[Frontiers in Bioscience 2, d519-526, November 1, 1997]

ROLE OF MMTV INTEGRATION LOCUS CELLULAR GENES IN BREAST CANCER

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1. ABSTRACT

Mouse mammary tumorigenesis as a result of mouse mammary tumor virus (MMTV) integrations has helped to identify a wide variety of interesting genes that play a role in mammary development and tumorigenesis. Several such genes *int1/wnt1, wnt3, wnt 10B, int2/fgf3, fgf4*, *int3/notch* and *int6* have been shown to be genetically altered in naturally formed mammary tumors as a consequence of MMTV integration. Some of these genes have been well characterised and examined in *in vivo* breast cancer transgenic models for their potential for tumorigenesis. Overexpression of one or more of these genes have resulted in a striking proliferation of mammary gland epithelium of both female and male transgenic mice. Our own studies have demonstrated overexpression of *int5/aromatase* in mammary glands of virgin and postlactational females leads to the induction of various preneoplastic and neoplastic changes that are similar to early breast cancer, that may, in turn, increase the risks for developing breast cancer. Therefore, further understanding of these genes should provide new insights to their involvement and mechanism of action in breast cancer.

2. INTRODUCTION

Breast cancer is one of the most common malignancy in women with the incidence rate as high as one in every eight women (1). Several factors are associated with this disease such as genetics, life style, menstrual and reproductive history, long term treatment with estrogens. Genetic abnormalities include germline mutations, gene amplifications, rearrangements, overexpression, deletions or point mutations. The genes thought to be involved in human breast cancer encode growth factors, receptors, nuclear transcription factors, cell cycle regulatory proteins, tumor suppressor proteins and others. It is possible that these factors alone or in combination could provide a selective environment for the clonal outgrowth of mammary epithelial cells containing mutations.

Unlike retroviruses that carry a transduced oncogene *(v-myc, v-erb, v-Ha-ras* etc.), other retroviruses including avian leukosis virus (ALV), murine leukemia virus (MLV) and mouse mammary tumor virus (MMTV) does not carry a transduced oncogene and tumorigenesis is due to insertional mutagenesis. Tumorigenesis by these viruses mainly depends on the host cellular oncogenes, transcriptionally activated or otherwise mutated as a consequence of proviral integration. MMTV is considered as a biological carcinogen that induces tumor development as a consequence of insertional mutagenesis (2, see latest review for more information). MMTV is of special interest in studying the mechanism of mammary tumorigenesis. MMTV-infected mice develop initially preneoplastic hyperplastic alveolar nodules (HAN). These nodules, and also the primary tumor that develop within these hyperplasias, are hormone-dependent and are evoked by pregnancy and regress after parturition. After several cycles of pregnancy, tumors arise that eventually become hormone independent (3). MMTV induces mammary tumors by acting as an insertional mutagen, and activating transcription of the nearby genes. MMTV acts as a insertional mutagen that causes the deregulation of expression of adjacent cellular genes (named as *int* genes) in mammary tumors. Molecular analysis of proviral integration site in tumors has led to the discovery of a number of cellular proto-oncogenes such as several members of the fibroblast growth factor (FGF) family, w*nt* genes and others like *NOTCH* gene. Tumorigenesis by exogenous MMTV is accompanied by insertion of the proviral MMTV into the host genome. Several animal models have been used to study this mechanism. Inbred strains with high incidence of mammary tumors such as C3H, GR, BR6 and RIII mouse strains are intentionally used (4,5). MMTV in these mice is transmitted in two ways: In the GR mouse strain, the virus is transmitted endogenously via the germ line since these

Received 10/14/97 Accepted 10/17/97
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Table 1. Location, nature of the gene product and expression of *int* genes

