Kumar et al., 1990).

FUTURE TRANSGENIC MOUSE MODELS AND THEIR UTILITY

The existing transgenic mouse models for mammary tumors are informative, but they do not yet reflect the complexity of the events leading to tumor formation in humans. In all cases, spontaneously occurring events have to complement the expression of exogenously introduced oncogenes before full malignant transformation can be observed. This is not surprising if we assume that five or six independent steps must cooperate (Weinberg, 1989). Two lines of experiments will be required to complete the picture. First, it will be necessary to investigate the combinations of oncogenes and tumor suppressor genes which are affected in an individual tumor cell. Not all of the mutations described above are simultaneously involved in tumor formation, but subsets can be defined in which specific mutations occur together (Callahan et al., 1992) and possibly complement each other. In human tumors, one of these involves LOH on chromosomes 11p, 17p, and 18q, the other one LOH on 1p, 13q, and 18q (Cropp et al., 1990; Sato et al., 1990). Similar observations have been made in mouse mammary tumors (Peters, 1990). In addition, the genes affected through LOH will have to be identified. Second, these combinations of oncogenes and tumor suppressor genes will have to be used to derive transgenic mouse models. The necessity to inactivate various tumor suppressor genes, the shear number of cooperating mutations to be analysed, and tissue specific targeting of these events might make this a demanding task.

Although the reconstruction of the events leading to breast cancer in humans will not be trivial in transgenic mice, the models might become valuable. Extensive efforts have already been made to find associations between specific onco-

gene mutations and disease characteristics of breast cancer patients. Particularly important is the identification of mutations which predipose the breast cancer patients to early onset of disease, relapse, and metastasis. For example, associations have been described between amplification of the int-2 locus local recurrence and distal metastasis, LOH on 1p with shortened survival periods after relapse, and LOH at 3p, 11p, 13q, 17p, 17q, and 18q with aggressive tumors (Callahan et al., 1992). Once the associations between the genetic lesions, their biochemical consequences, and the tumor phenotypes can be made, it is conceivable that targeted treatment modalities can be designed and tested in transgenic animals. This applies to tumor prevention experiments in genetically prediposed animals due to loss of a tumor suppressor gene as well as to tumor treatment in animals in which tumors have been induced by dominantly acting oncogenes.

REFERENCES

1. Adams JM, Cory S (1991): Transgenic models of tumor development. Science 254:1161–1167.
2. Ali IU, Liderau R, Theillet C, Callahan R (1987): Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science 238:185–188, 1987.
3. Ali IU, Meissner S, Liderau R, Callahan R (1989): Allelic deletion of c-erbA proto-oncogene in human breast carcinoma signifies possible recessive mutation in members of the steroid/thyroid hormone receptor gene family. J Natl Cancer Inst 81:1815–1820.
4. Andres AC, Schoenenberger CA, Groner B, Hennighausen L, LeMeur M, Gerlinger P (1987): Ha-ras oncogene expression directed by a milk protein gene promoter: Tissue specificity, hormonal regulation, and tumor induction in transgenic mice. Proc Natl Acad Sci USA 84:1299–1303.
5. Andres AC, van der Valk MA, Schoenenberger CA, Flueckiger F, LeMeur M, Gerlinger P, Groner B (1988): Ha-ras and c-myc oncogene expression distinctly interferes with morphological and functional differentiation of mammary epithelial cells in single and double transgenic mice. Genes Dev 2:1486–1495.
6. Andres AC, Bchini O, Schubaur B, Dolder B, LeMeur M, Gerlinger P (1991): H-ras induced transformation of mammary epithelium is favoured by increased oncogene expression or by inhibition of mammary regression. Oncogene 6:771–779.
7. Baker SJ, Markowitz S, Fearon ER, Willson JKV, Vogelstein B (1990): Suppression of human colorectal carcinoma cell growth by wild type p53. Science 249:912–915.
8. Ball RK, Ziemiecki A, Schoenenberger CA, Reichmann E, Redmond SMS, Groner B (1988): v-myc alters the response of a cloned mouse mammary epithelial cell line to lactogenic hormones. Mol Endocrinol 2:133–142.
9. Bartek J, Bartkova J, Kyprianou N, Lalani EN, Staskova Z, Shearer M, Chang S, Taylor-Papadimitriou J (1991): Efficient immortalisation of luminal epithelial

cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus. Proc Natl Acad Sci USA 88:3520–3524.
10. Bchini O, Andres AC, Schubaur B, Mehtali M, LeMeur M, Lathe R, Gerlinger P (1991): Precocious mammary gland development and milk protein synthsis in transgenic mice ubiquitously expressing human growth hormone. Endocrinology 128:539–546.
11. Bieche I, Champeme MH, Merlo G, Larsen CJ, Callahan R, Lidereau R (1990): Loss of heterozygosity of the L-myc oncogene in human breast tumors. Hum Genet 85:101–105.
12. Bradbury JM, Sykes H, Edwards PAW (1991): Induction of mouse mammary tumors in a transplantation system by the sequential introduction of the myc and ras oncogenes. Int J Cancer 48:908–915.
13. Bouchard L, Lamarre L, Tremblay PJ, Jolicour P (1989): Stochastic appearance of mammary tumors in transgenic mice carrying the MMTV c-neu oncogene. Cell 57:931–936.
14. Callahan R, Campbell G (1989): Mutations in human breast cancer: An overview. J Natl Cancer Inst 81:1780–1786, 1989.
15. Callahan R, Cropp CS, Merlo GR, Liscia DS, Cappa APM, Lidereau R (1992): Somatic mutations and human breast cancer: A status report. Cancer 69:1582–1588.
16. Cropp CS, Liderau R, Campbell G, Champene MH, Callahan R (1990): Loss of heterozygosity on chromosome 17 and 18 in breast carcinoma: Two additional regions identified. Proc Natl Acad Sci USA 87:7737–7741.
17. Dandekar S, Sukumar S, Zarbl H, Young LJT, Cardiff RD (1989): Specific activation of the cellular Harvey-ras oncogene in dimethylbenzanthracene induced mouse mammary tumors. Mol Cell Biol 6:4104–4108.
18. DeCaprio JA, Ludlow JW, Lynch D, Furukawa Y, Griffin J, Piwnica-Worms H, Huang CM, Livingston DM (1989): The product of the retinoblastoma susceptibility gene has properties of a cell cycle regulatory element. Cell 58:1085–1095.
19. Edwards PA, Ward JL, Bradbury JM (1988): Alteration of morphogenesis by the v-myc oncogene in transplants of mammary gland. Oncogene 2:407–412.
20. Fearon E, Cho KR, Nigro JM, Vogelstein B (1990): Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247:49–56.
21. Greenberg NM, Reding TV, Duffy T, Rosen JM (1991): A heterologous hormone response element enhances expression of rat β-casein promoter driven chloramphenicol acetyltransferase fusion genes in the mammary gland of transgenic mice. Mol Endocrinol 5:1504–1512.
22. Huang HJS, Yee JK, Shew JY, Chen PL, Bookstein R, Friedman T, Lee E, Lee WH (1988): Suppression of the neoplastic phenotype by replacement of the Rb genein human cancer cells. Science 242:1563–1566.
23. Hynes NE, Taverna D, Harwerth IM, Ciardiello F, Salomon DS, Yamamoto T, Groner B (1990): Epidermal growth factor receptor, but not c-erbB-2, activation prevents lactogenic hormone induction of the β-casein gene in mouse mammary epithelial cells. Mol Cell Biol 10:4027–4034.
24. Hollingsworth RE, Lee WH (1991): Tumor suppressor genes: New prospect for cancer research. J Natl Cancer Inst 83:91–96.

25. Iwamoto T, Takahashi M, Ito M, Hamaguchi M, Isobe K, Misawa N, Asai J, Yoshida T, Nakashima I (1990): Oncogenicity of the ret transforming gene in MMTV ret transgenic mice. Oncogene 5:535–542.

26. Kumar R, Medina D, Sukumar S (1990): Activation of H-ras oncogenes in preneoplastic mouse mammary tissues. Oncogene 5:1271–1277.

27. Leder A, Pattengale PK, Kuo A, Stewart T, Leder P (1986): Consequences of widespread deregulation of the c-myc gene in transgenic mice: Multiple neoplasms and normal development. Cell 45:485–495.

28. Liotta LA, Steeg PS (1991): Clues to the function of Nm23 and Awd proteins in development, signal transduction, and tumor metastasis provided by studies of *Dictostelium discoideum*. J Natl Cancer Inst 82:1170–1172.

29. Malkin D, Li FP, Strong LC, Fraumeni JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky M (1990): Germ line mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250:1233–1238.

30. Mangues R, Seidman I, Pellicer A, Gordon JW (1990): Tumorigenesis and male sterility in transgenic mice expressing a MMTV N-ras oncogene. Oncogene 5:1491–1497.

31. Matsui Y, Halter SA, Holt JT, Hogan BLM, Coffey RJ (1990): Development of mammary hyperplasia and neoplasia in MMTV TGF-alpha transgenic mice. Cell 61:1147–1155.

