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REVIEW ARTICLE

From chromosomal abnormalities to the identification of target genes in mouse models of breast cancer

Victoria T. Fabris*

Laboratory of Hormonal Carcinogenesis, Instituto de Biología y Medicina Experimental (Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET), Buenos Aires, Argentina

Cytogenetic studies of breast cancer cells have identified numerous chromosomal imbalances, including gains in human chromosome regions 1q, 4p, 8q, and 20q and losses in regions 1p, 3p, 6q, 11q, 16q, 17p, and 22q. Mouse models have been developed to study the mechanisms of mammary carcinogenesis, and in most cases, the corresponding karyotypes have been reported. Here, I summarize the cytogenetic findings and the candidate genes that are involved in mammary tumorigenesis. The most commonly altered chromosomes in mouse breast cancer models are chromosomes 4 and 11, which are orthologous to human chromosomes that are also affected by chromosomal abnormalities in human breast cancer. The genes that are affected by chromosomal imbalances in mouse models have also been found to participate in human breast cancer. In addition, the amplification and overexpression of several new genes in mouse models have subsequently been confirmed in human breast cancer. In this review, I compile information on the available karyotypes for mouse breast cancer models.

Keywords Breast cancer, mouse model, cytogenetics, karyotype, carcinogenesis

Breast cancer is one of the most frequent cancers among women and is one of the major causes of cancer death. In the United States, it is estimated that 232,340 new cases of breast cancer and 39,620 new breast cancer deaths occurred in 2013 (1).

In humans, cytogenetic studies of cancers of hematological origin have provided key information required to understand the evolution of the disease. These types of studies have been delayed in breast cancer and other solid tumors, primarily because of the limited technical approaches to deal with isolated metaphases and the complexity of the karyotypes that have been encountered (2). However, the development of new molecular cytogenetic techniques has contributed to the identification of different chromosomal rearrangements. The most frequent alterations that have been reported in human breast cancer include gains in chromosome regions 1q, 4p, 8q, and 20q and losses in chromosome regions 1p, 3p, 6q, 11q, 16q, 17p, and 22q. Moreover, the amplification of chromosomal region 17q and the overexpression of HER-2/*neu*, which maps to this region, are of prognostic and therapeutic value in human breast cancer (3).

Several different mouse models have been developed to study the mechanisms of mammary carcinogenesis. Studying the chromosomal and genetic alterations that occur in these models may provide a better understanding of the disease. There are substantial differences between mouse and human chromosomes. Despite these karyotype differences, syntenic regions have been mapped, and correlations can be made between the regions that are affected by genetic changes in humans and in mouse models. The karyotype of mouse mammary tumor cells has been studied in most of the available models using different cytogenetic techniques. It is likely that comparing the data that have been obtained from all of these mouse models with the data available from human breast cancer studies may help dissect the pathways that are involved in breast cancer development.

This review will summarize both the cytogenetic findings and the target genes that are located in the regions that are involved in chromosomal alterations in different mouse models of breast cancer. Specifically, the focus will be on chromosomal aberrations that are found in murine mammary tumor cells. This approach allows the identification of

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* Corresponding author.
E-mail address: victoriafabris@gmail.com.

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candidate genes that may participate in carcinogenesis. The information that has been gleaned from these mouse models regarding the mechanisms and new pathways that are involved in mammary tumorigenesis are also discussed.

Cytogenetics of mouse models

Spontaneously derived mammary tumor models

The SP1 tumor cell line is derived from a spontaneous nonimmunogenic and nonmetastatic intraductal mammary carcinoma that arose in an 18-month-old CBA/J female mouse. Tumor cells were cultured in vitro, and the cell line was subsequently maintained by syngeneic transplantations in vivo (4). This mouse model is especially well-suited for the study of metastasis and immunogenicity. The cytogenetic profile of SP1 cells in culture is described in Table 1. Metaphase cells were hypotetraploid, displaying a mean of 67 chromosomes. The karyotypes included a chromosomal alteration that was derived from chromosome 3 and an unidentified small acrocentric chromosome (marker chromosome). The in vitro treatment of SP1 cells with 2′-deoxy-5-azacytidine (SP1-Az) or hydroxyurea (SP1-HU) conferred a metastatic phenotype. The metastatic SP1-Az and SP1-HU cells maintained the hypotetraploid karyotype and retained the alterations that were observed in the nonmetastatic SP1 cells (Table 1). In addition, for both treatments, the cells acquired two isochromosomes, which were derived from chromosome 4 and chromosome 8 (Table 1). An isochromosome of the small marker was observed only in the SP1-Az cells.

The increase in the copy number of chromosome 8 that was observed in the metastatic SP1 cells was due to the presence of isochromosome 8. Interestingly, although both drugs affect DNA methylation through different mechanisms, they induced similar karyotypic alterations (5).

In another study, Elliot et al. (6) compared the karyotypes of nonmetastatic SP1 cells, which were inoculated subcutaneously, to metastatic cells, which were obtained by the successive inoculation of SP1 cells into the mammary gland. The nonmetastatic SP1 cells also showed a hypotetraploid chromosome number (Table 1). However, karyotypic differences were found between these data and those reported by Frost et al. (5), which could be due to different in vivo/in vitro growth conditions of the SP1 cells used by both groups. Several cell subpopulations with different chromosomal abnormalities were identified in the SP1 cell line used by Elliot et al. (6). A marker chromosome derived from chromosome 12 was identified in all of the SP1 cells. In addition, a translocation of chromosome 8 and an isochromosome of chromosome 2 were found in some subclones of the SP1 cells (Table 1). Moreover, the SP1 cells that grew from the subcutaneous inoculations or in the mammary gland displayed differences in their cytogenetic aberrations. The metastatic SP1 cells (SP1-M) maintained the abnormalities of the SP1 cell line and exhibited new chromosomal alterations, such as translocations between chromosomes 1 and 19 and between chromosomes 10 and 18 (Table 1). An analysis of cells from lung metastases revealed the presence of both the aforementioned abnormalities and new chromosomal alterations such as translocations that were derived from chromosome 3 (6).

In summary, the analysis of cytogenetic markers has been useful in the study of clones that were selected during the metastatic process in the SP1 tumor model. The gain of mouse chromosome 8 was the most important chromosomal change reported by Frost et al. (5) and may participate in the metastatic phenotype. Nevertheless, Elliot et al. found that no chromosomal alterations were associated with metastasis (6). It should be mentioned that the complexity of the karyotype invariably increased during tumor progression.

The M05 tumor model, which originated from a virgin female BALB/c mouse, is another spontaneous mammary carcinoma with a reported karyotype (7). The M05 tumor is a semidifferentiated mammary carcinoma with areas of papillary differentiation. Two different cell lines were generated from the primary culture of one M05 tumor: a cell line with epithelial characteristics (LM05-E) and a fibroblastic cell line (LM05-F). The M05 tumor and the cell lines express hormone receptors and are estrogen-responsive; however, only the fibroblastic cell line gives rise to sarcomatoid tumors. LM05-E cells are only tumorigenic when co-injected with fibroblastic cells. The results of karyotype studies of the cell lines are shown in Table 1. LM05-E cells were tetraploid and displayed a gain of chromosome 19 and losses of chromosomes 1 and 4. Translocations that were derived from chromosome 8 and chromosome 15 were also found in these cells. LM05-F cells had a different karyotype and displayed a mode of 126−129 chromosomes and several unidentified translocations. LM05-F cells were observed to have copy number gains of chromosomes 15 and 19 and losses of chromosomes 12, 13, 14, and X. The differences in karyotypes between the epithelial and the fibroblastic cell lines suggest that the lines originated from different cells. These data indicate that LM05-F does not derive from the epithelial mesenchymal transition (EMT) of an LM05-E cell (8).

