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Expression of activated oncogenes in the murine mammary gland: transgenic models for human breast cancer

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Abstract

Breast cancer is the leading cause of death among non-smoking women and thus has been the focus of intensive research. It has been generally accepted that the deregulation of oncogenes or their regulators play a pivotal role in progression of this prevalent disease. For example, amplification and overexpression of a number of oncogenes has been observed in a proportion of primary breast cancer biopsies. More recently, there has also been reports of inactivation tumor supressor genes in human breast cancer. While there is compelling evidence for a role of these genes in breast cancer tumor progression due to limitations inherent in these studies it is difficult to establish a direct causal association between expression of a certain oncogene and tumor progression. For this reason many groups have employed the transgenic mouse as a model system to directly study effects of oncogene expression in the murine mammary gland. This review will attempt to highlight some of the important lessons and potential applications that have emerged from the study of oncogene expression in the mammary epithelium of transgenic mice. The utility of the transgenic system to assess the transforming potential of oncogenes, to investigate the multi-step nature of malignant progression, and to be used as models for therapeutic intervention will be discussed.

Introduction

Oncogenesis is generally viewed as a multistep process that likely involves both the activation of oncogenes and the loss of function of tumor supressor genes. This view is supported by tissue culture experiments which show that oncogenes can collaborate to transform primary cells [1–2] and tumor supressor genes can cause reversion of transformed cells [3]. Although these experiments have yielded important information regarding the action of oncogenes and their supressors, they do not view malignant transformation in its natural *in vivo* context. For example, tissue culture experiments cannot easily reveal the role of humoral or cell mediated factors may play a role in malignant progression. For this reason, several groups have been interested in creating useful transgenic mouse models to study the genetic requirements for this malignancy (see Table 1). In the design of many of these transgenic experiments an important consideration in the choice of the oncogene was its relevance to human breast cancer. Given the large body of evidence supporting the role oncogenes and their regulators in tumor progression, breast tumor biopsies and derived cell lines have been examined for amplification and overexpression of oncogenes. Using southern, northern blot analyses and immunohistochemistry the overexpression and amplification of several classes of oncogenes in human breast cancer has been demonstrated. Interestingly, these oncogene products appear to af-

fect different components of the signal transduction pathway including transcription factors [5–7] growth factors [8–9] and growth factor receptors [10–14]. In some of these studies these genetic aberrations appear to be correlated with the clinical prognosis of the disease. For example, the extent of amplification of the c-erbB-2/c-neu gene is inversely correlated with survival of the patient [10–11]. Although other groups have reported amplification of c-erbB-2/c-neu in similar proportion of primary human breast cancers no correlation between amplification status and prognosis was reported [15–18]. While the reasons for this discrepancy are not clear, variables such as heterogeneity in the composition of tumor samples and differences in reagents used in the analysis would certainly influence the outcome of the results.

Given the inherent limitations of these clinical studies, a number of laboratories have turned to the transgenic mouse as an experimental system in which the action of an oncogene can be readily assessed in a tissue specific manner. Targeted expression of oncogenes to the mammary epithelium of transgenic mice has been achieved by fusing activated oncogenes to a variety of mammary specific transcriptional elements. In particular, the Mouse Mammary Tumor Virus Long Terminal Repeat (MMTV LTR) and the Whey acidic protein gene (Wap) promoter have extensively employed to direct oncogene expression to the mammary gland [19–20]. Although both these regulatory sequences are transcriptionally active in the mammary epithelium their behaviour differs in several respects. Because the MMTV LTR contains transcriptional elements that can respond to both glucocorticoids and androgens the range of tissues that the LTR is active in is broader than the Wap promoter. For example, MMTV dependant expression can be detected in the parotid gland, components of the male reproductive tract including the epididymis, seminal vesicle, and prostate gland and occasionally in the lymphoid system [19]. By contrast the Wap promoter is expressed at high levels only in mid pregnant and lactating mammary gland and thus is restricted to differentiated mammary epithelium [20–22].

