Breast Cancer Research and Treatment **39**: 33-44, 1996.

MMTV-induced mutations in mouse mammary tumors: Their potential relevance to human breast cancer

Robert Callahan
Oncogenetics Section, Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20892, USA

Key words: mouse model system, mutations, MMTV

Summary

In mouse mammary tumor virus (MMTV) infected mice, three identifiable stages of mammary tumorigenesis can be biologically defined: preneoplastic hyperplastic nodules, malignant tumor, and distant metastatic lesions (primarily in the lung). MMTV is a biological carcinogen which induces somatic mutations as consequence of its integration into the host cellular genome. Each stage of mammary tumorigenesis appears to result from the clonal outgrowth of cells containing additional integrated proviral MMTV genomes. This phenomenon has provided the basis for an approach to identify genes which, when affected, may contribute to progression through the different stages of mammary tumorigenesis. Eight different genes (*Wnt1*, *Wnt3*, *Wnt10b*, *Fgf3*, *Fgf4*, *Fgf8*, *Int3*, and *Int6*) have been shown to be genetically altered in multiple mammary tumors as a consequence of MMTV integration. Although the significance of the human homologs of these genes as targets for somatic mutation during human breast carcinogenesis is only now being explored, it is clear that this work has led to a new appreciation of the complexity of the genetic circuitry that is involved in the control of normal mammary gland growth and development. It seems likely that some of the mutations induced by MMTV, and the signaling pathways in which these target genes take part, will be relevant to the progression from preneoplastic lesions to distant metastasis in human breast cancer.

Introduction

The etiology of breast cancer is influenced by a variety of factors such as menstrual and reproductive history, family history, long term treatment with estrogens, diet, and previous atypical benign breast disease [1-3]. It seems probable that these factors could provide a selective environment for the clonal outgrowth of mammary epithelial cells which contain somatic mutations. One could imagine that some of these mutations could uncouple normal mammary gland development or contribute to the development of neoplasia. Alternatively, they could provide atypical non-malignant as well as malignant cells with a selective growth advantage, the means to metastasize to distant organ sites, or evasion of host immunosurveillance. Because of the hetero-

Address for correspondence and offprints: Dr. Robert Callahan, National Cancer Institute, Building 10, Room 5B50, Bethesda, MD 20892, USA; *Tel:* 301-496-2367; *Fax:* 301-402-0711

Table 1. Common insertion sites (*Int* genes) for MMTV

Int gene	Normal expression in adult tissues[a]	Chromosome[b]	
		Mouse	Human
Int1/Wnt1	Testis	15	12q13
Int2/Fgf3	None	7	11q13
Int3	All	17	6p21.3
Hst/Fgf4	None	7	11q13
Wnt3	Thalamus, brain, hair root, skin	11	17q21-22
Int6	All	15	8q22
Wnt10b	Virgin but not pregnant mammary gland	15	12q13
Fgf8	Ovary and testis	19	10q*

a. [33,36,43,50,68,70,71]
b. [50,80-82], and for human *Int6* (Miyazaki et al, manuscript in preparation)
* Predicted location

geneity and multiplicity of factors necessary for the development of most epithelial tumors, the current view is that multiple somatic mutations act in concert to produce an invasive carcinoma with the ability to metastasize to distant organ sites.

A preview of the complexity of somatic alterations which occur during human breast tumor progression was provided by earlier cytogenetic analysis of primary human breast tumor cells in culture [4]. The alterations include aneuploidy (either gain or loss of entire chromosomes) and rearrangements affecting chromosomes 1q (translocations), 6q (deletions and translocations), 7p (translocations, pericentric inversions, and isochromosomes), and 11q (translocations). More recently, in a study using comparative genome hybridization methodology to map regions of the genome with increased DNA sequence copy number (amplification), 26 chromosomal subregions were found to be affected in primary human breast tumors and breast tumor cell lines [5]. Although genetic analysis of primary breast tumor DNAs has demonstrated amplification of known proto-oncogenes such as FLG (8p12), MYC (8q24), BEK (10q24), 11q13 (*Fgf3*/PRAD1/Cyclin D), 15q24-q25 (IGFR-1/FES), and ERBB2 (17q12), it seems probable that amplification of other, as yet unknown, genes contributes to malignant progression in breast cancer (reviewed in [6]).

Loss of heterozygosity (LOH) represents another common genetic alteration in primary human breast tumors and occurs as a consequence of either interstitial deletions, chromosome loss, or aberrant mitotic recombinational events. It is thought that LOH reveals within the affected region of the genome the presence of a recessive mutation in the remaining allele of a "tumor suppressor" gene (reviewed by [7,8]). Tumor suppressor genes are believed to be involved in the normal suppression of cellular proliferation during development [8]. Commonly one normal allele is lost as a result of LOH, while the other allele contains either a small deletion or a point mutation which inactivates the gene product. At the present time, 11 chromosome arms have been found to be frequently affected by LOH in breast tumors, including chromosomes 1p [9], 1q [10], 3p [11], 6q [12,13], 7q [14], 11p [15,16], 8q (our unpublished data), 13q [17], 16q [18], 17p [19], 17q, and 18q [20]. Moreover, it is not uncommon for multiple regions within these chromosome arms to be independently affected by LOH.

A major thrust of research in this area is the identification of the target genes affected by LOH or DNA amplification. However, the apparent complexity of genetic alterations which are found in primary human breast tumors and the possibil-

ity that some them are a consequence of tumor progression rather than a cause make this a daunting challenge. Therefore several different types of approaches are being applied to this problem, including surveys of directional cDNA libraries [21,22] and positional cloning [23]. Another approach is based on the mouse model system in which the mouse mammary tumor virus (MMTV) is the biological carcinogen that induces tumor development as a consequence of insertional mutagenesis. In this article I will summarize the current status of this latter approach and its implications for human breast cancer research.