Chemically induced mammary tumor models

The administration of dimethylbenz[a]anthracene (DMBA) to virgin female (BALB/c × DBA/2) F1 mice induces mammary carcinomas with a latency of approximately 7 months. However, the combination of a synthetic analogue of progesterone, medroxyprogesterone acetate (MPA), and DMBA (MPA + DMBA) decreased the latency to 3 months and increased the incidence of mammary carcinomas. Tumors that were induced by DMBA or MPA + DMBA were mostly type B adenocarcinomas, according to Dunn's classification, and tumors that were induced by MPA + DMBA showed a normal diploid karyotype. However, a loss of heterozygosity (LOH) analysis of tumor cells from DMBA-treated mice showed that 25% and 20% of cells had chromosomal imbalances on chromosomes 4 and 8, respectively. In addition, 30% of DMBA-induced tumors showed LOH on chromosome 11 near the *Trp53* locus (Table 1). To study chromosomal changes that occur during tumor progression, syngeneic transplants of DMBA-induced tumors were performed. An increase in chromosomal aberrations was observed in transplanted tumors, and LOH was observed on chromosomes 4, 6, 7, 12, and 14. LOH on chromosome 4 was found in 66% of tumor cells, and a detailed study of this chromosome revealed the loss of the entire chromosome. The authors proposed that the loss of the *p16INK4a/Cdkn2* gene,

Table 1 Cytogenetic description of the different mouse models of breast cancer

Mouse model	Ploidy (MN)	Chromosomal abnormalities	Freq.	Cytogenetic techniques	Candidate genes	Ref.
Spontaneously						
SP1						
SP1	3n (67)	Der(3)	nd	G-banding	nd	(5)
SP1-HU and	3n (73)	Der(3); i(4); i(8)				
SP1-Az						
SP1	3n (72)	i(2); Der(8); Der(12)	nd	G-banding	nd	(6)
SP1-M	3n (65)	Rb(1;19); i(2); Der(8); Rb(10;18); Der(12)				
M05						
LM05-E	3n (76)	-1; -4; Der(8); Der(15); +19; i(19)	nd	G-banding and FISH	nd	(8)
LM05-F	5n (126)	-12; -13; -14; +15; +19; -X	nd			
Chemically induced						
DMBA						
DMBA	2n (40)	Del(4)	25	LOH	*p16INK4a*	(9)
		Del(8)	20			
		Del(11)	30			
		Del(12); Del(19)	15			
DMBA + MPA	2n (40)	Normal karyotype	nd			
1,3-Butadiene	nd	Del(11)	100	RFLP and SSLP	*Trp53*	(10)
		Del(14)	59			
Hormonally induced						
MPA	2n-4n (38-72)	Rb(2;10); t(X;2); t(4;7); t(4;12;5); t(Dp6;8); t(6;2)	6	G-banding and FISH	nd	(19)
		+3; +4; -8; -12	35			
		Der(4); Der(7); i(10); i(14)	12			
		+5	23			
		+6; -9; -10	29			
		-16	47			
		-X	53			
Genetically Engineered Mice						
MMTV-*myc*						
myc/p53$^{+/+}$	2n (42)	Dic(4); t(X;11); +18; -X	100	SKY	*Trp53*	(24)
myc/p53$^{+/-}$	2n-4n (41-64/69)	+2; +6; -9; -10; t(11;11); Del(11); Del(17); -19; +3; -6; +8; -16; -18	50		*Brca1* *ErbB-2*	
		t(5;11); Del(10); t(Dp11;1)	75			
		t(X;3)	15			
myc/p53$^{+/+}$ cell lines	2n-3n (nd)	Del(2); -4; Dic(4); +6; Del(8); +11; -17; t(X;11)	nd	CGH and SKY	*Brca1*	(25)
MMTV-*neu*						
unactivated *neu*	nd	Del(3)	29	LOH	*p16INK4a*	(28)
		Del(4)	82		*Mom-1*	
activated *neu*	nd	Del(4)	50	LOH	*p15; p16*	(30)
		Del(8)	21		*PITSLRE*	
		Del(19)	32		*Mom-1*	
					Mdgi	
					Cx37	
					Cdh1	
					Cdh15	
					Pten	

(*Continued on the next page*)

Table 1 (*Continued*)

Mouse model	Ploidy (MN)	Chromosomal abnormalities	Freq.	Cytogenetic techniques	Candidate genes	Ref.
Endogenous promoter-*neu*	2n-4n (40-87)	+2; +6; +15; +19	17	CGH and SKY	*p16INK4a* *Tp73*	(39)
		-4; Del(4)	58			
		Del(8)	41			
		Del(9); +11; Dp(11); DMs	67			
		Del(18)	8			
		Del(14)	33			
MMTV-v-Ha-*ras*	nd	Del(4)	38	SSLP	*Loh-3*	(43)
WAPRAS	2n (42)	+1; +15	32	R-banding and FISH	nd	(45)
		+2; +17	16			
		+7	11			
		+12	21			
		+19	26			
MMTV-*Brca1*$^{Ko/Co}$	2n-6n (nd)	+1; +2; +5; +9; -10; +13; -16; +18	26	CGH and SKY	*ErbB-2* c-*Myc* *Trp53* *Rb1*	(48)
		Del(1); Del(3); t(6;13); t(11;13); t(9;6)	9			
		+3; Del(4); -12; Del(11)	33			
		+6; Del(14)	40			
		+11	60			
		+15	53			
		+X	46			
		t(6;11); t(7;11)	18			
Wnt-1						
Wnt-1/p53$^{+/+}$	2n (40)	Normal karyotype	nd	CGH	*Trp53* *Fgf3*	(55)
Wnt-1/p53$^{+/-}$	2n (40)	-3; -7; -9; -13	25			
Wnt-1/p53$^{+/-}$ (LOHp53)	2n-3n (40-65)	-4; -8; -9; -10; -14	38			
		+10	25			
		-11	63			
		-13	50			
Wnt-1/p53$^{-/-}$	2n-4n (40-74)	-2; +2; +10; +14; -16	14	CGH	*Trp53* *Fgf3*	(55)
		+7	29			
		-X	43			
MMTV-PyV-mT	nd	-4	42	CGH and SKY	*Sept9*	(67)
		+11	46			
		+15	38			
		+17	23			
C3(1)/SV40 T-antigen	nd	+6	68	CGH	*Kras*	(68)

Abbreviations: MN, modal chromosome number; Freq., frequency expressed as a percentage of mammary carcinomas containing the chromosomal abnormality; Ref., reference number; Der, derivative chromosome; i, isochromosome; Rb, Robertsonian translocation; Del, deletion; t, translocation; Dp, duplication; +, gain of copy number; -, loss of copy number; Dic, dicentric chromosome; DMs, double minute chromosomes; RFLP, restriction fragment length polymorphism; SSLP, simple-sequence length polymorphism; nd, not determined.

which maps to the middle portion of chromosome 4, and the inactivation of the remaining allele could promote tumor growth during successive transplantation (9). The middle portion of mouse chromosome 4 is homologous to human chromosome 9p21, which is a region that was found to be affected by LOH in human breast cancer (10). However, the inactivation of the *p16INK4A* gene by allelic loss was observed in only a few cases of breast cancer (11).