Transgenic mice as models for multistep carcinogenesis

The analyses of transgenic mouse strains bearing

Table 1. Comparison of MMTV and WAP oncogene transgenic strains

Construct	Pathology[a]	Tumor occurrence[b]	Onset[b]	References
MMTV/c-myc	m.gl. tumors, lymphomas	stochastic	325 days	[24–26]
MMTV/v-Ha-ras N-ras	m.gl. tumors, lymphomas, sal. gl. tumors, har. gl. hyperplasia, lung tumors, epididymal hyperplasia	stochastic	168 days	[26, 29, 30]
MMTV/activated c-neu	m.gl. tumors, sal. gl. hyperplasia, epididymal hyperplasia	single-step or rapid progression	89 days	[60]
MMTV/activated c-neu	m. gl. tumor, epididymal hyperplasia, har. gl. tumors	stochastic	5–10 months	[61]
MMTV/c-erbB-2/c-neu	lymphomas, facial adenocarcinoma	stochastic	ND	[62]
MMTV/ret	m. gl. adenocarcinoma	stochastic	6–7 months	[63]
MMTV/int-1	m. gl. hyperplasia, m. gl. tumors, sal. gl. tumors	stochastic	3–7 months	[43]
MMTV/int-2	m. gl. hyperplasia, m. gl. tumors, prostate and epididymal hyperplasia	stochastic	> 18 months	[31, 52]
MMTV TGF α	m. gl. tumors	stochastic	3–5 months	[56]
MMTV/SV 40 large T	lymphomas, lung and kidney adenocarcinomas, m. gl. adenocarcinomas	stochastic	ND	[71]
MMTV/E1A and E1B	gastric tumors		ND	[72]
Wap/c-myc-/v-Ha-ras*	m. gl. tumors, sal. gl. tumor*	stochastic	3–6 months	[20–22]

[a] abbreviations: mammary gland = m. gl., salivary gland = sal. gl., harderian gland = har. gl.
[b] refers to mammary tumor.

activated oncogenes have provided important insight into the *in vivo* requirements for tumorigenesis. Generally the expression of an activated oncogene in a tissue specific manner results in the appearance of clonal malignancies after a certain latency period [23]. These observations argued that in addition to oncogene expression, full malignant transformation required additional genetic events. The first example of transgenic strains behaving in this manner were the MMTV/c-myc mice [24–26]. Initially, the MMTV/c-myc showed no morphological abnormalities and as evidenced by their ability to nurse their young appeared to possess normal mammary gland function. However, beginning at 4 to 5 months of age, multiparous transgenic carriers began to develop solitary mammary adenocarcinomas. By 325 days of age 50% of all female carriers had developed mammary adenocarcinomas. As expected the male MMTV/c-myc transgenic mice did not develop mammary tumors. Because the mammary tumors arose with variable onset in these strains and appeared adjacent to normal tissue also expressing the transgene, it was argued that expression of c-myc while necessary was not sufficient for tumorigenesis [24–26]. Rather, these results suggested that additional genetic events were required for malignant transformation. The importance of the transgenic mouse as a powerful system to assess the tissue specific transforming properties of oncogenes is further highlighted by the fact that these studies predicted a role for c-myc in mammary tumorigenesis prior to its detection in human breast cancer [5].

While most of the MMTV/c-myc strains expressed the transgene in the mammary epithelium one particular line expressed the transgene in a broad range of tissues including the salivary glands, testis, lung, brain and male reproductive tract [25]. As a consequence these transgenic mice developed testicular and lymphocytic tumors. The B and T cell tumors that arose in this strain were particularly instructive because it was possible to directly assess their clonality by inspecting the structures of the immunoglobulin and T cell receptor chains. Consistent with the apparent clonal origin of mammary adenocarcinomas, these lymphoid tumors possessed uniquely rearranged immunoglobulin and T cell receptor chains indicating that they had arisen from a clonal antecedent. The clonal nature of these B and T cell tumors again demonstrated that further genetic events were required for progressing cells towards full malignancy. In contrast to the mammary and lymphoid tissue, expression of c-myc transgene in the salivary glands had no apparent phenotypic consequence. The inability of c-myc to perturb salivary epithelial cell proliferation reflects the redundancy of growth control mechanisms that exist *in vivo* and further stresses the importance of the tissue context in tumor progression.