32. Merlo G, Siddiqui J, Cropp CS, Callahan R (1989): Frequent alteration of the DF3 tumor-associated antigen gene in primary human breast carcinomas. Cancer Res 49:6966–6971.

33. Miyamoto S, Guzman RC, Shiurba RA, Firestone GL, Nandi S (1990): Transfection of activated Ha-ras protooncogenes causes mouse mammary hyperplasia. Cancer Res 50:6010–6014.

34. Muller WJ, Sinn E, Pattengale PK, Wallace R, Leder P (1988): Single step induction of mammary adenocarcinoma in transgenic mice bearing activated c-neu oncogene. Cell 54:105–115.

35. Muller WJ, Lee FS, Dickson C, Peters G, Pattengale P, Leder P (1990): The int-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 9:907–913.

36. Nielsen LL, Discafani CM, Gurnani M, Tyler RD (1991): Histopathology of salivary and mammary gland tumors in transgenic mice expressing a human Ha-ras oncogene. Cancer Res 51:3762–3767.

37. Nusse R (1988): The int genes in mammary tumorigenesis and in normal development. Trends Genet 4:292–295.

38. Peters G (1990): Oncogenes at viral integration sites. Cell Growth Diff 1:503–510.

39. Pittius CW, Sankaran L, Topper YJ, Hennighausen L (1988): Comparison of the regulation of the whey acidic protein gene with that of a hybrid gene containing the whey acidic protein gene promoter in transgenic mice. Mol Endocrinol 2:1027–1032.

40. Redmond SM, Reichmann E, Muller RG, Friis RR, Groner B, Hynes NE (1988): The transformation of primary and established mouse mammary epithelial cells by p21-ras is concentration dependent. Oncogene 2:259–265.

41. Ross SR, Hsu CLL, Choi Y, Mok E, Dudley JP (1990): Negative regulation in correct tissue-specific expression of mouse mammary tumor virus in transgenic mice. Mol Cell Biol 10:5822–5829.

42. Sato T, Tanigami H, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y (1990): Allelotype of breast cancer: Cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res 50:7184–7189.

43. Sandgren EP, Luettke NC, Palmiter RD, Brinster RL, Lee DC (1990): Overexpression of TGF-alpha in transgenic mice: Induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell 61:1121–1135.

44. Schoenenberger CA, Andres AC, Groner B, van der Valk M, LeMeur M, Gerlinger P (1988): Targeted c-myc expression in mammary glands of transgenic mice induces mammary tumors with constitutive milk protein gene transcription. EMBO J 7:169–175.

45. Seemayer TA, Cavenee WE (1989): Biology of disease: Molecular mechanisms of oncogenesis. Lab Invest 60:585–599.

46. Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P (1987): Coexpression of MMTV v-ras and MMTV c-myc genes in transgenic mice: Synergistic action of oncogenes in vivo. Cell 49:465–475.

47. Steeg PS, Bevilaqua G, Kopper L, Thogeirsson UP, Talmadge JE, Liotta LA, Sobel ME (1988): Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 80:200–204.

48. Stewart TA, Pattengale PK, Leder P (1984): Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV myc fusion genes. Cell 38:627–637.

49. Strange R, Aguilar-Cordova E, Young LJ, Billy HT, Dandekar S, Cardiff RD (1989): Ha-ras mediated neoplastic development in the mouse mammary gland. Oncogene 4:309–315.

50. Sturzbecher HW, Maimets T, Chumakov P, Brain R, Addison C, Simonis V, Rodge K, Philip R, Grimaldi M, Court W, Jenkins JR (1990): p53 interacts with p34 cdc2 in mammalian cells: Implications for cell cycle control and oncogenesis. Oncogene 5:795–801.

51. Suda Y, Aizawa S, Furuta Y, Yagi T, Ikawa Y, Saitoh K, Yamada Y, Toyoshima K, Yamamoto T (1990): Induction of a variety of tumors by c-erbB-2 and clonal nature of lymphomas even with the mutated gene. EMBO J 9:181–190.

52. Theillet C, Liderau R, Escot C, Hutzell P, Brunet M, Gest J, Schlom J, Callahan R (1986): Frequent loss of a H-ras-1 allele correlates with aggressive human primary breast carcinomas. Cancer Res 46:4776–4781.

53. Tremblay PJ, Pothier F, Hoang T, Tremblay G, Brownstein S, Liszauer A, Jolicoeur P (1989): Transgenic mice carrying the mouse mammary tumor virus ras fusion gene: Distinct effects in various tissues. Mol Cell Biol 9:854–859.

54. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE (1988): Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell 55:619–625.

55. Weinberg RA (1989): Oncogenes, antioncogenes, and the molecular basis of multistep carcinogenesis. Cancer Res 49:3713–3721.