Background: The model system

Many of the classical inbred strains of *Mus musculus* were intentionally derived from stocks of mice that had a high incidence of mammary tumors (C3H, GR, BR6, and RIII mouse strains) and have been inbred for the past 50 to 60 years for a high incidence of mammary tumors (reviewed in [24,25]). Mice of each strain congenitally transmit highly infectious MMTV through the milk to their offspring. C3H and GR mice also contain genetically transmitted proviral genomes (*Mtv1* and *Mtv2*, respectively) which encode infectious viruses that are also expressed in the milk. Parous C3H females develop pregnancy-independent mammary tumors. In addition, MMTV(C3H)-infected females frequently develop mammary preneoplastic hyperplastic alveolar nodules (HAN), whereas uninfected females infrequently develop HANs [26-28]. When HANs are passaged in syngeneic mice, mammary tumors frequently develop within them in a stochastic manner. The GR, BR, and RIII females have a high incidence of pregnancy-dependent mammary tumors, or plaques, which after one or more parities develop pregnancy-independent mammary tumors. In addition, it is not uncommon to find metastatic lesions in the lungs of MMTV-infected mammary tumor bearing mice. This has provided an experimental approach to dissect, at the genetic level, the mutational events that contribute to each stage of tumor development.

The target genes in high incidence inbred mouse strains

MMTV is a nonacute transforming retrovirus. An obligate step in the replication of the MMTV virus is the integration of a DNA copy of its proviral genome into the host cellular genome, an event which is potentially mutagenic for the host cell. In this regard MMTV has been shown to act as a insertional mutagen that causes the deregulation of expression of adjacent cellular genes (designated *Int* loci) in mammary tumors (Figure 1) [29]. To identify affected cellular genes the viral genome has been used as a molecular tag. Using this approach six *Int* loci (*Int1/Wnt1, Int2/Fgf3, Wnt3, Hst/k-Fgf/Fgf4, Wnt10b,* and *Fgf8*) have been identified in mammary tumors of high incidence inbred mouse strains or MMTV infected transgenic mouse strains (Table 1) [30-34]. The mechanism by which MMTV activates the expression of the *Int* genes mentioned above, is primarily a consequence of the effect of enhancer sequences within the long terminal repeat (LTR) of the integrated MMTV proviral genome on the transcriptional promoter of the adjacent affected gene (Figure 1).

The *Wnt* genes are members of a family of 12 or more genes related to the *Drosophila* segmented polarity gene *wingless* (*wg*) (reviewed in [35,36]). The *Wnt1* and *Wnt3* genes are not normally expressed in the mammary gland, but are expressed at specific sites and times during embryonic development or in other adult tissues. However, several of the other *Wnt* gene family members are expressed at defined times during mammary gland differentiation and development. *Wnt1* encodes cysteine-rich secreted glycoproteins of 41-44 kDa which are associated with extracellular matrix and cell surfaces. Based on studies of the *Drosophila* mutant *wg*, (the homolog of *Wnt1*), there is evidence that one function of *Wnt1* is to increase Ca^2-dependent cell