In another set of experiments, the c-myc protooncogene was placed under the transcriptional control of the Wap promoter/enhancer and several transgenic strains were derived [21]. Consistent with the behaviour of the endogenous Wap promoter transgene expression was strictly regulated by the presence of lactogenic hormones. Consequently, mice bearing the Wap/c-myc transgene had to go through at least two lactational periods before tumors arose in these strains. Like the MMTV/c-myc mice, the Wap/c-myc mice developed solitary tumors that appeared in a stochastic fashion. Furthermore, these tumors arose adjacent to normal epithelium which also expressed the transgene. While the expression of the Wap/c-myc transgene in the morphologically normal epithelium was dependant on lactogenic hormones, tumors which arose in these strains continue to grow and express the transgene in the absence of hormone stimulation. In a similar manner the expression of a number of milk proteins appear to be coordinately rendered hormone independent within these tumors. Taken together, these observations argue that initiation of these hormone independent tumors requires enhanced c-myc expression in combination with secondary mutation(s).

The behaviour of the c-myc oncogene early in mammary differentiation (MMTV/c-myc) and later in epithelial differentiation (Wap/c-myc) is remarkably similar. Despite the fact that c-myc is uniformly expressed throughout the mammary epithelium the tumors which develop in both transgenic strains appeared clonal in origin, thus suggesting the involvement of secondary genetic

events. Conceivably, these alterations could involve the activation of additional proto-oncogenes or loss of supressor gene function which in collaboration with the action of the activated c-myc participate in cellular transformation.

One clue as to the possible nature of one of these complimenting events came from a set of earlier chemical mutagenesis experiments. In these experiments activation of the Ha-ras-1 oncogene was found to be a common early event in the initiation of rat mammary carcinomas [27–28]. These results suggested that activation of ras may be an important step in mammary carcinogenesis. To directly assess the oncogenic potential of activated ras in the mammary epithelium transgenic mice bearing either a Wap/ras [20, 22], MMTV v-Ha-ras [26, 29] and MMTV/N-ras [30] transgenes were established. In most of the derived MMTV/v-Ha-ras and MMTV/N-ras transgenic strains the tissue specificity of expression followed a pattern observed in the c-myc mice including the mammary epithelium, the salivary glands, the spleen and the thymus as well as the Harderian lachrymal gland. Transgenic animals expressing a MMTV/activated ras oncogene in the mammary epithelium developed mammary adenocarcinomas beginning as early as one month age. By 168 days 50% of female transgenic mice had developed mammary tumors [26]. Although these MMTV/v-Ha-ras transgenics exhibited accelerated tumor kinetics relative to the MMTV/c-myc mice, like the myc transgenic animals these tumors appeared to be clonal in origin and arose adjacent to morphologically normal epithelium which also expressed the transgene. Again these observations suggested that in addition to activated ras expression other secondary events were required for full malignant transformation. Surprisingly in contrast to the MMTV/c-myc mice, male MMTV/v-Ha-ras also developed mammary adenocarcinomas albeit with longer latency [26, 29]. It is conceivable that the appearance of mammary tumors in male transgenic mice could be due to early onset of oncogene expression in male mammary epithelium prior to its regression. If activated ras expression interfered with mammary epithelial regression, these epithelial cells could then be susceptible to the oncogenic action of v-Ha-ras.