It has been demonstrated by others that the carcinogen DMBA induces increased expression of cyclin D1 and c-Myc in mammary tumors (12). The upregulation of these oncogenic pathways, together with the inactivation of certain genes, such as *p16INK4A,* by chromosomal deletions, could promote the progression of DMBA-induced mammary tumors.

The carcinogen 1,3-butadiene induces lung and mammary carcinomas in (C57BL/6 × C3H/He) F1 mice (13). LOH studies were performed in these tumors to evaluate the possible inactivation of tumor suppressor genes during carcinogenesis. All chromosomes except chromosome 8 were affected by LOH in these mammary tumors. Allelic losses on chromosome 11, which harbors the *Trp53* gene, and chromosome 14, which harbors the *Rb1* gene, were the most frequent alterations observed in these mammary tumors (Table 1) (14). However, the *Trp53* locus, but not the *Rb1* allele, was inactivated by chromosomal deletion. These data suggested that the tumor suppressor gene *Trp53* may participate in mammary carcinogenesis induced by 1,3-butadiene.

In addition to the loss of p53 function by mutation and chromosomal deletion, the carcinogen 1,3-butadiene induces mutations in the *Hras1* (a member of the *ras* proto-oncogene family) and *Catnb* (a member of the Wnt signaling pathway) genes (15). Together, the deregulation of these genetic pathways may contribute to the development of mammary carcinomas induced by the carcinogen 1,3-butadiene.

Interestingly, the 1,3-butadiene–induced lung carcinomas, but not the 1,3-butadiene-induced mammary carcinomas, showed LOH on chromosome 4. As mentioned previously, the loss of chromosome 4 was observed in DMBA-induced mammary tumors. The differences in the cytogenetic findings between chemically induced mammary tumors may be a result of either the differential action of these carcinogens in the mammary gland or the genetic background of the mouse strains that were used in both models.

Hormonally induced mammary tumors

The continuous administration of MPA to BALB/c mice induced ductal mammary carcinomas with a latency of 1 year and an incidence of 79% (16). The carcinomas are maintained by syngeneic transplantation. These carcinomas show high levels of estrogen receptors (ERs) and progesterone receptors (PRs) and usually metastasize in axillary lymph nodes and lungs. Initially, the tumors are hormone dependent (HD) and require an exogenous hormone supply. Occasionally, tumors can begin to grow in the absence of progestins, thereby giving rise to hormone-independent (HI) variants that still express hormone receptors (17). HD tumors and some HI variants are responsive to endocrine therapy (18). Thus, the MPA breast cancer model is especially well-suited to the study of tumor progression. Cytogenetic analysis of MPA-induced mammary carcinomas revealed aneuploidy and chromosomal translocations. All of the tumors, either HD or HI, with a diploid chromosome number were responsive to the hormonal therapy. No correlation between a chromosomal alteration or the total number of chromosome aberrations and the HD or HI phenotype was observed. However, recurrent abnormalities were found in both HD and HI tumors and are shown in Table 1. Gains of chromosomes 3, 4, and 6 and losses of chromosomes 16 and X were observed in all of the tumors, and other chromosomal gains and losses were found in some of the tumors. Several translocations, which most frequently involved chromosomes 2, 4, 6, and 7, were identified. Moreover, the t(4;7) was observed in two different HI tumors (19).

Interestingly, some of the HI variants had a similar karyotype to that of the HD tumors, which suggested that the acquisition of an HI phenotype might occur independently of changes in the karyotype. The differences observed between the parental HD and the derived HI tumors may appear later during the successive in vivo transplantations.

Gene expression profiles were generated from both HD and HI tumors, and several genes were observed to be differentially regulated in both variants (20). Interestingly, some of the genes that were upregulated in the HI tumors were located on chromosomes that were gained only in the HI tumors. The most relevant genes that were associated with tumorigenesis were the *Insig1* (insulin-induced gene 1) and *Cxcl9* (chemokine C-X-C motif ligand 9) genes, which are located on chromosome 5, and the *Fbp2* (fructose bisphosphatase 2), *Plk2* (Polo-like kinase 2), and *Adcy2* (adenylate cyclase 2) genes, which map to chromosome 13.

Mammary tumors arising in genetically engineered mice

The cytogenetic study of breast cancer models in genetically engineered mice (GEM), which involves the overexpression of an oncogene or the deletion of a tumor suppressor gene, has demonstrated that secondary genetic events participate in mammary tumorigenesis. Many of these types of chromosomal abnormalities have been identified, which has led to the identification of candidate genes that may collaborate with the initial modified gene to drive the growth of mammary tumors.

Myc

The *MYC* oncogene is upregulated in 80% and amplified in 20–30% of human breast cancer samples. The MYC protein regulates the transcription of several genes and is associated with cell cycle progression and apoptosis (21). High expression levels of the c-*myc* oncogene under the control of the mouse mammary tumor virus (MMTV) promoter in the mammary gland of FBV background mice induced mammary carcinomas with a latency of approximately 6 months. The MMTV–c-*myc* transgenic mouse was one of the first mouse models of breast cancer (22). Activation of the Ras pathway caused by point mutations in the *Kras* proto-oncogene in MMTV–c-*myc*-induced mammary carcinomas cooperates with the c-*myc* oncogene in the maintenance of mammary tumor growth (23). In addition, other oncogenes or tumor suppressor genes could be deregulated by chromosomal abnormalities in this transgenic model, which may collaborate in the progression of mammary carcinomas.

The histology and karyotype of tumor cells from MMTV–c-*myc* transgenic mice can be modulated by p53. MMTV–c-*myc*/p53$^{-/-}$ transgenic mice had increased areas of lobular hyperplasia in the mammary glands. In contrast, the mammary glands of MMTV-c-*myc*/p53$^{+/+}$ or *myc*/p53$^{+/-}$ mice had multiple areas of ductal hyperplasia and some terminal ducts with lobular hyperplasia. A detailed study of the progression of these mammary carcinomas was prevented because MMTV–c-*myc*/p53$^{-/-}$ mice quickly developed lymphoma. However, MMTV–c-*myc*/p53$^{+/+}$ and *myc*/p53$^{+/-}$ mice developed mammary carcinomas with the same latency period and histology. Cytogenetic analysis of the mammary tumors in MMTV–c-*myc*/p53$^{+/+}$ or–c-*myc*/p53$^{+/-}$ mice mostly revealed structural abnormalities of chromosome 11. As shown in Table 1, MMTV-c-*myc*/p53$^{+/+}$ tumors are diploid with a few alterations, including a translocation between chromosomes X and 11, a dicentric chromosome derived from chromosome 4, trisomy of chromosome 18, and a loss of chromosome X. In contrast, the tumors induced in c-*myc*/p53$^{+/-}$ mice were polyclonal, had chromosome numbers in the triploid or tetraploid range, and had several translocations, which principally involved chromosome 11. Chromosomal deletions and other abnormalities that were observed in these tumors are shown in Table 1 (24). Significantly, the distal portion of chromosome 11, which was involved in most of the translocations observed in this model, is homologous to human chromosome 17 and contains genes that are involved

in cell growth suppression and transformation. The genes *Trp53* (band 11B2-C), *Brca1* (band 11D), and *ErbB-2* (band 11D) are commonly mutated or amplified in human breast cancer and map to this region. The loss of one p53 allele in c-*myc*/p53$^{+/-}$ tumors could promote alterations that involve chromosome 11, which is where both the *Trp53* and *Brca1* genes are located. Both genes are involved in genomic instability and could participate in the increase in ploidy and the chromosomal alterations that have been observed in these tumors compared with those in c-*myc*/p53$^{+/+}$ tumors. However, the *Trp53* gene was not affected by mutations or chromosome translocations, and only one breakpoint involved the *Brca1* gene. These data suggest that other unidentified genes located on chromosome 11 could cooperate with c-*myc* in mammary tumorigenesis (24).