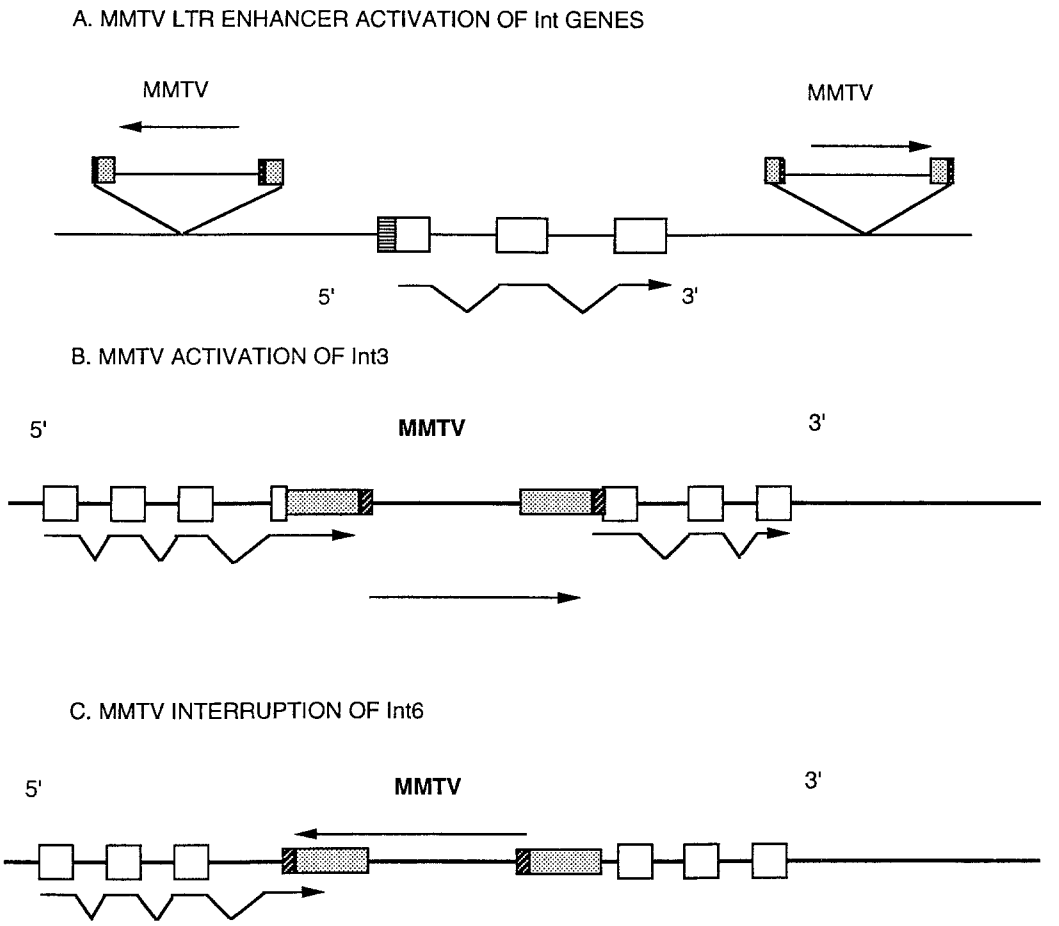

Figure 1. Modes of activation of expression of host genes by MMTV. The stippled and the diagonally hatched boxes represent the U3 and U5 portions of the MMTV LTR. The open boxes correspond to exons of the target gene. The box with horizontal hatches corresponds to the promoter of the target gene. The transcriptional orientation of the MMTV proviral genome and the target gene is indicated.

adhesion through a cellular signal transduction pathway that regulates the β-catenin intracytoplasmic (or intracellular) pool size and stabilizes its binding to cadherin (reviewed in [35,37]).

The *Fgf3*, *Fgf4*, and *Fgf8* genes are members of the fibroblast growth factor (*Fgf*) gene family [38-40]. Like *Wnt1* and *Wnt3* genes, they too are only expressed during early embryonic development or adult tissues other the mammary gland [41,42]. Members of the *Fgf* family vary in length, but are homologous to one another within a core of 120 amino acid residues. *Fgf*s exhibit an overlapping, but not identical, range of biological activities that can act as mitogens, chemoattractants, and mediators of cellular differentiation. *Fgf*s are also potent angiogenic factors *in vivo* (reviewed in [43,44]).

The biological consequences of *Wnt1* and *Fgf3* expression on mammary gland development and tumorigenesis have been evaluated in transgenic mouse strains containing either MMTV LTR activated *Wnt1* or *Fgf3* transgenes [45-47]. Mammary glands of *Wnt1* transgenic virgin females resemble the hormonally stimulated glands normally observed in pregnant animals except that there are increased numbers of terminal branches

Table 2. Frequency of MMTV insertion into *Int* loci[a]

Mouse strain	Number of tumors	Frequency of insertions (%)				
		Int1/Wnt1	*Int2/Fgf3*	*Wnt1+Fgf3*	*hst/FGF4*	*Int3*
C3H/OuJ	28	57	3.5	18	0	0
BALB/cfC3H	30	23	23	7	3	0
RIII	29	14	31	27.5	0	0
BALB/cfRIII	40	15	7.5	17.5	0	0
CZECHII	45	24	2	2	0	18
CZECHIIfC3H	30	30	13	0	0	3

a. [25] and our unpublished data.

and alveoli producing a diffuse lobular-alveolar hyperplasia. Focal mammary tumors arise from within these hyperplasias that are indistinguishable from the MMTV-induced disease. This is consistent with the pregnancy-independent nature mammary tumorigenesis in C3H mice.

In contrast, mammary glands of virgin *Fgf3* transgenic females appeared normal with only microscopic areas of ductal hyperplasia composed of focal aggregates of cells. Mammary hyperplasia was most evident during pregnancy. Three patterns of proliferation were observed: ductal hyperplasias, papillocystic forms, and nodular solid aggregates of cells. After parturition the hyperplastic areas either regressed or remained static, becoming more pronounced in subsequent pregnancies. In this regard the lesions resemble the pregnancy-dependent lesions of the BR6 mice. Focal mammary tumors arose in a fraction of the mice at a late age. The most common tumors were mixtures of ductal hyperplasias composed of irregular, anastomosing, bilayered tubules that exhibited no signs of lactational activity. The hormone dependence of these tumors has been not established.

Since mammary tumors arise in the *Wnt1* and *Fgf3* transgenic mice in a stochastic manner, it seems that additional mutations or epigenetic events must be required for progression to malignancy. Some MMTV-infected mice develop tumors in which both *Wnt1* and *Fgf3* are activated by the viral genome, raising the possibility that these two activated genes collaborate in mammary tumorigenesis (Table 2). Two different lines of evidence provide support for this conclusion. First, the *Wnt1/Fgf3* bitransgenic mouse strain develops pregnancy-independent mammary tumors earlier and at a higher frequency than in either parental line [48]. Second, 45% of the mammary tumors (36 out of 80) arising in MMTV(C3H)-infected *Wnt1* transgenic mice contained a viral insertion at either *Fgf3* or *Fgf4* [49]. Moreover, another 10% of the tumors (8 out of 80) contained a virus integration at *Fgf8* [33]. Similarly, 8 of 35 (23%) mammary tumors from MMTV-(C3H)-infected *Fgf3* transgenic mice contained a viral insertion at *Wnt1* [50]. Another 2 tumors from this cohort contained a viral insertion at a new related gene, *Wnt10b*, although in these latter two cases, activation of *Wnt10b* expression as a consequence of viral integration was not demonstrated. Taken together, however, these results strongly imply that certain members of the *Wnt* and *Fgf* gene families collaborate in the deregulation of normal control of growth and differentiation toward malignant mammary tumorigenesis when activated by MMTV. The common theme in these studies is that the target genes for MMTV activation are normally not expressed or are expressed only at low levels in mammary epithelium.

