Mammary cancer in transgenic mice expressing insulin-like growth factor II (IGF-II)

P Bates, R Fisher, A Ward, L Richardson, DJ Hill and CF Graham

Correspondence: CF Graham
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Cancer Research Campaign Growth Factors, Zoology Department, South Parks Road, Oxford OX1 3PS, UK; 2Lawson Research Institute, 268 Grosvenor Street, London, Ontario, Canada N6A 4V2.

Summary The effect of insulin-like growth factor II (IGF-II) on tumour development in the mouse mammary gland was studied. To promote extra IGF-II expression in the mammary gland, sheep β-lactoglobulin regulatory elements were attached to the coding regions of the mouse Igf-2 gene and injected into the pronuclei of mouse zygotes. Mammary tumours developed in each of the four independent lines of mice which expressed transgene IGF-II in the gland. Tumours from two of the lines grew after transplantation to both male and female hosts. Primary tumours contained stromal and epithelial regions, but the tumours were dominated by mammary adenocarcinoma after transplantation. The tumours expressed high levels of IGF-2 mRNA transcribed from the integrated transgenes.

Keywords: insulin-like growth factor II; transgene; mammary tumours; breast cancer

There is no direct evidence that either insulin or the insulin-like growth factors (IGFs) contribute to cancer growth in the human breast (reviewed by Yee, 1992; Callahan and Salomon, 1993). Genetic evidence is also lacking because the IGF genes and the IGF receptor genes are not frequently amplified in mammary carcinomas and the two major familial susceptibility genes map elsewhere (Hall et al., 1990; Berns et al., 1992; Milazzo et al., 1992; Hebert et al., 1994; Wooster et al., 1994). However there is accumulating evidence that IGF-II can promote the growth of human breast cancer cell lines in culture and in xenografts (e.g. Brunner et al., 1992), and that part of this action is mediated through the type I IGF receptor (Peyrat and Bonneterre, 1992). This circumstantial evidence suggested the present experimental study.

The purpose was to find out if enhanced IGF-II expression in the mouse mammary gland caused tumours. We report that this is the case for transgenic mice, in which IGF-II expression is controlled by the sheep β-lactoglobulin regulatory elements. Although IGF-II is found at low concentration in the normal milk of humans, cows and rats (Francis et al., 1988; Donovan et al., 1991a,b; Breier et al., 1993), it now joins a growing list of oncogene products and growth factors whose excess leads to tumour formation in the mouse breast (reviewed by Wang et al., 1994; Webster and Muller, 1994).

Materials and methods

Transgene construction

The Igf-2 locus and the constructs are illustrated in Figure 1. Sheep β-lactoglobulin (BLG) promoter sequences were subcloned using a SalI site, located approximately 4 kb 5' of the transcript initiation site, and a XhoI site (formerly a PvuII site, converted using an oligonucleotide linker) within the BLG untranslated leader sequences (Ali and Clark, 1988; Harris et al., 1988; Whitelaw et al., 1992). The SalI-XhoI fragment was ligated at a 5' SalI site within a genomic subclone encompassing all three Igf-2 coding exons (Ward et al., 1994), derived from a larger fragment (Rotwein and Hall, 1990). Before pronuclear microinjection, the BLG/Igf-2 gene fusion was purified from pUC19 vector sequences as an approximately 8 kb EcoRI fragment, utilising sites located about 3 kb 5' of the BLG transcript initiation site and immediately 3' of Igf-2 genomic sequences. A probe for detecting transgenic mice on Southern blots consisted of a 692 bp XbaI to KpnI fragment containing part of exon 6 from the genomic clone (Rotwein and Hall, 1990).

Figure 1 Transgene design. The genomic Igf-2 locus (Rotwein and Hall, 1990) is displayed with transcript initiation sites (filled arrows), exons (open boxes, numbered 1 to 6), and first in-frame translational start (open triangle) and stop (filled triangle) codons indicated. The transgene construct comprises approximately 3 kb of the 5' promoter sequence from the sheep β-lactoglobulin gene (BLG) joined with the three Igf-2 coding exons, to form a transcriptional fusion (stippled rectangles mark the BLG part). The region spanning the BLG/Igf-2 sequence junction, subcloned to generate a probe construct for use in ribonuclease protection assays, has been magnified. Transcript initiation sites are given for both the BLG gene promoter (filled arrow), and the bacteriophage SP6 polymerase promoter (open arrow) used to synthesise antisense RNA probes. Regions of probes protected from RNAase degradation following hybridisation with endogenous (125 bp) and transgene (183 bp) transcripts are also shown. The probe used to detect the transgenes in DNA on Southern blots is shown at the top, together with the XbaI fragment from the endogenous (approximately 1.8 kb) and transgene locus, the latter fragment varying in size with the integration site.
Ribonuclease protection assays