In addition to mammary tumors, the MMTV/v-Ha-ras mice exhibited several other growth disturbances. Interestingly, a proportion of the MMTV/v-Ha-ras transgenic animals developed an uniform, bilateral enlargement of the Harderian gland [26, 29–30]. Histological examination of these growths revealed extensive epithelial hyperplasia involving the entire gland. Consistent with this diagnosis these growths could not be propagated in nu/nu mice or syngeneic recipients. Besides harderian gland hyperplasia, tumors involving the lymphoid, salivary glands, and lung were also observed [26, 29–30]. On the basis of these observations it was concluded that v-Ha-ras while capable of eliciting a non-neoplastic proliferative effect in the Harderian gland required the collaboration of other events to initiate further malignant transformation. By contrast to the results obtained with the MMTV/activated ras transgenic strains Wap/activated ras transgenics exhibited a low incidence of tumor formation [20, 22]. However, the Wap/ras mice did demonstrate impaired mammary gland function as evidenced by their reduced capacity to express both β casein and whey acidic protein mRNAs [22]. The differential tumorigenic potential exhibited by the MMTV/ras and Wap/ras transgenic strains illustrates an important point regarding the ability of an oncogene to transform a given cell type. Because the MMTV/ras gene is expressed early in mammary differentiation its expression may be targeted to a susceptible stem cell population that has undergone terminal differentiation in the Wap/ras transgenic mice. Conceivably the differentiation state of the mammary epithelium could account for the different oncogenic properties the activated ras transgene in these mice. Indeed, the transforming potential of the int-2 gene in the mammary epithelium [31] or the c-myc gene in the lymphoid compartment [32] appears to be dependant on the differentiated state of the cell.

Because full malignant transformation of primary cells *in vitro* require the complementary action of two oncogenes [1, 2] it was important to determine whether coexpression of activated ras and myc was sufficient to transform the entire mammary epithelium *in vivo*. By crossing separate strains MMTV/v-Ha-ras and MMTV/c-myc [26] or

Wap/v-Ha-ras and Wap/c-myc [22] it is possible to derive transgenic mice that coexpress both transgenes in the mammary epithelium. Despite the different promoters driving coexpression of myc and ras, the outcome of these experiments were remarkably similar. Although the type of tumors that arose in the dual carriers did not differ from their single oncogene siblings, the dual carriers developed tumors with dramatically accelerated kinetics. However, both the stochastic and clonal nature of these tumors suggested that further genetic events were required. Thus, in contrast to the primary tissue culture system, the nature of events required for tumor formation *in vivo* are likely more complex.

Growth factors, their receptors and mammary tumorigenesis

While transgenic mice have provided important insight in the tissue specific transforming properties of oncogenes another useful system to study the role of oncogenes in mammary tumor progression are the retroviruses. Retroviruses such as mouse mammary tumor virus (MMTV) do not carry dominantly acting oncogenes but rather induce tumor formation by integrating next to and transcriptionally activating sets cellular genes termed Wnt-1 (also known as Wnt-1, [33]), int-2, int-3 and int-4 [34]. In the case of the int-1 and int-2 activation, insertion of the retrovirus occurs outside the coding sequences, usually upstream of the gene in the opposite transcriptional orientation or downstream in the same orientation suggesting the requirement for viral transcriptional enhancer function [35–38].

Despite its frequent activation in mammary tumors Wnt-1 is not normally expressed in the mammary gland [39–41]. Rather, its expression appears to be restricted to the neural tube of midgestation embryos and the testis of mature males. Although experiments have suggested that Wnt-1 can act as epithelial growth factor in cell culture [42] there was little direct evidence supporting its role in tumor formation *in vivo*. Consequently, transgenic mice bearing a MMTV/Wnt-1 fusion gene were established [43]. While these transgenic mice showed a similar pattern of tissue specific expression as exhibited by other MMTV/oncogene strains unusually high levels of transcripts were detected in the mammary fat pads of both male and virgin female. Histological examination of mammary epithelium derived from male and female transgenic animals revealed the presence of extensive epithelial hyperplasia involving the entire mammary gland. The global hyperplasia observed in the Wnt-1 females also had profound effect on mammary gland function in that female carriers were unable to nurse their young. At approximately 3 months of age, solitary mammary adenocarcinomas began to arise from this hyperplastic tissue in both male and female animals. By contrast to the global hyperplasia observed in the mammary epithelium, the tumors appeared to be clonal in origin. Two important findings have emerged from these observations. First, like many of the other MMTV/oncogene strains expression of Wnt-1 is not sufficient for full transformation of the epithelium tissue. And second, that Wnt-1 expression can interfere with the normal mammary gland development and regression.