Evidence that members of these two gene families do interact in the normal regulation of development in other tissues is beginning to emerge. For instance, in *Wnt7a*$^{-/-}$ null mice Parr and McMahon [51] found that the forelimbs lacked normal dorsal structures and that *Fgf4* expression was also reduced. In other studies

[52], loss of Sonic hedgehog (*Shh*) expression could only be rescued when both the *Fgf4* and *Wnt7a* proteins were present. In studies of Xenopus mesoderm induction, *XWnt8* has been shown to be a "competence modifier" that alters the response of blastula animal caps to *Fgf2* [53]. This suggests that activation of *Fgf3*, *Fgf4*, *Fgf8*, *Wnt1*, *Wnt3*, and possibly *Wnt10b* interferes with or overrides normal controls of mammary gland growth and development imposed by other members of these gene families which are normally expressed in the mammary gland. A second conclusion that can be reached is that activation of members of the *Wnt/Fgf* gene families alone is not sufficient to induce malignancy in the mammary gland and points to the necessity for additional mutations or epigenetic events.

The frequency with which *Wnt* and *Fgf* genes are activated by MMTV in mammary tumors seems to be dependent on the host inbred mouse strain and the strain of virus (Table 2) [25,54]. For instance, in the low incidence BALB/c mouse strain MMTV(C3H) induces pregnancy-independent tumors as in the donor strain. Similarly, the MMTV(RIII) virus induces pregnancy-dependent tumors in BALB/c mice. However, the frequency of mammary tumors in which *Wnt1*, *Fgf3*, and *Fgf4* are activated by MMTV integration in these strains is significantly different from the frequency found in tumors of the parental strains (Table 2). This suggests that during inbreeding of the high incidence mouse strains, mutations were fixed in the germline that either provide a selective growth advantage to mammary epithelial cells having activated *Wnt/Fgf* genes or broaden the host range within the mammary epithelium for MMTV infection and replication. In this regard, it seems relevant that the *Wnt1* transgenic mouse strain develops hyperplasias of mammary epithelium prior to tumorigenesis. However, surveys of C3H HANs have shown that *Wnt1* is only infrequently rearranged by MMTV [55]. This suggests that in the context of the C3H genetic background, activation of *Wnt1* plus possibly an *Fgf* gene is sufficient to induce a malignancy in the mammary gland. In this scenario the additional mutation(s) required for malignancy are probably already present in the germline.

Mammary tumorigenesis in feral mice

MMTV has been detected in feral strains of mice (reviewed in [56]). Although mammary tumorigenesis does not play an important role in the zoological history of feral mice in their natural habitat, when brought into a laboratory setting they do develop mammary tumors [56-58]. Analysis of these mice which have not been bred for a high incidence of mammary tumors has led to four significant observations which are relevant to the mouse as a model system to detect mutations that contribute to breast cancer. (1) Feral mice are generally hemizygous for genetically transmitted (endogenous) MMTV genomes. This means that due to random assortment at each generation some fraction of the offspring lack endogenous MMTV genomes. From a practical point of view mice devoid of endogenous MMTV genomes make it possible to unambiguously detect acquired MMTV genomes in mammary tumors. (2) Even in this setting, *Wnt1* and *Fgf3* are activated by MMTV insertion at a frequency comparable to MMTV-infected low incidence mouse strains (Table 2). (3) Like the high incidence inbred mouse strains, stages in mammary tumorigenesis can be biologically separated — premalignant mammary hyperplastic nodules can be transplanted serially in related mice to produce hyperplastic mammary gland within which malignant tumors and distant metastases develop [25]. (4) New target genes for MMTV have been identified which appear to contribute to different stages of progression in mammary tumorigenesis and which are unrelated to members of the *Wnt* or *Fgf* gene families [25,56].

A feral mouse model system for MMTV induced mammary tumorigenesis

A colony, designated CZECH II, was derived from a single breeding pair of *M. musculus musculus* trapped in Czechoslovakia [57]. The CZECH II mice lack endogenous MMTV genomes but do contain an infectious strain of MMTV that is transmitted congenitally through the milk [57,59]. This colony has a 20% incidence of pregnancy-independent mammary adenocarcinomas that are histopathologically similar to those induced by MMTV (C3H). The frequency with which *Wnt1* was activated by MMTV was similar (24%) to that observed in BALB/cfC3H (30%) or CZECHIIfC3H (30%) mammary tumors, whereas MMTV-induced activation of *Fgf3* and *Fgf4* was significantly less frequent (Table 2). Like high incidence inbred mouse strains, CZECHII mice also develop mammary preneoplastic HANs which we have developed into mammary hyperplastic outgrowth lines (HOGs). A survey of DNA from 31 CZECHII HOGs revealed that 22.6 % (7 out of 31) had MMTV-induced rearrangements of *Wnt1*, while none had rearrangements of either *Fgf3* or *Fgf4* (E. Kordon and G.H. Smith, personal communication). Since the frequency of MMTV-induced rearrangements of *Wnt1* is similar in both CZECHII HOGs and mammary tumors, it seems likely that in the setting of this mouse strain, activation of *Wnt1* is primarily an early event in tumorigenesis which disrupts regulatory controls of normal mammary gland development leading to lobular hyperplasia. Mammary tumors arising from within these HOGs frequently contain additional MMTV proviral genomes. Based on the results obtained with the MMTV-infected *Wnt1* transgenic mice, it seems probable that members of the *Fgf* gene family will be found to be activated by MMTV in tumors derived from *Wnt1* positive HOGs. Moreover, relative to the results of similar studies of C3H HOGs where rearrangements of *Wnt1* rarely occur [55], the observations made in CZECHII HOGs serve to highlight the impact of the host genetic background on the frequency and consequences of MMTV activation of a particular *Int* gene in the context of malignant progression.