GENE	LOCATION		GENE PRODUCT	EXPRESSION IN NORMAL TISSUES
	Mouse	Human		
Int1/Wnt1	15	12	Morphogen	Testis
Int2/Fgf3	7	11	Growth factor	None
Int3/Notch	17	6	Notch related protein	All
Int4/Notch	11	17	wnt related protein	None
Int5	9	15	Aromatase	Breast, Ovaries, Testis
Int6	11			All
Fgf4/Hst	7	11	Growth Factor	None
Wnt 10b	15	12		Mammary glands
Fgf8	19	10		Ovaries, Testis

mice contain an endogenous MMTV locus on chromosome 18, *Mtv-2,* which expresses the MMTV provirus at high levels. In most other susceptible mouse strains, like C3H and BR6, MMTV expression in the mammary gland of an infected female is markedly increased during lactation under the influence of steroid hormones. As a result, MMTV is secreted in the mother's milk and transmitted exogenously to the offspring, probably through the gut epithelium (3). Parous C3H females develop pregnancy-independent mammary tumors. In addition, MMTV (C3H)-infected females frequently develop mammary preneoplastic HANs, whereas uninfected females infrequently developed HANs (6-8). 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 (2). In addition, metastatic lesions in the lungs of MMTV-infected mammary tumor bearing mice have been detected.

3. *Int/wnt* GENES

3.1 Biological effects of *int* genes

Most of the *int* genes fall into two groups: the *wnt-1 /int-4* family and the *int-2/hst* family. Although the two groups show no sequence similarity to each other, they may have similar functions: besides being implicated in mouse mammary tumorigenesis, both are thought to have essential functions in pattern formation in early embryogenesis genes (9,10). The location and gene products of all *int* genes are shown in table 1. The *wnt-1* gene is related to the Drosophila gene wingless, and probably encodes a growth factor. Overexpression of the *wnt-1/int-1* gene from the MMTV-LTR promoter/enhancer has resulted in a striking proliferation

of mammary gland epithelium of both female and male transgenic mice. The *int-2* protein is a member of the fibroblast growth factor (FGF) family proteins. When the *int-2* gene was expressed in transgenic mice from the MMTV promoter, hyperplasia was seen after one or more pregnancies. The *int-3* gene is related to the notch family of genes from Drosophila and encodes a transmembrane protein that is probably a receptor (11). The MMTV virus integrates in the middle of the gene and thereby separates the extracellular and intracellular domains. They may alleviate negative regulation of the function of the receptor by its ligand binding domain, and thereby potentiate its oncogenic function (11,12). *Int-5* originally named *int-H*, was found to be rearranged in three chemically induced hyperplastic alveolar nodules and the tumors that arose from these precancerous mammary hyperplasias after transplantation (13, 14). The highly conserved *int-6* gene, cloned as a common MMTV insertion site in Czech 2 hyperplastic outgrowth lines, encodes a protein of unkown function (15). An interesing aspect is that all proviruses in *int-6*, mapped thus far, have integrated in a reverse orientation in one of the introns of the gene. The presence of a cryptic transcription stop signal in the reverse sequence of the MMTV LTR leads to premature transcripton termination, resutling in novel *int-6*/LTR chimeric mRNA species which encode different forms of truncated *int-6*. The biological significance of these aberrant *int-6* proteins is still unclear.