Perhaps one of the most intriguing aspects of these studies relates to the fact that the Wnt-1 gene while capable of causing extensive epithelial proliferation is not normally expressed in the mammary gland. The dramatic effects of Wnt-1 on mammary gland development raises the interesting possibility that a related member of the Wnt-1 family is normally involved in mammary differentiation and proliferation. One explanation that may account for these observations is that Wnt-1 and its putative homologues are capable of signalling cellular proliferation via a common receptor on the mammary epithelial cell. Consistent with this hypothesis several Wnt-1 related genes have recently been isolated and have been shown to be expressed during specific stages of mammary gland development [44]. Given the potent transforming properties of the Wnt-1 product, these related family members should be viewed as potential mammary oncogenes.

Like the Wnt-1, int-2 transcripts are not normally expressed in the adult animal, but rather appear

to be expressed in the developing embryo at specific sites and times [39, 45–46]. The int-2 gene has recently been shown to be a member of the basic fibroblast growth factor gene family sharing 35 to 55% amino acid homology to other family members [47–51]. To establish a direct role for int-2 in mammary tumorigenesis, transgenic strains bearing two different MMTV/int-2 recombinants were derived [31, 52]. In one of these transgenic stains sequences within the MMTV LTR portion of the transgene required for expression in virgin epithelium are deleted (truncated LTR) [31, 53] while the other strains expressed the transgene in virgin tissue [52]. Although high levels of transgene specific RNA can be detected in lactating mammary epithelium derived from both strains transgenic mice harbouring the truncated MMTV LTR/int-2 fusion gene do not express detectable quantities of the transgene product in virgin epithelium. These latter mice appear to exhibit normal mammary gland function. However, histological examination of the mammary epithelium from these mice revealed an extensive well differentiated hyperplasia involving the entire mammary epithelium. Despite the high levels of transgene transcript detected in these truncated LTR strains, the appearance of mammary tumors was rare and occurred only after long latency (up to 18 months). The low tumorigenic potential exhibited by these MMTV/int-2 transgenic strains is surprising in view of the close association between insertional activation of this locus and mammary tumorigenesis. This paradox might be explained in the following manner. For example, the differentiated state of the mammary epithelial cell may play an important role in the oncogenic potential of int-2. If int-2 is not expressed in the appropriate cell type its tumorigenic potential may be altered. Indeed, consistent with this hypothesis int-2 is not expressed in these strains until the mammary epithelium is induced to differentiate. Thus like Wnt-1, the primary consequence of int-2 expression in the mammary gland is to interfere with normal mammary gland development.

In contrast to the transgenic strains bearing the truncated LTR/int-2 construct other strains expressing the transgene in virgin epithelium [52] exhibited a profound lactation defect due to extensive tissue hyperplasia. While experience with these MMTV/int-2 strains is limited it might be predicted that the early onset of int-2 expression (expression in virgin epithelium) will result in a higher tumor incidence in this strain. Future studies with these mice should provide important insight into relationship between differentiation and tumorigenesis.

Another growth factor that has been implicated in the regulation of mammary epithelial proliferation and differentiation is TGF α. Elevated expression of TGF α has been observed in rat mammary tumors [54] as well as human breast cancer [55]. To establish a direct role for TGF α in mammary tumorigenesis, a cDNA encoding TGF α was placed under the transcriptional control of either the MMTV [56] or metallothinein (MT) promoter [57–58]. Global expression of the TGF α transcripts throughout the mammary gland in females in one strain of MMTV/TGF α mice resulted in a lactation defect due to extensive aveolar hyperplasia. As observed with the MMTV/Wnt-1 mice clonal mammary adenocarcinomas began to appear around 3 months of age. However, in contrast to the MMTV/Wnt-1 transgenic mice male transgene carriers derived from this strain exhibited no apparent growth disturbance.