In a follow-up study, the authors found that c-*myc* could induce genomic instability regardless of p53 status. The authors described several MMTV−c-*myc*/p53$^{+/+}$ tumors that contained numerous chromosomal abnormalities, as observed in the c-*myc*/p53$^{+/-}$ cell lines. The deregulation of c-*myc* expression may contribute to the deregulation of the cell cycle and of cellular division and could be involved in the occurrence of chromosomal alterations. Although all chromosomes displayed either gains or losses, a comparative genomic hybridization (CGH) analysis of the cell lines derived from the tumors revealed that the main alterations included the partial or total loss of chromosome 4 and the partial or total gain of chromosomes 6, 8, and 11 (Table 1). Subsequently, spectral karyotyping (SKY) confirmed translocations involving chromosome 11, specifically the terminal bands 11D−E; in one case, the breakpoint included the *Brca1* gene on band 11D, which was detected by fluorescence in situ hybridization (FISH). A dicentric chromosome derived from chromosome 4 and deletions in some chromosomes were also described (25). The loss of chromosome 4, particularly bands 4D−4 E, was the most evident alteration that had not previously been observed in the parental tumors (24), most likely because of the selection of a particular clone during the in vitro culture of the derived cell lines (25).

Neu

The HER-2/*neu* oncogene is amplified and overexpressed in 30% of human breast carcinomas (26). MMTV-*neu* transgenic mice, which overexpress the inactivated rat HER-2/*neu* (c-*erbB-2*) proto-oncogene in the FVB mouse background, develop metastatic mammary carcinomas with a long latency period (27). LOH analysis of the mammary tumors in this model revealed allelic imbalances, which were located primarily on chromosome 4 and included the loss of the entire chromosome in some cases, and less frequently on chromosome 3 (Table 1) (28). The MMTV-*neu* transgenic mouse model described by Bouchard et al. (29) in (BALB/c × C57BL/6) F1 mice was also used to study new genes that could act in combination with the activated *neu* oncogene to promote mammary tumorigenesis. LOH analysis primarily showed loss of the entire chromosome 4 and deletions in the terminal regions of chromosomes 8 and 19 (Table 1) (30). Tumor suppressor genes that cooperate with the *neu* proto-oncogene to drive mammary tumors could be located on these chromosomes.

Mouse chromosome 4 is homologous to regions of human chromosomes 1p, 6q, 8q, and 9p, which contain tumor suppressor genes that are involved in many cancers. These candidate genes may be deleted in this transgenic mouse model. Moreover, deletions in human chromosomes 1p and 9p were observed in human breast carcinomas (31,32). The middle portion of mouse chromosome 4 (bands 4C3−C6) is homologous to human 9p21, which harbors the tumor suppressor genes *p15INK4B/CDKN2B* and *pl6INK4A/CDKN2A*. Both of these cyclin-dependent kinase inhibitors regulate the progression of the cell cycle and cell proliferation. Moreover, low expression of p16INK4A is frequently observed in human breast cancer (10,33). In addition to chromosomal deletion, DNA methylation of the *pl6INK4A* gene was found to be the most frequent mechanism of inactivation in breast cancer, although deletions of this gene were observed in several human cancers (34). Some reports identified other tumor suppressor genes located on the 9p21−p22 region that have been proposed to participate in combination with the *pl6INK4A* gene (35).

The distal portion of mouse chromosome 4, which is orthologous to human 1p35−p36 and is frequently deleted in human breast cancer, harbors the tumor susceptibility locus *Mom-1* (modifier of Min-1) and the candidate tumor suppressor gene *PITSLRE*, which is deleted in neuroblastomas. The *Mom-1* locus was correlated with colon cancer development in the multiple intestinal neoplasia mouse model (Min), which contains the *Apc* gene mutation. The phospholipase A2 gene, *Pla2s/Pla2g2a*, is a candidate gene that maps to this locus (36). Other candidate tumor suppressor genes that are located in the deleted region of mouse chromosome 4 include *MDGI* (mammary-derived growth inhibitor) and *Cx37* (connexin 37). Moreover, the E- and M-cadherin (*Cdh1* and *Cdh15*, respectively) genes on mouse chromosome 8 and a locus near *Pten* on mouse chromosome 19 have also been suggested to participate in *neu*-induced mammary tumorigenesis (30).

Several cell lines were derived from tumors that were induced in MMTV-*neu* transgenic mice (27) and exposed to 17-β-estradiol or phytoestrogens. Estrogen treatment shortened the latency period, and the resulting tumors were more aggressive than the tumors that arose in control or soy-fed transgenic mice. CGH analysis showed gains and losses in the copy number of some chromosomes. The most frequent alterations observed in tumor cell lines from transgenic estrogen-treated mice were a gain of chromosome 10 and a loss of the entire chromosome 4. The tumors that arose in the untreated transgenic mice exhibited only a loss of chromosome 4 (37).

The conditional expression of an activated form of the *neu* oncogene under the control of the endogenous *neu* gene promoter in the mammary gland resulted in the formation of mammary carcinomas with a latency of approximately 13 months (38). Several cell lines were developed from these tumors, and analyses by molecular cytogenetic techniques (CGH and SKY) are shown in Table 1. Most of the cell lines were diploid, and some were in the diploid-triploid range. Although most of the chromosomes were affected by numerical abnormalities, the deletion of the terminal region of chromosome 4 with a breakpoint at band 4C3 and deletions close to the centromere of chromosomes 8, 9 and 14 were the most consistent alterations found. In addition, the

duplication of the chromosomal region 11D and multiple double minute chromosomes (DMs) were observed (Table 1). The results of FISH, which probed for the HER-2/*neu* gene on chromosome 11D, confirmed the amplification of this oncogene in the DMs. In contrast, the *Trp53* and *Brca1* genes, which are also located on chromosome 11, were not affected by chromosomal deletions, indicating that structural alterations in these genes were not involved in tumorigenesis in this mouse model. The genomic amplification of HER-2/*neu* was accompanied by a deletion of the distal portion of chromosome 4. As mentioned previously, this region is orthologous to human chromosomes 1p32−p36 and 9p, which are also affected by copy number loss in human breast cancer. Putative tumor suppressor genes have been identified in these regions that may be involved in the development of mammary tumors by the amplification of the *neu* oncogene (28,30), including *p16INK4A* on human chromosome 9p21. Another candidate tumor suppressor gene suggested by the authors of this model is *TP73*, which is member of the *TP53* gene family located on human chromosome 1p36 (39).