3.2 Int/wnt/fgf genes in naturally occurring mammary tumors

Six *int* loci *(int1/wnt1, int2/fgf3, wnt3, hst/K-fgf/fgf4, wnt*10b, and *fgf*8) have been identified in mammary tumors of high incidence in inbred mouse strains or MMTV-infected transgenic mouse strains (16-20). Most of the *int* genes activated by MMTV are not involved in other types of tumors and have been discovered by using MMTV cDNA as a screening probe to clone integration loci. When multiple insertions in the same locus are found in individual tumors, there is usually an oncogene in the vicinity. At *int1/wnt1* and *int2/fgf3* loci, proviruses were found at each end of the gene that is usually pointing away from the gene itself. The typical orientation and the large distance of some proviruses from the *int1/wnt1* or *int2/fgf3* promoter indicate that the transcriptional activation of the *int1/int2* genes is mediated by enhancers in the MMTV genome, acting on *int1/wnt1* and *int2/fgf3* promoters. The promoters of *int1/wnt1* and *int2/fgf3* genes have been mapped and are indeed similarly active in tumors as well as where the genes are expressed, except for rare cases of proviral insertions within the promoter. In some tumors, several different *int* genes are activated, either in the same clonal population of cells or in different clones that may cooperate with each other. The *int2/fgf3* gene is amplified in up to 19% of human breast carcinomas. In most cases, the *int2/fgf3* amplification is associated with co-amplification of a cluster of genes. Most of the oncogenes activated by MMTV proviral insertions encode secreted molecules that belong either to the *wnt* gene family or to the *fgf* growth factor family. The members of the *fgf* and *wnt* family have various characters in common. They appear to be involved in many crucial developmental decision, functioning as short-range intercellular signaling molecules, and both FGF and Wnt proteins can associate tightly with extracellular components of the extracellular matrix. Proviral insertion either upstream or downstream of the gene could simultaneously activate (MMTV) transcription from three dissimilar *int2/fgf3* promoters. In some tumors, the activating provirus lies within the transcription unit and disrupts the structures of the various RNAs. Insertions in the 5' region of the gene had complex effects depending on the orientation and position of the provirus relative to three promoters and intron-exon boundaries. These data strongly implicate the normal

product of the *int-2/fgf-3* gene, which is related to the fibroblast growth factor family, as a contributory factor in virally induced mammary tumors (21).

The mouse *wnt* family includes at least 10 genes that encode structurally related secreted glycoproteins. *Int1/wnt1* and *int4/wnt3* were originally identified as oncogenes activated by the insertion of MMTV in virus-induced mammary adenocarcinomas, although they are not expressed in the normal mammary gland. Another w*nt* gene, *wnt2,* was found to be amplified in progressed (hormone-independent) GR mouse mammary tumors (22). Three out of eight FGF growth factor family of genes have been shown to be implicated in MMTV-induced tumorigenesis *(fgf-3/int-2, fgf-4/hst* and *fgf-8*). In human and in mouse, *fgf-3/int-2* and *fgf-4/hst* are closely linked, and a minority of the proviral insertions found in this region activate *fgf-4/hst* rather than *fgf-3/int-2. Fgf8* has been shown to be activated by proviral insertions in MMTV-induced mammary tumors from *wnt1/int1* transgenic mice. All three share a number of common structural features. They contain a signal peptide but lack a transmembrane domain, implicating that they are secreted.

Int3 is a common insertion site of MMTV which is frequently activated in MMTV induced tumors in the CZECH II mouse strain and Jyg strain. It is related to the *Drosophila* gene *Notch.* The *Notch* gene is best known as a gene involved in cell-cell interactions affecting neuronal differentiation. Activation of *int3* in mouse mammary tumors occurs in the configuration of inserted provirus with respect to the gene. All proviral insertions map in the middle of the gene disrupting the transcriptional unit in such a way that the upstream transcription is terminated in the left LTR of the proviruses, and the downstream part of the gene is activated by promoter insertion from the right LTR (11, 23). Activation of *int3* is the result of a truncation of the extracellular domain, since expression of a transgene expressing the cytoplasmic domain leads to mammary hyperplasia (24). Recent studies have shown that *int6* gene can be converted into a putative dominant negative oncogene after retroviral insertion. Binding of HTLV-l tax oncoprotein to *int6* caused its redistribution from the nuclear domain to the cytoplasm. Thus, *int6* is a component of promyelocytic leukemia nuclear bodies and Tax disrupts its normal cellular localization by binding to it (25). *Int6* gene, that is cloned as a common MMTV insertion in CZECH II has all provirus mapped have integrated in a reverse orientation in one of the introns of the gene. The presence of a cryptic transcription stop signal in the reverse sequence of the MMTV-LTR leads to premature transcription termination resulting in a novel *int-6*/LTR chimeric mRNA species which encode different forms of truncated *int6.* The biological significance of these aberrant int6 proteins is still unclear.