Due to the wide spectrum of tissues in which the mettalothionein promoter is active the other MT/TGF α strains exhibited other growth disturbances including heptacellular carcinomas and pancreatic metaplasia [57–58]. However, despite the low levels of mammary gland specific expression of TGF α observed in these mice, mammary adenocarcinomas were also detected. In addition to frank malignancies other developmental abnormalities in mammary gland morphogenesis were observed. In particular, one strain of MT/TGF α mice the mammary glands exhibited impeded morphogenetic penetration of the epithelial cells into the stromal fat pad [57]. Taken together, these sets of experiments argue that overexpression of TGF α while not sufficient for mammary carcinogenesis predisposes the epithelium to secondary events that are required for full malignant transformation.

The observation that overexpression of growth factors in the mammary epithelium can result in

malignant transformation argues that deregulation of their cognate receptors might also achieve the same end. Indeed, amplification and overexpression of the human c-erbB-2/c-neu tyrosine kinase is frequently observed in human primary breast cancers [10–11]. While overexpression of the c-neu protein appears to be the major mechanism of oncogenic activation in these human tumors c-neu can also be converted into an oncogene by a single mutation in the transmembrane domain [59]. Given the close correlation between expression of the human homologue of c-neu and malignant development of mammary tumors, it was important to establish whether expression of this oncogene was directly involved tumorigenesis. To accomplish this both the activated rat c-neu [60–61] and unactivated human c-erbB-2/c-neu [62] have been placed under the transcriptional control of the MMTV promoter/enhancer and introduced into the germline of mice. In several strains of MMTV/activated c-neu transgenic mice early onset of transgene expression was initially associated with a lactation defect followed by the synchronous appearance of tumors involving all mammary glands in every transgenic mouse examined, male and female [60]. Histological examination of these tumors and surrounding tissues revealed the complete absence of any morphologically normal mammary epithelium. Both the simultaneous occurrence and polyclonal nature of these tumors argued that in contrast to most transgenic tumor models, expression of activated c-neu alone was sufficient for malignant transformation of the mammary epithelium. However, because the clonality of mammary tumors cannot easily be assessed, it is also possible that rapid tumor progression is responsible for the observed phenotype. Interestingly, expression of the activated c-neu transgene in the parotid gland or epididymis led to a benign, bilateral hyperplasia without progressing to full malignancy. These results suggested again that the tissue context in which oncogene expression occurs is a major determinant in malignant progression.

By contrast to these results expression of activated c-neu in other strains of transgenic mice resulted in the stochastic appearance of mammary tumors [60–61]. The stochastic appearance of tumors in at least one of these lines (NK; [60]) can be attributed to nonuniform expression of the c-neu transgene as established by *in situ* hybridization. The differences in phenotype exhibited by the various transgenic strains may reflect differences in the level expression of the activated c-neu tyrosine kinase. For example, it is conceivable that transformation of the primary mammary epithelium requires a critical threshold of tyrosine kinase activity. Perhaps in the stochastic MMTV/c-neu strains the level of c-neu tyrosine kinase activity is below this threshold and only in the tumors which arise is this threshold exceeded. Indeed, consistent with this view, MMTV/ret transgenic animals expressed relatively high levels of ret specific tyrosine kinase activity in the mammary tumors by comparison to adjacent normal epithelium [63].

Although point-mutated activated c-neu appears to a potent oncogene in the mammary epithelium, there is no evidence of its occurrence in human breast cancer [64]. Rather it appears that overexpression of the unaltered c-neu/c-erbB-2 product is the primary mechanism by which this oncogene contributes to malignancy. To test the oncogenic potential of unaltered human c-neu/c-erbB-2 in the mammary epithelium several transgenic strains of MMTV/c-erbB-2 were established [62]. While several adenocarcinomas of the lung and Harderian gland were noted no obvious growth disturbance in the mammary epithelium was described. Because no attempt was made to assess the levels of c-erbB-2 transcript in the mammary epithelium it unclear whether this absence of phenotype is a consequence of low levels of transgene expression or other factors. For example, it is conceivable that the human c-erbB-2 growth factor receptor cannot bind the rodent ligand and thus is unable to signal cell proliferation. Future studies with transgenic mice bearing MMTV/mouse c-neu or MMTV/rat c-neu fusion genes should allow this issue to be addressed.