In summary, high expression of both the activated and the inactivated HER-2/*neu* proto-oncogene in the murine mammary gland by the MMTV promoter can induce mammary carcinomas. In addition, the p16INK4A signaling pathway has been implicated in the development of mammary tumors induced by HER-2/*neu* overexpression, as determined using the MMTV-*neu*−*INK4A*$^{+/-}$ mouse model (40) and suggested by the cytogenetic data that were presented in this review.

Although the pattern of chromosomal aberrations varies in each HER-2/*neu* transgenic mouse model, the loss of chromosome 4 was the most common alteration. In contrast, rearrangements of chromosome 11 were a recurring alteration in the mammary tumors described by Montagna et al. (39). In this study, overexpression of the HER-2/*neu* proto-oncogene was under the control of the endogenous promoter, which likely induces different genetic changes. In addition, the differences observed in the alterations found in these models may be due to differences in the analysis techniques used to obtain the karyotypes; cytogenetic analyses by CGH and SKY provide a more complete description of the chromosomal alterations than the LOH technique.

Ras

Gene amplification of *RAS* is a rare event in breast cancer; however, overexpression of the ras protein is observed in cells that also overexpress p53 and/or HER-2/*neu* (41). In some cases, HER-2/*neu* and Ras may cooperate to drive tumor aggressiveness. (FVB/N × *Mus musculus castaneus*) F1 mice, which contain the MMTV/v-Ha-*ras* transgene, develop mammary tumors between 4 and 28 months of age (42). LOH studies revealed the loss of genomic markers on chromosome 4 in 40% of the mammary tumors, suggesting that either deletion or loss of one copy of mouse chromosome 4 had occurred (Table 1). As was mentioned previously, the deleted chromosome is orthologous to human chromosomes 1p32−p36 and 9p21−p22, which are also affected by LOH in many breast cancer samples. Several candidate tumor suppressor genes were postulated to map to these regions. Consistent with this hypothesis, the authors determined that the *Loh-3* locus on mouse chromosome 4 contained a novel putative tumor suppressor gene; inactivation of this gene may contribute to tumorigenesis in the *ras* mouse model (43). The characteristics of tumors induced in this transgenic model could be modulated by the p53 status. Specifically, MMTV-*ras*/p53$^{-/-}$ mice developed more salivary tumors than mammary carcinomas, and the tumors had a high histological grade and an increased proliferation rate compared with *ras*/p53$^{+/+}$− or *ras*/p53$^{+/-}$−induced tumors. The tumors that grew in *ras*/p53$^{-/-}$ mice displayed heterogeneous DNA and a high degree of aneuploidy (44).

A cytogenetic study conducted in mammary carcinomas induced by the human *HRAS* proto-oncogene under the control of the murine whey acidic protein (*WAP*) promoter (*WAPRAS* mouse model) revealed that some of the tumors had a normal diploid karyotype. However, most of the tumors were diploid and displayed chromosomal abnormalities. As shown in Table 1, trisomies of chromosomes 1, 15, 17, and 19 were frequently observed and chromosome 12 trisomy was occasionally found. In this transgenic model, the *HRAS* oncogene was inserted in mouse chromosome 1, as confirmed by FISH analysis. However, not all of the chromosomes that carried the activated oncogene were observed in trisomy. Moreover, chromosome 1 was duplicated, although this chromosome was not carrying the oncogene, suggesting that the trisomies are secondary chromosomal events of the activated oncogene (45).

Structural abnormalities were also observed; however, the chromosomes involved were not identified. Interestingly, in this particular mouse model, only chromosomal gains were observed, whereas chromosomal losses are more prevalent in human breast cancer. In the *WAPRAS* model, activated *ras* may promote gains of certain chromosomes that carry genes involved in proliferation. Moreover, some of the chromosomes in trisomy, such as mouse chromosomes 1 and 15, are homologous to human chromosome arms 1q and 8q, respectively, which have been observed to be gained in human breast carcinomas. Notably, *Ras* manipulation is useful for experimental models of mammary carcinogenesis; however, in contrast to *MYC* and HER-2/*neu*, the *HRAS* oncogene is rarely activated or amplified in human breast tumors (45).

BRCA

The *BRCA1* (human breast cancer susceptibility 1) gene is associated with DNA repair, and mutations in *BRCA1* confer a predisposition to familial breast and ovarian cancer (46). The conditional deletion of *Brca1* in the murine mammary gland results in tumor formation after nearly 1 year of latency (47). This model has been used as the basal-like breast cancer model (triple-negative mammary tumors). A deficiency in p53 has been shown to accelerate tumor development in this background. The mammary carcinomas were studied by molecular cytogenetic techniques, and all of the chromosomes exhibited copy number imbalances with different frequency levels. Tumors that carried the mutated *Brca1* gene in a p53$^{+/-}$ background did not show an increased number of chromosome alterations, as expected from the loss of one p53 allele. The tumors showed chromosomal instability and heterogeneity of the alterations, with numerous chromosomal abnormalities observed only in some cells. CGH and SKY data revealed that most of the

alterations involved mouse chromosome 11, including translocations and deletions. The tumors frequently showed a gain of the terminal portion of chromosome 11 (specifically band 11D–E, which is orthologous to human 17q11-qter), which contains the *ErbB-2* gene, an amplification of the chromosome 15 bands 15D2-D3 (orthologous to human 8q24), which contain the c-*Myc* gene, and a gain of chromosome X. Loss of all or part of chromosomes 4, 12, and 14, including the *Rb1* gene on band 14D3, was also observed (Table 1). The most common structural alterations involved translocations of chromosome 11 with different partners (Table 1). These abnormalities resulted in the duplication of the distal portion of chromosome 11, which harbors the *ErbB-2* gene. However, most of the duplications involved a region that was distal to this oncogene. In one case, the translocation breakpoint was in the *Trp53* locus on chromosome 11. The other identified chromosomal alterations are listed in Table 1 (48).

The *Brca1* conditional knockout mouse model described by Brodie et al. (49) showed aneuploidy and the amplification of certain genomic regions, leading to the overexpression of the ErbB-2, c-Myc, and cyclin D1 gene products (49). The deregulation of the expression of these proteins is known to cooperate with Brca1 in the progression of mammary carcinomas (50). Expression array analysis of tumors in p53 heterozygous mice with the *Brca1* gene deleted in the mammary gland showed amplification of a locus on chromosome 6 that contains the *Met* and *Capza2* genes as DMs. A more detailed study by quantitative PCR and protein expression revealed that only the overexpression of Met was due to the chromosomal amplification. However, *MET* amplification is not observed in human breast cancer (51).

The inactivation of *Brca1* and/or *Bard1* in the mammary gland demonstrates the tumor suppressor role of the BRCA1/BARD1 heterodimer in mammary tumorigenesis. Tumor cells from *Brca1*-or *Bard1*-mutant mice showed a triploid karyotype with complex structural abnormalities, which is indicative of chromosomal instability resulting from *Brca1* or *Bard1* gene inactivation. Several translocations involving up to four chromosomes (some with more than two centromeres), Robertsonian translocations, intrachromosomal rearrangements, telomeric fusions and acentric chromosomal fragments were observed in the *Bard1* mouse model (52).