3.3 Int-5: MMTV integrations in hormone/carcinogen-induced HAN model

Gray *et al* (26) and others (27,28) have shown that mammary tumors induced by 7,12-dimethylbenz (a)-anthracene (DMBA) or mammotrophic hormones (MTH) also contain one or more newly integrated MMTV proviral DNA copies. Not only the tumors, but also preneoplastic lesions, the HAN, contain MMTV provirus. HAN are at a greater risk for tumor development than are corresponding normal mammary alveolar cells and serve as a source of pre-neoplastic or pre-malignant changes in cancer. Three different high cancer-incidence hyperplasias (D2, C4, C5) had provirus integrated at the same genomic locus, designated as *int5* (formerly called *int-H).* Integration of the MMTV proviral DNA at the *int5* locus is associated with tumorigenicity and "turn off" of casein, which is a marker of terminal differentiation. These data indicate an insertional mechanism for chemical carcinogenesis and implicate *int5* as a cellular proto-oncogene. Recently, we have cloned *int5* both from mouse pre-cancerous D2 HAN genomic DNA and its normal allele from BALB/c genome (14). The localization of *int5* on chromosome 9 unequivocally distinguishes this gene from any other *int* genes. Unlike other *int* genes, *int-5* was identified and cloned from a chemically induced HAN model. The MMTV provirus integrated at *int5* in D2 neoplasms was clearly a novel integration and the activation of latent endogenous virus in BALB/c mice as a result of hormonal or chemical treatment might have led to these novel integrations. *Int5* is unique, and also different from other oncogenes implicated in breast cancer, because of its possible role in early neoplastic changes leading to HAN formation. Our studies have shown that the cellular gene at the MMTV integration site in the *int5* locus is identical to the gene encoding aromatase (CYP 19), a member of the cytochrome P450 gene superfamily. MMTV is integrated within the 3' untranslated region of the aromatase gene and this integration is responsible for the overexpression of this gene (hereafter called as *int5/aromatase).* Aromatase catalyzes the conversion of androgens to estrogen, which is the rate-limiting step in estrogen biosynthesis. *Int5/aromatase* is expressed in normal mammary gland and overexpressed in mammary tumors. Using a cell line derived from the D2 tumor (generated using D2 HAN that carried novel MMTV integration in the *int5* locus), we have demonstrated the effect of the aromatase substrate, androstenedione, on the proliferation of tumor cells. Proliferative effects of androstenedione were blocked by aromatase inhibitors, such as fadrozole hydrochloride, aminoglutethimide, arimidex, providing evidence for the role of *int5/aromatase* in this process. Furthermore, the androstenedione-mediated proliferation was inhibited by the addition of anti-estrogen ICI 164, 384, suggesting that the estrogen formed from the conversion of androstenedione by *int5/aromatase* acts like a mitogen to stimulate the growth of D2 tumor cells. We have also demonstrated the formation of estrogens in these cells using androstenedione as a substrate. These results suggested that the overexpression of this gene may be responsible for mammary tumorigenesis. This was the first demonstration of integration of MMTV in a cellular gene that plays a role in hormone-dependent breast cancer (14, 24-31). Estrogens are the most important hormones involved in supporting the growth of hormone-dependent breast cancers. Breast tumors from postmenopausal women maintain a high estrogen content, even though the plasma levels of estradiol fall to low levels following menopause. Maintenance of high tumor estrogen concentrations reflects the *in situ* estradiol production from plasma estrogen precursors. One pathway for *in situ* synthesis involves the conversion of androstenedione to estrone/estradiol catalyzed by aromatase. Several workers have demonstrated the presence of intra-tumoral aromatase activity in breast carcinomas and have also shown a significant correlation between the aromatase activity and clinical response to endocrine therapy with aromatase inhibitors (32-34).