New *Int* genes in CZECHII HOGs and mammary tumors

Activation of the *Int3* locus was first detected in the CZECHII mouse mammary tumors [59]. The locus was defined by the integration of an MMTV proviral genome within a 500bp region of the cellular genome of five independent mammary tumors corresponding to an exon of the target gene (Figure 1). The *Int3* locus is located in the class II region of the major histocompatibility (*MHC*) locus on chromosome 17 [60,61]. In each case the transcriptional orientation of the integrated viral genome was in the same direction, which was the same as that of the target gene. A 2.3kb species of RNA was detected in tumors containing a virus-induced rearrangement of *Int3*. This RNA species was not detected in tumors where the locus was intact or in the normal mammary gland. Nucleotide sequence analysis of the *Int3* gene revealed that it is related to the *Drosophila Notch* gene [62]. However, *Int3* is not the murine homolog of *Notch*; rather, it is one of a four member gene family [63-65]. The *Drosophila Notch* encodes a receptor protein that is involved in cell fate determinations during development [66]. Expression of *Int3* as well as *Notch1* and *Notch2* RNA can be detected in mammary glands of virgin, pregnant, and lactating mouse mammary glands (unpublished data). So far *Notch1* and *Notch2* have not been found to be rearranged by MMTV (unpublished data). All of the viral integration events within *Int3* occurred within an exon encoding the transmembrane domain of the encoded protein. This results in the overexpression of the portion of the gene encoding the intracellular domain of the protein. Experiments in which the same region of the *Drosophila Notch* gene are overexpressed demonstrated that this represents a gain-of-function mutation, mimicking the consequences of the

interaction between the Notch protein and its ligand [67].

Transgenic mice which express MMTV-activated *Int3* as a transgene develop a profoundly altered mammary gland, and within 4 to 6 months 100% have focal mammary tumors [68,69]. In virgin females the mammary ductal epithelium minimally penetrates the mammary fat pad. During the first pregnancy the mammary fat pad fills with ductal epithelium, but there is little lobular-alveolar development. The tumors appear as focal outgrowths derived from intraductal hyperplasias which are common within virgin and parous females. Since *Int3* is expressed during normal mammary gland development, activation of this gene by MMTV appears either to deregulate normal development controls, leading to hyperplasia from which tumors develop, or to provide a force towards malignancy. Interestingly we have not found *Int3* to be rearranged in our panel of HOGs (E. Kordon and G.H. Smith, personal communication). It has only been found to be rearranged by MMTV in feral mouse [59,70] and 2 BR6 inbred mouse mammary tumors [54]. We conclude that the effect of expression of the truncated *Int3* protein on mammary gland development and tumorigenesis is exquisitely dependent on the timing of its expression relative to mammary gland development.

A second common insertion site, designated *Int6*, for MMTV has been detected in a CZECHII HOG and two independent CZECHII mammary tumors [71]. The *Int6* gene is expressed in all adult tissues which have been tested, including the mammary gland, and as early as day 8 of embryonic development. It encodes a 55 kDa cytoplasmic protein which is unrelated to any of the gene products in the GenBank. The *Int6* gene has been highly conserved through evolution. The amino acid sequence of the mouse and human *Int6* gene products are identical (unpublished data), and related sequences can be detected in *Drosophila* and *C. elegans*

Like *Int3*, the MMTV proviral genome integrates within *Int6*. However, in each case the virus integrates within an intron of the *Int6* gene in the opposite transcriptional orientation (Figure 1). This results in the expression of a truncated-chimeric *Int6* transcript from the rearranged allele. The chimeric transcript is composed of *Int6* exon, intron, and MMTV LTR sequences. *Int6* transcription terminates at a cryptic termination signal within the MMTV LTR in the minus strand orientation. In the *Int6* positive HOG and tumors the unaffected allele was determined by nucleotide sequence analysis to be normal. This suggests that either the truncated gene product of the rearranged allele is biologically activated or that MMTV induces a dominant-negative mutation of *Int6*. The fact that MMTV rearrangement of *Int6* was initially detected in a CZECHII HOG is consistent with its being an early event in mammary tumorigenesis and suggests that examination of tumors derived from this HOG will lead to the identification of an MMTV-induced mutation which complements *Int6* in mammary tumor progression.

Implications for human breast cancer research

One of the major problems in identifying and addressing the impact of somatic mutations on the evolution of breast carcinogenesis is a fundamental lack of information on the identity of the signaling pathways which regulate the growth and development of the mammary gland. It seems likely that the target cells which are susceptible to carcinogenic mutations are those which have been incompletely committed to a particular fate of differentiation, i.e. stem cells [72-75]. However, again the number of molecular tags which identify these cells are limited. The MMTV/mouse model system has provided a productive and experimentally amenable approach, relative to other strategies, to identify genes and signaling pathways which when altered by mutation contribute to the deregulation of normal mammary gland development leading subsequently to mammary tumorigenesis. It seems likely that further analysis of MMTV induced HOGs, HOG-derived tumors, and subsequent distant metastases

will lead to the identification of additional MMTV-induced genetic alterations which taken together define pathways of mutations that drive malignant progression to its endpoint, metastasis.

The mouse model system does, however, have some potentially important limitations, relative to human breast cancer, which should be recognized. For instance there are endocrinological, hormonal, and obvious life style differences between the two biological systems. In addition, within the scientific community there has also been another perceived limitation, namely that the histopathological descriptions of mouse mammary tumors do not correspond to the most frequent forms of human breast tumors, i.e. invasive ductal carcinomas. However, in this regard it seems relevant that Wellings [76] found that many of the human mammary lesions observed are localized to the terminal ductal lobular unit. One of these, atypical lobular type A lesions (ALA), are morphologically similar to the mouse mammary HAN lesions. At the present time the question of whether the genetics of mouse mammary tumorigenesis is directly relevant to human breast cancer remains largely unanswered. *Wnt1* and *Wnt3* appear not be frequently rearranged or amplified in invasive ductal carcinomas (IDC) of the breast, but other forms of breast cancer have not been extensively studied [77,78]. Similarly, *Fgf3/Fgf4* are frequently co-amplified in IDC of the breast, but whether they are expressed as a consequence is controversial (reviewed in [79]). The human homologs of the other *Int* genes have not, as yet, been tested for genetic alterations in human breast tumors.