Conditional knockout of the *BRCA2* (human breast cancer susceptibility 2) gene in the mammary gland also induced mammary carcinomas after a latency of approximately 1 year, with an incidence of 77%. The tumors were invasive solid carcinomas and adenoacanthomas and had normal parenchyma and glandular growth at the periphery of the tumor. The tumors were nonmetastatic and showed an intermediate nuclear grade. Some of the tumors expressed ERα, and most tumors were PR negative (PR^-). Cytogenetic analysis revealed aneuploidy and chromosomal abnormalities, and most cells were in the hyperdiploid to tetraploid range. Translocations, dicentric chromosomes, DMs, and chromosome fragments were observed; however, the chromosomes involved in the rearrangements were not reported (53).

Wnt-1

Wnt-1 transgenic mice develop mammary carcinomas, and the latency, histology, and chromosomal instability of these tumors are modulated by p53 status. In *Wnt-1* transgenic mice, the absence of both alleles of *Trp53* shortens the latency, decreases fibrosis and increases the number of mitoses observed in the tumor cells. The presence or absence of p53 also modulates the expression of target genes involved in cell cycle regulation and cell differentiation. The expression of cyclin G1, c-kit, and p21WAF/CIP1 increased, whereas cyclin B1 expression decreased in *Wnt-1*/$p53^{+/+}$ tumors compared with that in p53 null tumors. In contrast, the expression of alpha smooth muscle actin, cytokeratin 19, and kappa casein, which are involved in differentiation, increased in *Wnt-1*/$p53^{+/+}$ tumors compared with that in $p53^{-/-}$ (54). Tumors in the *Wnt-1*/$p53^{+/+}$ mice showed a normal diploid karyotype. In tumors that arose in *Wnt-1*/$p53^{+/-}$ mice, the loss of the remaining p53 allele led to chromosomal alterations, and the tumor cells were highly aneuploid. The cytogenetic findings are listed in Table 1. The loss of genetic material on chromosome 11, which contains the *Trp53* gene, in combination with losses on chromosomes 4, 8, 9, 13, and X and copy number gains of chromosomes 7 and 10, may provide a growth advantage to these cells and could contribute to tumor progression. Most of the *Wnt-1*/$p53^{-/-}$ tumors were subtetraploid and had dicentric chromosomes and regions of gene amplification. The chromosomes affected by gains and losses are listed in Table 1. Although aneuploidy was observed in p53 homozygous tumors, there was only one case of a gene amplification that is known to cooperate with *Wnt-1*, amplification of the *int-2/FGF-3* gene, which maps to mouse chromosome 7 (55).

Trp53

Mutations in *TP53* were observed in 20–40% of human breast carcinomas. The *Trp53* gene cooperates with several oncogenes and tumor suppressor genes, as observed in the mouse models described previously, to contribute to mammary tumorigenesis. Moreover, tumors that arose in p53 heterozygous mice frequently lost the remaining p53 allele. To study possible tumor suppressor genes that cooperate with p53 in mammary tumorigenesis, LOH analysis was conducted in radiation-induced mammary carcinomas from p53 heterozygous ($p53^{+/-}$) mice. The tumor cells showed frequent losses of regions on chromosomes 8, 11, and 12. Chromosomes 5, 14, and 18 were less frequently affected by LOH. In addition, the deleted locus on chromosome 8 contains several variants of the cadherin gene (*Cdh1*, *Cdh*3, *Cdh*5, *Cdh8, Cdh11,* and *Cdh16*). This region is syntenic to human chromosome 16q22, which also showed LOH in human breast cancer samples. The authors demonstrated that cadherin 1 and 5 were not expressed in the tumors, suggesting that these genes were affected by the allelic loss (56).

Trp53 knockout mice develop lymphomas early; therefore, it is not possible to study mammary tumorigenesis in this model. However, the transplantation of p53-null ($p53^{-/-}$) mammary epithelial cells into the cleared mammary fat pad of wild type mice allows for the development of $p53^{-/-}$ mammary tumors. Several transplantable outgrowth lines that displayed ductal morphology and expressed hormonal receptors were developed from the p53-null mammary glands. These lines gave rise to ductal carcinomas when transplanted into the murine mammary fat pad (57). However, only a low percentage of the tumors retained the

hormonal receptors (58). The p53-null outgrowth lines are highly aneuploid. Array CGH and FISH evaluation of the chromosomal imbalances in one of these lines grown in vitro and the tumors derived from the p53-null mammary glands revealed amplification of the centromeric region of chromosome 8 (band 8A1) to be the most important alteration. Karyotype analysis of this cell line revealed several rearrangements derived from chromosome 8, including a large acrocentric chromosome and a small metacentric chromosome that both contained a homogeneously staining region (HSR) that hybridized to a probe for the amplified region of chromosome 8. This region is orthologous to human chromosome 13q34 and contains the genes *Cul4a*, *Lampl*, *Tfdp1* and *Gas6*, which were found to be amplified and overexpressed in human breast cancer samples (59).