3.4 Characterization of *int* genes using *in vivo* breast cancer transgenic models

Transgenic animal models have been used to study breast transformation for a number of years and have yielded

valuable information on the subject. The *int* genes were first discovered because of adjacent integration of the MMTV proviral DNA. As described earlier, this virus does not carry a transduced cellular oncogene like many of the well known oncogenic retroviruses, but rather acts as an insertional mutagen. Overexpression of the *int1/wnt1* gene from the MMTV-LTR promoter/enhancer resulted in a striking proliferation of mammary gland epithelium of both female and male transgenic mice. Mean tumor onset time was around 5 months for transgenic females, and by 7 months of age more than 80% had developed tumors. Transgenic male animals develop tumors less frequently and later in life (35). Transcriptional activation of *int1/wnt1* gene by proviral insertion mutations is thought to be a key step in mammary tumor induction by MMTV. *Int1/wnt1* gene appears not to be expressed in normal mammary glands from pregnant or lactating mice (17,36). Studies by Tsukamoto *et al* (35) have shown that transgenic mice with MMTV-LTR on 5' end of *int1/wnt1* gene had hyperplasia compared to non-transgenic littermates. It is suggested that transcriptional activation of *int1/wnt1* and associated hyperplasia are initiating events in multi step carcinogenesis.

Overexpression of *wnt1/int1* in the mammary gland of MMTV-*wnt1* transgenic mice caused massive proliferation of mammary epithelial cells, resulted in hyperplastic glands (35). Also it has been shown that mammary glands of *wnt1/int1* transgenic virgins are similar to hormonally-stimulated glands in pregnant animals with increased number of terminal buds and alveoli showing hyperplasia. MMTV-*wnt1* or MMTV-*fgf* transgenic mice develop mammary tumors after a variable latency period indicating that activation of either *wnt1/int1* or *fgf* alone is not sufficient for complete malignancy (3). Tumors develop faster in bitransgenic mice for *wnt1/int1* and *fgf-3/int2*, compared to either gene alone. This clearly shows that co-operation of these two growth factors in tumorigenesis (37). *Int-2/fgf-3* transgenic mice infected with MMTV showed activation of *wnt1/int1* and a new *wnt* gene family member, *wnt10b.* On the contrary, *wnt1/int1* transgenic mice infected with MMTV developed many tumors with proviral insertions in members of the Fgf family. The virus associated with *int2/fgf3* insertions is from the RIII strain. When *int2/fgf3* gene was expressed in transgenic mice from the MMTV promoter, hyperplasia was seen after one or more pregnancies. About one half of transgenic females developed tumors before the age of one year. Some virgin females also developed tumors, but at a lower frequency than multiparous females (38). While transgenic females display a pronounced mammary gland hyperplasia, expression of MMTV *fgf3/int2* in the prostate gland of male carriers results in a benign epithelial hyperplasia, indicating that *fgf3/int2* can act as a growth factor in different epithelial tissues (3). In this case, microscopic ductal hyperplasia was observed in gland of virgin transgenic females. Ductal hyperplasia, papillocystic forms and nodular solid aggregates of cells were observed during pregnancy. Some lesions regress and some remained static and one's that remained static become more pronounced in subsequent pregnancies.

Integration of MMTV provirus into the *int3/notch* locus promotes the transcription and translation of flanking cellular *int3/notch* sequences sharing significant homology with the intracellular domain of the neurogenic *Notch* gene of Drosophila. Transgenic mice were generated (24) with the DNA fragment consisting of the MMTV-LTR and flanking cellular *int3/notch* sequences. Poor differentiation of mammary and salivary adenocarcinoma were prominent in transgenic mice between 2 and 7 months of age. All females *int3/notch* mice were lactational deficient and mammary glands were arrested in development. Jhappan *et al* (24) have also shown that all male *int3/notch* transgenic mice were sterile, apparently the result of severe hyperplasia of the epididymis. Thus overexpression of *int3/notch* gene in *in vivo* may cause deregulation of normal developmental controls and hyperproliferation of glandular epithelia. These findings also suggest that the activated *int3/notch* gene product may have been a contributing factor in the development of Czech II mouse mammary tumors in which the MMTV-induced rearrangement in the *int3/notch* locus (39).