Whatever the explanation for the different phenotypes exhibited by the various MMTV/activated c-neu strains it is clear that under certain circumstances transformation of the primary epithelial cell by activated neu requires few if any additional genetic events. Given the close correlation be-

tween overexpression of c-neu in human breast cancer and the results of these transgenic mouse experiments the-neu/c-erbB-2 gene should be viewed as a major target for diagnosis and therapy.

Transgenic models for the action of tumor supressor genes

In addition to the activation of dominantly acting oncogenes there is increasing evidence to suggest that malignant progression involves the inactivation of growth supressor genes. In breast cancer there is compelling evidence to suggest that loss of function of the retinoblastoma and p 53 tumor supressor genes are pivotal events in tumor progression [65–68]. Interestingly, the products of the p 53 and retinoblastoma (Rb) genes have recently been shown to specifically associated with SV40 large T antigen and the adenovirus E1A and E1B proteins [69–70]. Because the regions within these viral T antigens responsible for association with these growth supressor genes are required for viral mediated transformation, it has been proposed that these viral proteins antagonize the function of these tumor supressor products and thus promote cell proliferation. To elucidate the role of p 53 and Rb in breast cancer several groups have taken advantage of the ability of these viral proteins to associate and interfere with the function of both p 53 and retinoblastoma proteins and have derived both MMTV/SV40 Large T and MMTV/E1(E1A + E1B) transgenic strains [71–72]. Although, high levels of SV40 large T antigen were detected in the mammary epithelium of these mice, the major growth disturbances occurred in the kidney, lung and lymphoid tissues. By contrast the mammary epithelium was relatively resistant to the oncogenic effects of SV40 large T antigen developing focal adenocarcinomas only after long latency. Consistent with these results the MMTV/E1 mice failed to exhibit any mammary epithelial growth disturbance. Taken together these observations suggest that inactivation of growth supressors such as p 53 and Rb are not sufficient for mammary tumorigenesis but require the concerted action of other genetic events.

Conclusions

The analyses of transgenic strains expressing activated oncogenes in the mammary epithelium can potentially teach us much about the multi-step nature of breast cancer. With the exception of the activated c-neu oncogene, deregulated expression of one or even two oncogenes are not sufficient for malignant transformation. Thus one of the major challenges that remains is the identification of these cooperating genetic alterations. It is evident by crossing different oncogene bearing transgenic strains one can potentially recapitulate the steps necessary achieve full malignant transformation. By introducing the appropriate combination of activated oncogenes and inactivated supressor genes into the germline of mice the role and relevance of these genes in mammary carcinogenesis can be better understood.

Other approaches in identification of these secondary events have recently been developed. For example, one strategy that has been employed takes advantage of the fact that certain retroviruses such as Moloney Murine Leukaemia virus (MoMuLV) cause cancer by insertional activation of proto-oncogenes. If one infects transgenic strains bearing an activated oncogene such as pim-1 with MoMuLV there is dramatic acceleration of tumor formation due to proviral activation of c-myc or N-myc [73]. Indeed consistent with the prediction that myc and pim-1 were cooperating crosses between myc and pim-1 transgenics results in the embryonic appearance of tumor [74]. Conceivably, a similar approach could be utilized to identify collaborating genes in the MMTV and Wap/oncogene strains.

The study of these oncogene bearing transgenic strains has also provided important insight in the tissue specific transforming potential of certain activated oncogenes. While genes such as c-myc and SV40 large T antigen clearly require collaborating events expression of the activated c-neu apparently require few if any additional genetic events to induce malignant transformation. Thus the transgenic mouse can be used as useful *in vivo* test of the tissue specific transforming potential of a particular oncogene.

Although much has been learned from the transgenic mouse concerning the role of oncogenes in breast cancer much remains to be accomplished. For example, transgenic models dealing with metastasis still remain to developed. Clearly, the future possibility exists that these transgenic mice might be employed as useful models to assess the efficacy of drugs that might interfere with malignant progression or as sensitive tests for carcinogens. Ultimately, future studies with these and other strains of transgenic mice will provide important insight into the molecular basis of breast cancer.

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