Cell cycle and mitotic checkpoints

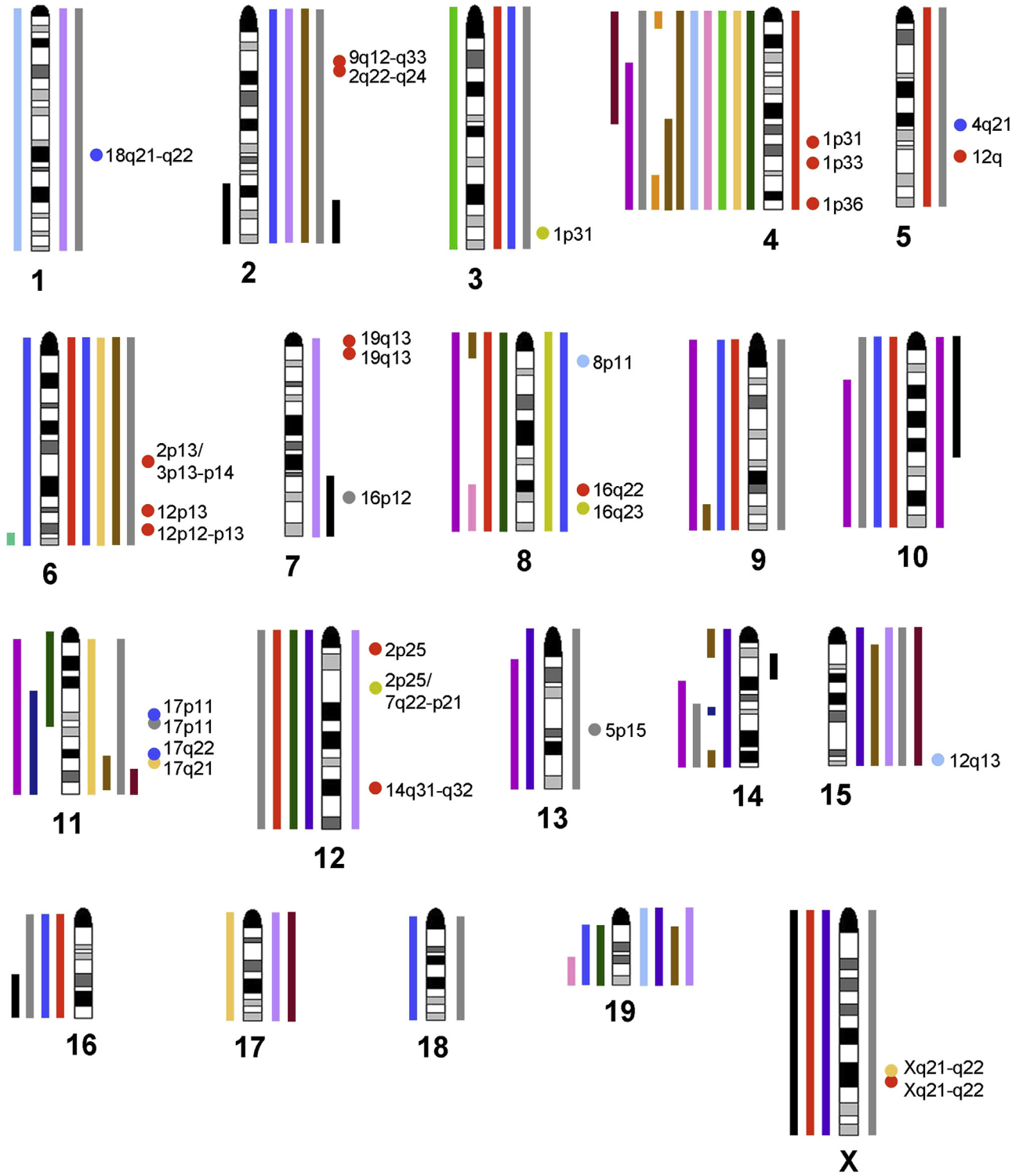
The use of mouse cell lines that overexpress genes involved in mitotic chromosome segregation has helped to clarify the role of aneuploidy in tumorigenesis. For example, the overexpression of the *ESPL1* gene, which encodes separase, in FSK-3 cells (a nontumorigenic diploid mouse mammary epithelial cell line) gave rise to tumors that expressed EMT markers and had a mesenchymal morphology. Separase is an endopeptidase that is involved in sister chromatid separation during anaphase and is overexpressed in human breast cancer samples (60). The overexpression of separase induced premature sister chromatid separation, chromosome bridges, and delays in chromosome migration during anaphase. Increases in the chromosome number, aneuploidy, and chromosomal abnormalities were observed in tumors from mice that were transplanted with separase-overexpressing FSK cells. The most frequent alterations included trisomy of chromosomes 8, 11, and 15; amplification of certain regions of chromosomes 8 and 11 that included the genes *NFATC3* and *DDX28* on chromosome 8, and *RGS9* and *AXIN2* on chromosome 11; monosomy of chromosome 10; and translocation of chromosomes 2 and 11 (61).

Another protein that has been implicated in mitosis is Aurora-B kinase, which is involved in the correct association of the kinetochores with the microtubules and is frequently overexpressed in breast cancer. To explore the role of Aurora-B kinase in tumorigenesis, nonneoplastic murine mammary epithelial cells (NMuMG cell line) were transfected with the *Aurkb* gene. The overexpression of Aurora-B in this cell line induced tetraploidy by premature chromatid separation and resulted in a few chromosomal rearrangements, including translocations involving chromosomes 3, 10, and 19. Tetraploid cells overexpressing Aurora-B gave rise to invasive mammary tumors when injected into nude mice. These tumors showed trisomies of chromosomes 15 and 19 and deletions on chromosome 1. Genes that are involved in tumorigenesis, such as the peroxiredoxin 6, *Mark-1*, tumor suppressor candidate 4 (*Npr2-like*), and *Cdh1* genes, are located in the deleted regions. In addition, the amplified regions included the genes that encode the casein kinase 1 isoform-α and the β platelet-derived growth factor receptor precursor (62).

The deregulation of *CDC25 A*, a gene that encodes a cell cycle–promoting phosphatase that activates cyclin-dependent kinases, may also contribute to aberrant mitosis and genomic instability. Murine mammary glands that overexpressed human *CDC25 A* exhibited alveolar hyperplasia and the proliferation of mammary epithelial cells. However, these animals did not develop mammary tumors. The MMTV-*CDC25 A* model was used to study the relation between this cell cycle gene and the *ras* and *neu* oncogenes in mammary tumorigenesis. The double transgenic MMTV-*CDC25 A* mouse crossed with either MMTV-H-*ras* or MMTV-*neu* mice developed mammary tumors with a shorter latency period and a more invasive phenotype than those of the single transgenic animals. The mammary tumors induced in MMTV-*CDC25 A*;MMTV-*neu* mice were evaluated by array CGH and conventional cytogenetics. The tumors displayed a tetraploid karyotype; deletions in the terminal region of chromosome 4, the centromeric region of chromosome 2, and the terminal portion of chromosome 13; and an insertion on chromosome 17. A translocation between chromosomes X and 18, t(X;18); chromosomes 10 and 15 gains; and the loss of chromosome X were observed. Array profiling identified candidate genes with low expression levels that were located in the deleted regions of chromosome 4, including *Jun*, *Casp9,* and *Trp73*. The distal portion of mouse chromosome 4 is orthologous to human chromosome 1p31–p36, which shows a copy number loss in human breast cancer. Conversely, most of the tumors induced in MMTV-*neu* mice exhibited a normal diploid or tetraploid karyotype, with the t(X;18) as the sole abnormality identified (63). The karyotype described in MMTV-*neu*-induced tumors disagrees with the data obtained from the LOH analysis performed by others using the MMTV-*neu* mouse model, as described above (29,30). The discrepancies observed in both transgenic mouse models can be explained by the different genetic background of the mouse strain that was used.

Polyomavirus middle T antigen

The expression of polyomavirus middle T antigen (PyV-mT) in the mammary gland results in the development of mammary tumors at 5 weeks of age (64). Despite the short time before tumor appearance, the expression of the *PyV-mT* gene induces hyperplastic focal premalignant lesions in the mammary gland that are more suitable to a multistep than a single-step model of carcinogenesis (65). PyV-mT interacts with growth factor signaling pathways such as the PI3 kinase pathway to promote cell proliferation. Several cell cycle proteins, such as cyclin D1, Cdk2, and zinc finger transcription factors, were overexpressed in PyV-mT mammary tumors (66). The cell lines derived from these tumors were analyzed by CGH and SKY to identify secondary genetic changes that may participate in mammary carcinogenesis in this model. Although all of the chromosomes were affected by copy number changes, the loss of the distal portion of chromosome 4 and gain of chromosomes 11, 15, and 17 were the most frequent alterations observed (Table 1). The copy number increase in chromosome 11 was limited to band 11E2. The SKY results revealed that the amplification of this region was due to duplications and translocations of chromosome 11 with different partners. Several bacterial artificial chromosome (BAC) clones were used as probes for FISH to identify the genes that were amplified on chromosome 11. A clone containing the *Sept9* gene hybridized to the amplified regions, and high levels of *Sept9* expression were observed


18q21-q22
1
9q12-q33
2q22-q24
2
1p31
3
1p31
1p33
1p36
4
4q21
12q
5
2p13/
3p13-p14
12p13
12p12-p13
6
19q13
19q13
16p12
7
8p11
16q22
16q23
8
9
10
17p11
17p11
17q22
17q21
11
2p25
2p25/
7q22-p21
14q31-q32
12
5p15
13
14
12q13
15
16
17
18
19
Xq21-q22
Xq21-q22
X
SP1 (5)
LM05-E (6)
LM05-F (6)
DMBA (9)
Butadiene (10)
MPA (19)
MMTV-myc (24)
MMTV-myc (25)
MMTV-neu (28)
MMTV-neu (30)
Neu model (39)
MMTV-ras (43)
WAPRAS (45)
MMTV-Brca1Ko/Co (48)
Wnt-1 p53+/- LOH (55)
Wnt-1 p53-/- (55)
MMTV-PyV-mT (67)
SV40 T-antigen (68)

in tumors that carried the 11E2 amplification (67). The *Sept9* gene encodes septin, a protein that participates in vesicle transportation and cytokinesis. Notably, this gene was also amplified in the MMTV−c-*myc* (25) and *Brca1* mouse models (48), which also displayed gains of the terminal portion of chromosome 11. In addition, *Sept9* was linked to the expression of the apoptotic genes *Thsp1* and *Bax*, which were downregulated in amplified *Sept9* mammary tumors in the MMTV-PyV-mT model (67).