Our recent studies (40) have shown that the cellular gene at the mouse mammary tumor virus integration site in the *int5* locus is aromatase. We have generated transgenic mice that overexpress *int5/aromatase* under the control of mouse mammary tumor virus enhancer/promoter, and demonstrated for the first time that increased mammary estrogens due to the overexpression of *int5/aromatase* in mammary glands of virgin and postlactational females leads to the induction of various preneoplastic and neoplastic changes that are similar to early breast cancer, that may, in turn, increase the risk of developing breast cancer. The preneoplastic/neoplastic changes continues to progress in postlactational females that overexpress *int5/aromatase*. In fact when these animals are exposed to carcinogens like DMBA, there is increased incidence of mammary tumorigenesis suggesting that indeed increased mammary estrogen increases the risk of developing breast cancer due to carcinogen exposure (unpublished data). Overexpression of *int5/aromatase* in males leads to the increased mammary growth and hyperplastic and dysplastic changes that are similar to gynecomastia (early male breast cancer). These observations indicate that increased mammary estrogen alone without the influence of circulating ovarian estrogen may be sufficient to induce early breast cancer.

4. FERAL MOUSE MODEL

MMTV has also been detected in feral strains of mice (41). Since these mice are hemizygous for endogenous MMTV, some fraction of the offspring lack endogenous MMTV genome. This makes it possible to unambiguously detect acquired MMTV genomes in mammary tumors. Even in this setting, *wnt1/int1* and *fgf3/int2* are activated by MMTV insertion at a frequency comparable to MMTV-infected low incidence mouse strains. The CZECH II mice lack endogenous MMTV genomes but do contain an infectious strain of MMTV that is transmitted congenitally through the milk. 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 to that observed in BALB/cfC3H or CZECHII/C3H mammary tumors, whereas MMTV-induced activation of *fgf3/int2* and *fgf4* was significantly less frequent (2). Like high incidence inbred mouse strains, CZECH II mice also develop mammary preneoplastic HANs which has been developed into mammary hyperplastic outgrowth lines (HOGs; 2). The frequency of MMTV induced rearrangements of *wnt1* is similar in both CZECHII HOGs and mammary tumors. It is predicted that in this setting, activation of *wnt1* may be an early event in tumorigenesis which may disrupt regulatory controls of normal mammary gland development leading to lobular hyperplasia. It is also thought that the mammary tumors arising from within

these HOGs frequently contain additional MMTV proviral genomes. Based on the results obtained (2) with the MMTV-infected *wnt1* transgenic mice, it is possible that members of the *fgf* gene family to be activated by MMTV in tumors derived from *wnt1* positive HOGs.

5. INSERTIONAL MUTAGENESIS-MECHANISMS

The important feature of the MMTV provirus is that the 3' LTR contains an open reading frame that encodes a superantigen (SAg), and, after reverse transcription and integration of the provirus into the genome, the viral superantigen is presented on the cell surface along with MHC class II molecules. The ability of an integrated provirus to activate the transforming potential of a flanking gene is in all cases mediated by the transcriptional control on the integration site and the transcriptional orientation of the provirus with respect to the cellular gene. These sequences are capable of initiating, enhancing and/or terminating transcription of host sequences, resulting in high levels of messenger RNAs encoding the intact protein or the production of aberrant transcripts encoding mutant proteins (3). Activation of the gene by the promoter insertion mechanism requires the integration of a provirus in the same transcriptional orientation as the target gene. Initiation of transcription occurs from the viral promoter in either the 5' or the 3' LTR, which replaces the function of the normal promoter. Viral deletions are frequently associated with activation by the promoter insertion in case the 3' LTR promoter is used. This results often in removal of the 5' LTR, suggesting that transcriptions driven by the 5' promoter and proceeding into the 3' LTR may negatively influence transcription from the 3' LTR-promoter (42). When the 5' LTR promoter is used, transcription results in the formation of fusion transcripts containing both viral and cellular sequences, due to frequent read-through at the 3' LTR polyadenylation site and subsequent splicing using the heteronuclear mRNA splice donor or cryptic splice donor sites.