In the short term, the MMTV/mouse model system provides an opportunity to identify the genes (or gene families) encoding signaling pathways which are involved in normal mammary gland development. It seems likely that some of these mutations induced by MMTV or the genes involved in the particular signaling pathways will be relevant to human breast cancer. Since the *Int6* type of viral insertion (Figure 1) can be functionally similar to LOH, it seems reasonable that the identification of genes affected by this type of virus-induced mutation could be prime candidates for mutation in human breast tumors.

References

1. Gompel G, van Kerkem C: The breast. *In:* Silverberg S (ed) Principles of Surgical Pathology. Wiley Medical, New York, 1983
2. Harris JR, Hellman S, Canellos GP, Fisher B: Cancer of the breast. *In:* DeVita VT (ed) Cancer Principles and Practice of Oncology. J.B. Lippincott, Philadelphia, 1982
3. Lynch HT, Albano WA, Danes S, Layton MA, Kimberling WJ, Lynch JF, Cheng SC, Costello KA, Mulcahy GM, Wagner CA, Tindall SL: Genetic predisposition to breast cancer. Cancer 53:612-622, 1984
4. Trent JM: Cytogenetic and molecular biologic alterations in human breast cancer: a review. Breast Cancer Res Treat 5:221-229, 1985
5. Kallioniemi A, Kallioniemi O-P, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW, Waldman FM: Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic mapping. Proc Natl Acad Sci USA 91:2156-2160, 1994
6. Callahan R, Salomon DS: Oncogenes, tumour suppressor genes and growth factors in breast cancer: Novel targets for diagnosis, prognosis and therapy. Cancer Surveys 18:35-56, 1993
7. Hollingsworth RE, Lee W-H: Tumor suppressor genes: new prospect for cancer research. J Natl Cancer Inst 83:91-96, 1991
8. Knudson AG: Hereditary cancers: clue to mechanisms of carcinogenesis. Br J Cancer 59:661-666, 1989
9. Bieche I, Champeme MH, Merlo G, Larsen CJ, Callahan R, Lidereau R: Loss of heterozygosity of the L-*myc* oncogene in human breast tumors. Hum Genet 85:101-105, 1990
10. Merlo GR, Siddiqui J, Cropp CS, Liscia DS, Lidereau R, Callahan R: Frequent alteration of the DF3 tumor-associated antigen gene in primary human breast carcinomas. Cancer Res 49:6966-6971, 1989
11. Ali IU, Meissner S, Lidereau R, Callahan R: Allelic deletion of c-*erb*A-2 proto-oncogene in human breast carcinoma signifies possible recessive mutation in member(s) of steroid/thyroid hormone receptor family. J Natl Cancer Inst 81:1815-1820, 1989
12. Devilee P, van Vliet M, van Sloun P, Kuipers-Dijkshoorn N: Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. Oncogene 6:1705-1711, 1991
13. Orphanos V, McGown G, Hey Y, Boyle JM, Sant-

ibanez-Koref M: Proximal 6, a region showing allele loss in primary human breast cancer. Br J Cancer 71:290-293, 1995

14. Bieche I, Champeme MH, Matifas F, Hacene K, Callahan R, Lidereau R: Loss of heterozygosity on chromosome 7q associated with aggressive human primary breast tumours. Lancet 339:139-143, 1992
15. Ali IU, Lidereau R, Theillet C, Callahan R: Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science 238:185-188, 1987
16. Theillet C, Lidereau R, Escot C, et al: Frequent loss of a H-*ras*-1 allele correlates with aggressive human primary breast carcinomas. Cancer Res 46:4776-4781, 1986
17. Varley J, Armour J, Swallow JE, Jeffreys AJ, Ponder BAJ, T'Ang A, Fung YKT, Brammar W, Walker RA: The retinoblastoma gene is frequently altered leading to loss of expression in primary breast tumors. Oncogene 4:725-729, 1989
18. Devilee P, van den Broek M, Kuipers-Dijkshoorn N, Kolluri R, Meera Khan P, Pearson P, Cornelisse C: At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. Genomics 5:554-560, 1989
19. Merlo G, Venesio T, Bernardi A, Canale L, Gaglia P, Lauro D, Cappa APM, Callahan R, Liscia DS: Loss of heterozygosity on chromosome 17p13 in breast carcinomas identifies tumors with a high proliferation index. Am J Pathol 140:215-223, 1992
20. Cropp C, Lidereau R, Campbell G, Champeme M-H, Callahan R: Loss of heterozygosity on chromosome 17 and 18 in breast carcinoma: two new regions identified. Proc Natl Acad Sci USA 87:7737-7741, 1990
21. Morrison BW, Leder P: neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-tumors. Oncogene 9:3417-3426, 1994
22. Adams SM, Helps NR, Sharp MGF, Brammar WJ, Walker RA, Varley JM: Isolation and characterization of a novel gene with differential expression in benign and malignant human breast tumours. Human Mol Genet 1:91-96, 1992
23. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rosteck P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A, Skolnick MH: Isolation of a strong candidate for the 17q-linked breast and ovarian cancer susceptibility gene. Science 250: 66-71, 1994
24. Weiss R, Teich N, Varmus H, Coffin J: Molecular biology of tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor NY, 1982, pp 16-18
25. Marchetti A, Robbins J, Campbell G, Buttitta F, Squartini F, Bistocchi M, Callahan R: Host genetic background effect on the frequency of mouse mammary tumor virus-induced rearrangements of int-1 and int-2 loci in mouse mammary tumors. J Virol 65: 4550-4554, 1991
26. Medina D: Preneoplastic lesions in mouse mammary tumorigenesis. Methods Cancer Res 7:3-53, 1973
27. DeOme KB, Faulkin LJ, Bern HA, Blair PB: Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. J Natl Cancer Inst 78:751-757, 1959
28. Daniel C, DeOme K, Young L, Blair P, Faulkin L: The in vivo life span of normal and preneoplastic mouse mammary glands: a serial transplantation study. Proc Natl Acad Sci USA 61:53-60, 1968
29. Varmus HE: Recent evidence of oncogenesis by insertional mutagenesis. Cancer Surv 1:309-320, 1982
30. Dickson C, Smith R, Brookes S, Peters G: Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region int-2. Cell 37:529-536, 1984
31. Nusse R, Varmus HE: Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 31:99-109, 1982
32. Peters G, Brookes S, Smith R, Placzek M, Dickson C: The mouse homolog of the *hst*/k-FGF gene is adjacent to *int*-2 and activated by proviral insertion in some virally induced mammary tumors. Proc Natl Acad Sci USA 86:5678-5682, 1989
33. MacArthur CA, Shankar DB, Shackleford GM: Fgf-8, activated by proviral insertion, cooperates with the Wnt-1 transgene in murine mammary tumorigenesis. J Virol 69:2501-2507, 1995
34. Roelink H, Wagenaar E, Lopes da Silva S, Nusse R: Wnt-3, a gene activated by proviral insertion in mouse mammary tumors, is homologous to int-1/Wnt-1 and is normally expressed in mouse embryos and adult brain. Proc Natl Acad Sci USA 87:4519-4523, 1990
35. Klingensmith J, Nusse R: Signaling by *wingless* in Drosophila. Dev Biol 166:396-414, 1994
36. Nusse R, Varmus HE: Wnt genes. Cell 69:1073-1087, 1992
37. Hinck L, Nathke IS, Papkoff J, Nelson WJ: β-catenin: a common target for the regulation of cell adhesion by Wnt-1 and Src signaling pathways. Trends Biochem Sci 19:538-542, 1994
38. Delli Bovi P, Curatola A, Kern F, Greco A, Ittmann