SV40 T-antigen

The expression of the SV40 T-antigen protein under the control of the 5′- flanking region of the C3(1) component of the rat prostatic steroid−binding protein (PSBP) produces both mammary and prostate tumors in virgin mice. The mammary tumors are hormone-dependent in the early stages and then progress to hormone-independent invasive ductal carcinomas, which primarily metastasize to the lungs, on loss of ER α expression. A CGH study of these tumors revealed that the most consistent chromosomal abnormality is a gain of the distal region of chromosome 6 (Table 1) and the consequent overexpression of the *Kras* gene, which maps to this region (68).

Discussion

Breast cancer, like other cancer types, is the result of a multistep process. Specifically, a series of genetic and epigenetic changes give cells the ability to ignore homeostatic rules, to gradually increase in number, and to recruit the proper microenvironment that favors cells with the ability to invade and to disseminate. The cytogenetic study of tumors has revealed chromosomal abnormalities that may deregulate genes involved in driving carcinogenesis and has detected other alterations that may promote tumor progression or behave as passenger alterations.

Figure 1 summarizes the chromosomal abnormalities observed in the different mouse mammary tumor models. As shown in the figure, all chromosomes display a chromosomal abnormality. Chromosome 4 was found to be one of the most commonly altered chromosomes in most of the models studied. Most of the mammary carcinomas that were induced in chemically treated mice and in genetically engineered mouse models had a loss of chromosome 4 (9,28,30,39,43,48). In contrast, the MPA model displayed both a gain and translocations that involved mouse chromosome 4 (19). This deviation from the previously mentioned models might be related to the fact that these carcinomas are ductal mammary carcinomas expressing high levels of hormone receptors. Hormone treatment may induce different chromosomal alterations that activate other pathways to develop mammary tumorigenesis. The proximal region of mouse chromosome 4 is orthologous to human chromosomes 8q and 6q, the middle portion corresponds to human chromosomes 9q and 9p, and the distal region is orthologous to human chromosome 1p32−p36. These chromosomal regions were found to be altered in human breast cancer, and some candidate genes implicated in mammary tumorigenesis were mapped to these chromosomes, including the *p16INK4A* gene on human chromosome 9p21. Another chromosome that is frequently altered in mouse models is chromosome 11 (Figure 1), which is orthologous to human chromosome 17q and carries the *ERBB2*, *BRCA1,* and *TP53* genes, which are relevant in breast cancer. These findings validate the use of these mouse models for the discovery of new target genes that may be involved in breast cancer (revised in (69)).

As shown in Figure 1, mouse chromosomes 1, 7, 13, 17, and 18 are less frequently observed with gain or loss of chromosomal material. In addition, the chromosomes 5, 7, 8, 11, 12, and X are involved in translocations in two or more breast cancer models (Figure 1).

New mechanisms involved in mammary tumorigenesis can be initially discovered through the cytogenetic analysis of mouse models, and then the role of these genes can be confirmed in human breast cancer. For example, the amplification and overexpression of the septin-encoding *Sept9* gene was observed in mammary carcinoma cells from the MMTV-PyV-mT model (67). Later, the amplification and participation of this gene was also confirmed in human breast cancer (70). In addition, the genomic amplification of mouse chromosome 8A1 in p53-null outgrowth mammary tumor lines led to the identification of candidate genes that were amplified and overexpressed in human breast cancer samples (59). A more extensive study evaluated the amplification of the chromosomal region 13q34, which is orthologous to mouse chromosome 8A, in familial and sporadic human breast cancer samples and determined that the overexpression of the *TFDP1* and *CUL4A* genes, which map to the amplified region, was associated with tumor proliferation (71).

In summary, diverse aspects of human breast cancer are represented by the different mouse models presently available. Mouse models of breast cancer have also been classified based on their gene expression profiles in luminal and basal-like carcinomas (72). The DMBA model and most of the tumors that are induced in genetically modified mice (e.g., MMTV-*Wnt-1*, MMTV-*Brca1*$^{Ko/Co}$, *Trp53*$^{+/-}$ transplantable tumors) are classified as basal-like carcinomas because the tumors are hormone receptor-negative and express basal/myoepithelial markers. Surprisingly, although MMTV-*neu*, MMTV-*myc*, and MMTV-PyV-mT tumors are ER- and PR-negatives, these tumors do express luminal

Figure 1 Chromosomal alterations in the different mouse models of breast cancer. Bars on the right side of the chromosome ideograms indicate gain of chromosomal material and bars on the left side indicate loss of chromosomal material. The circles indicate the location of the breakpoints of chromosomal translocations (t) and derivative chromosomes (Der): SP1 model: Der(3H3), Der(8E1), Der(12B); LM05-E model: Der(8A2), Der(15F); MPA model: t(4C6-C7;12A2), t(12E;5 F), t(4D1;7A2), t(4E2;7A1), t(Dp6F-G;8D), t(6D;2B-C1), t(XE;2B); MMTV-*myc*: t(5E2;11B), t(XE;11B5-C), t(Dp11 C;1E2); MMTV-*Brca1* model: t(11B;13 C), t(11B;7 F). The homologies on human chromosomes are shown for each breakpoint. Each color represents one mammary tumor model, and the corresponding reference number is indicated in parenthesis. (Color versions of these illustrations are available on the journal's website at www.cancergeneticsjournal.org.)

genes, including keratin 8/18, occludin, and tight junction proteins 2 and 3. In contrast, the MPA murine model represents luminal ER^+/PR^+ mammary carcinomas (17). Moreover, comparative cytogenetic data suggest that common genetic pathways occur in human and mouse mammary tumorigenesis, as syntenic chromosomal regions are amplified or deleted in human and murine mammary carcinomas.

However, it is important to remark that, in many cases, the genetically modified breast cancer models do not necessarily model human cancer; therefore, we must be cautious in extrapolating the results that are obtained from mouse models to humans. For example, the chromosomal changes observed in human breast carcinomas that carry either the amplified HER-2/*neu* or mutations in the *BRCA1* gene are not always the same as those observed in the corresponding transgenic or knockout mouse models. We hope that future studies characterizing the chromosome alterations observed in different mouse models of breast cancer, together with new approaches in genome sequencing, will help us understand the pathways that regulate breast cancer growth and metastasis.

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