6. RELEVANCE OF MAMMARY TUMOR VIRUS IN HUMAN BREAST CANCER

MMTV has been regarded as a potential model for human disease. Efforts to demonstrate the presence of viruses in human breast cancer through search for viral particles, immulogical cross-reactivity, or sequence homology have yielded contradictory results. Several lines of evidence, however, associate MMTV with human breast cancer. Detectable MMTV *env* gene-related antigenic reactivity has been found in tissue sections of human breast cancer (43-45), breast cancer cells in culture (46), human milk (47), in sera of patients (48), in cyst fluid (49), and in particles produced by a human carcinoma cell line (50). Sequence homology has also been found in human DNA under low stringency conditions of hybridization (51), and RNA related to MMTV has been detected in human breast cancer cells (52). The human homologue of the *int-2* locus has been found to be amplified and expressed in 15% of the breast cancers (53-56). Our own studies involving hormone/carcinogen-induced mammary tumor model has provided interesting insights into the role of *int5* novel MMTV integration and the nature of cellular gene involved in this integration locus. This is the first demonstration of integration of MMTV in a cellular gene that plays a role in hormone-dependent breast cancers. Wang *et al* (57) have detected mammary tumor virus env gene-like sequences in human breast cancer and suggest that these MMTV env gene-like sequences may play a role in the etiology of a large proportion of human breast cancer. Recent studies (58) have identified the human homologue of mouse *wnt10b* gene. The human *WNT10B* sequence was 88% and 95% identical to the murine gene at nucleotide and amino acid levels, respectively. In normal and benign proliferation of human breast tissue, *WNT10B* expression was found to be elevated in 3 of 50 primary breast carcinomas. These findings suggest that *WNT10B* gene may also be involved in human breast cancer, and show that there is differential expression of the *WNT10B* gene in benign and malignant disease. All the above studies clearly suggest that MMTV related genes may play a very improtant role in the etiology of human breast cancer. More detailed integrations may provide proof for definite role of MMTV in breast cancer.

7. CONCLUSIONS

Mouse mammary tumorigenesis caused by MMTV has provided a rich source of interesting genes that play a role in mammary development and tumorigenesis. Each stage of mammary tumorigenesis appears to result from the clonal out growth of cells containing additional integrated proviral MMTV genomes. Genes such as *int1/wnt1, wnt3, wnt10B, int2/fgf3, fgf4, int3/notch,* and *int6* have been shown to be genetically altered in naturally formed mammary tumors as a consequence of MMTV integration. Although the role of the human homologs of all of these oncogenes in human breast cancer is still poorly understood, it is clear that this work has led to new understanding of the importance of these genes in mammary gland growth, development as well as tumorigenesis. The fact that *fgf3/fgf4/hst* from the *int2* integration locus are frequently co-amplified in invasive ductal carcinomas of the breast clearly demonstrates the importance of these models in understanding the role of various genes in multistep process of breast tumorigenesis. Our studies involving hormone/carcinogen-induced mammary tumor model has provided interesting insights into the role of *int5* novel MMTV integration and the nature of cellular gene involved in this integration locus. This was the first demonstration of integration of MMTV in a cellular gene that plays a role in hormone-dependent breast cancers. The relevance of MMTV/*int* genes to human breast cancer have been studied. WNT10B expression has been detected in normal and benign porliferations of human breast tissue. Studies have also identified in breast cancer DNA a segment comprising LTR and env gene sequences, which were homologous to MMTV. It seems likely that some of the mutations induced by MMTV, and the signaling pathways in which the target genes take part, will be relevant to the progression from preneoplastic lesions to distant metastasis in human breast cancer. Further understanding of the functional role and their mechanisms of action could provide new insights into the importance of these genes in mammary and breast cancers.

8. ACKNOWLEDGMENT

This work is supported by NIH grant CA57559 (to RRT).

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