M, Basilico C: An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. Cell 50:729-737, 1987
39. Dickson C, Peters G: Potential oncogene product related to growth factor. Nature 326:833, 1987
40. Yoshida T, Miyagawa K, Odagiri H, et al: Genomic sequence of *Hst*/KS transforming gene encoding a protein homologous to fibroblast growth factors and the *Int*-2 encoded protein. Proc Natl Acad Sci USA 84:7305-7309, 1987
41. Jakobovits A, Shackelford GM, Varmus HE, Martin GR: Two protooncogenes implicated in mammary carcinogenesis, Int-1 and Int-2, are independently regulated during mouse development. Proc Natl Acad Sci USA 83:7806-7810, 1986
42. Wilkinson DG, Peters G, Dickson G, McMahon AP: Expression of FGF-related proto-oncogene Int-2 during gastrulation and neurulation in the mouse. EMBO J 7:691-695, 1988
43. Basillico C, Moscatelli D: The FGF family of growth factors and oncogenes. Adv Cancer Res 59:115-164, 1992
44. Goldfarb M: The fibroblast growth factor family. Cell Growth & Diff 1:439-445, 1990
45. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE: 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, 1988
46. Muller WJ, Lee FS, Dickson C, Peters G: The *int*-2 gene product acts as an epithelial growth factor in transgenic mice. EMBO J 9:907-913, 1990
47. Stamp G, Fantl V, Poulsom R, Jamieson S, Smith R, Peters G, Dickson C: Nonuniform expression of a mouse mammary tumor virus-driven int-2/Fgf-3 transgene in pregnancy-responsive breast tumors. Cell Growth & Diff 3:929-938, 1992
48. Kwan H, Pecenka V, Tsukamoto A, Parslow TG, Guzman R, Lin TP, Muller WJ, Lee FS, Leder P, Varmus HE: Transgenes expressing the Wnt-1 and Int-2 proto-oncogenes cooperate during carcinogenesis in doubly transgenic mice. Mol Cell Biol 12:147-154, 1992
49. Shackleford GM, MacArthur CA, Kwan HC, Varmus H: Mouse mammary tumor virus infection accelerates mammary carcinogenesis in Wnt-1 transgenic mice by insertional activation of int-2/Fgf-3 and hst/Fgf-4. Proc Natl Acad Sci USA 90:740-744, 1993
50. Lee FS, Lane TF, Kuo A, Shackleford GM, Leder P: Insertional mutagenesis identifies a member of the Wnt gene family as a candidate oncogene in the mammary epithelium of int-2/Fgf-3 transgenic mice. Proc Natl Acad Sci USA 92:2268-2272, 1995
51. Parr BA, McMahon AP: Dorsalizing signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb. Nature 374:350-353, 1995
52. Yang Y, Niswander L: Interaction between the signaling molecules Wnt7a and SHH during vertebrate limb development: Dorsal signals regulate anterior-posterior patterning. Cell 80:939-947, 1995
53. Christian JL, Olson DJ, Moon RT: XWnt-8 modifies the character of mesoderm induced by bFGF in isolated Xenopus ectoderm. EMBO J 11:33-41, 1992
54. Peters G: Oncogenes at viral integration sites. Cell Growth & Diff 1:503-510, 1990
55. Schwartz M, Smith G, Medina D: The effect of parity, tumor latency and transplantation on the activation of int loci in MMTV-induced transplanted C3H mammary preneoplasias and their tumors. Int J Cancer 51:805-811, 1992
56. Gallahan D, Escot C, Hogg E, Callahan R: Mammary tumorigenesis in feral species of the genus Mus. *In:* Potter M (ed) The Wild Mouse in Immunology [Current Topics in Microbiology and Immunology]. Springer Verlag, Heidelberg, 1986
57. Callahan R, Drohan W, Gallahan D, D'Hoostelaere L, Potter M: Novel class of mouse mammary tumor virus-related DNA sequences found in all species of Mus, including mice lacking the virus proviral genome. Proc Natl Acad Sci USA 79:4113-4117, 1982
58. Escot C, Hogg E, Callahan R: Mammary tumorigenesis in feral Mus cervicolor popaeus. J Virol 58:619-625, 1986
59. Gallahan D, Callahan R: Mammary tumorigenesis in feral mice: identification of a new int locus in mouse mammary tumor virus(CzechII)-induced mammary tumors. J Virol 61:66-74, 1987
60. Gallahan D, Kozak C, Callahan R: A new common integration region (int-3) for mouse mammary tumor virus on mouse chromosome 17. J Virol 61:218-220, 1987
61. Siracusa LD, Rosner MH, Vigano MA, Gibert DJ, Staudt LM, Copeland NG, Jenkins NA: Chromosomal location of the octamer transcription factors, Otf-1, Otf-2, and Otf-3, defines multiple Otf-3 -related sequences dispersed in the mouse genome. Genomics 10:313-326, 1991
62. Robbins J, Blondel BJ, Gallahan D, Callahan R: Mouse mammary tumor gene Int-3: a member of the Notch gene family transforms mammary epithelial cells. J Virol 66:2594-2599, 1992
63. Lardelli M, Dahlstrand J, Lendahl U: The novel Notch homolog mouse Notch3 lacks specific epidermal growth factor-repeats and is expressed in proliferating neuroepithelium. Mech Devel 46:123-136, 1994
64. Weinmaster G, Roberts VJ, Lemke G: A homolog of

Drosophila Notch expressed during mammalian development. Development 113:191-205, 1991
65. Weinmaster G, Roberts VJ, Lemke G: Notch2: a second mammalian Notch gene. Development 116:931-941, 1992
66. Muskavitch MAT: Delta-Notch signaling and Drosophila cell fate choice. Dev Biol 166:415-430, 1994
67. Struhl G, Fitzgerald K, Greenwald I: Intrinsic activity of the lin-12 and Notch intracellular domains in vivo. Cell 74:331-345, 1993
68. Smith GH, Gallahan D, Diella F, Jhappan C, Merlino G, Callahan R: Constitutive expression of a truncated INT3 gene in mouse mammary epithelium impairs differentiation and functional development. Cell Growth & Diff, in press
69. Jhappan C, Gallahan D, Stahle C, Chu E, Smith GH, Merlino GT, Callahan R: Expression of an activated Notch-related int-3 transgene interferes with cell differentiation and induces neoplastic transformation in mammary and salivary glands. Genes & Diff, in press
70. Sarkar NH, Haga S, Lehner A, Zhao W, Imai S, Moriwaki k: Insertional mutation of Int protooncogenes in the mammary tumors of a new strain of mice from the wild in China: Normal- and tumor-tissue-specific expression of Int-3 transcripts. Virology 203:52-62, 1994
71. Marchetti A, Buttitta F, Miyazaki S, Gallahan D, Smith GH, Callahan R: Int6, a highly-conserved widely-expressed gene is mutated by mouse mammary tumor virus in mammary preneoplasia. J Virol 69: 1932-1938, 1995
72. Kordon EC, McKnight R, Jhappan C, Hennighausen L, Merlino G, Smith GH: Ectopic TGFβ1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. Devel Biol 168:47-61, 1995
73. Medina D, Smith GH: In search of mammary gland stem cells. Protoplasma 159:77-84, 1990
74. Smith G, Medina D: A morphologically distinct candidate for an epithelial stem cell in mouse mammary gland. J Cell Sci 89:173-183, 1988
75. Smith G, Gallahan D, Zweibel J, Freeman S, Bassin R, Callahan R: Long-term in vivo expression of genes introduced by retrovirus-mediated transfer into mammary epithelial cells. J Virol 65:6365-6370, 1991
76. Wellings SR: A hypothesis of the origin of human breast cancer from the terminal ductal lobular unit. Path Res Pract 166:515-535, 1980
77. Roelink H, Wang J, Black DM, Solomon E, Nusse R: Molecular cloning and chromosomal localization to 17q21 of the human WNT3 gene. Genomics 17:790-792, 1993
78. Vande Vijver MJ, Petersen J, Mooi W, Lomans J, Verbruggen M, Vande Bersselaar A, Devilee P, Cornelisse C, Bos JL, Yarnold J, Nusse R: Oncogene activations in human breast cancer. *In:* Furth M, Greaves M (eds) Molecular Diagnostics of Human Cancer. Cold Spring Harbor Laboratory Press, NY, 1989, pp 385-391
79. Callahan R, Cropp C, Merlo GR, Diella F, Venesio T, Lidereau R, Cappa APM: Genetic and molecular heterogeneity of breast cancer cells. Clinica Chimica Acta 217:63-73, 1993
80. Nadeau JH, Davisson MT, Doolittle DP, Grant P, Hillyard AL, Kosowsky MR, Roderick TH: Comparative map for mice and humans. Mam Genome 3:480-536, 1992
81. Crosley PH, Martin GR: The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121:439-451, 1995
82. Miyazaki S, Kozak CA, Marchetti A, Buttitta F, Gallahan D, Callahan R: The chromosomal location of the mouse mammary tumor gene Int6 and related pseudogenes in the mouse genome. Genomics, in press