A probe construct was generated by transferring sequences spanning the BGL Igf-2 transgene cloning junction into the pGEM-4Z vector. The 224 bp fragment extended from an SpI site, 45 bp upstream of the BGL transcript initiation site, to a HindIII site within the first Igf-2 coding exon (exon 4), and it was inserted at the same sites in pGEM-4Z. This allowed the generation of a 267 nucleotide antisense RNA probe with SP6 RNA polymerase, following linearisation of the probe construct with HindIII. The probe was uniformly labelled by incorporation of 32P during the polymerase reaction (Melton et al., 1984), and RNAase protection assays were performed on samples of total RNA as previously described (Ward et al., 1994). The fragments protected from ribonuclease degradation were either 183 nucleotides (transgene) or 125 nucleotides long (endogenous). In all cases RNA integrity and loading was judged with a second probe which reacts with transcripts of the housekeeping gene encoding mouse glyceraldehyde 3-phosphate dehydrogenase (mGAP; Rathjen et al., 1990). Also included were controls with cellular RNA from NIH-3T3 cells (as a source of endogenous Igf-2 transcripts), or with yeast tRNA. The sizes of protected fragments were checked using deoxyribonucleotide sequence ladders (not shown).

Transgenesis and breeding

The transgene was injected into one pronucleus of zygotes which were the product of a cross between two F1, C57BL6/CBA parents, using standard techniques (Hogan et al., 1986; Allen et al., 1987). Thirteen percent of the young contained the transgene as judged by Southern blots of tail tip DNA at 4 weeks old. Amongst these eight transgenic founders, two did not transmit the transgene, a third had young which did not express the transgene and a fourth line died out. In the four breeding lines, the transgene behaved as a single Mendelian factor, with 3–10 copies of the transgene integrated into the heterozygous mice (Laura and Leroy 10 copies, Lesley 3 copies, Lorna 5 copies). These four founder transgenic mice gave rise to permanent lines, and the lines were maintained by breeding transgene heterozygotes by F1, C57BL6/CBA partners. Female transgene heterozygotes were poor mothers and there was excess mortality of their offspring during the lactation period (Fisher et al., unpublished). Each line was usually maintained by male transmission or by using normal mothers to wet nurse the offspring of transgenic mothers.

Tumour incidence and transplantation

The females were placed with normal F1, C57BL6/CBA males and the birth of each litter was recorded. The control mice were also transgenic and they were female founders and the daughters of male founders. These control transgenics were made and maintained on exactly the same genetic background as the experimental mice. The transgene constructs in the control mice consisted of the mouse Igf-2 regulatory elements attached to a firefly luciferase reporter gene and this gene was hardly expressed in the mammary gland (unpublished).

The mammary gland tumours were visible as external lumps, and they could also be found by palpation at early stages of their growth. When the lumps reached approximately 1 cm in diameter, the tumours were removed under Avertin anaesthesia. They were cut into 5 mm diameter lumps in solution A of Dulbecco and Vogt (1954), and these were transferred beneath the dorsal skin of F1, C57BL6/CBA hosts. To find out if the endocrinological changes of pregnancy altered tumour growth, some female hosts were allowed to mate. In this case the virgin and mating female hosts each received a tumour transplant weighing approximately 0.041 g.

Histology

Whole mounts of the fourth and fifth mammary gland were prepared (Edwards et al., 1988). In addition, mammary glands and tumours were fixed in 10% formal saline or Bouin’s fixative, sectioned at 8 μm after wax embedding and stained with haematoxylin and eosin. Tumours were also fixed, stained with osmium tetroxide, sectioned at 1 μm and stained with toluidine blue as described previously (Fletcher et al., 1978).

Plasma insulin-like growth factor assays

The IGFs were separated from the binding proteins by acid gel chromatography before immunoassay of IGF-1 and IGF-II (Hill, 1990). Human recombinant IGF-1 or IGF-II were used to construct standard curves (UBI, Lake Placid, NY, USA). About 10% of the IGF-II immunoreactivity eluted at <6–8 kDa. Each IGF-I and IGF-II plasma sample was measured within two separate assays. For IGF-I, the intra- and interassay coefficients of variation were 10% and 12% respectively, while the sensitivity was 0.4 ng ml⁻¹. The cross-reactivity of IGF-II in the assay was less than 1%. For IGF-II, the intra- and interassay coefficients of variation were 8% and 13% respectively, and the minimum level of detection was 5 ng ml⁻¹. The cross-reactivity with IGF-1 in the assay was less than 1%.

Results

Breeding history and tumour incidence

Mice from each of the four lines which expressed excess Igf-2 mRNA from the transgene in the mammary gland developed mammary tumours (Table I). These and subsequent tumours were found during routine breeding of females which were heterozygous for the transgene integration site. The tumours occurred in both female founder mice (Laura and Lorna), and in female descendants of the male founders (Leroy and Lesley).

No mammary tumours were found in control mice. The control group for the β-lactoglobulin:IGF-II transgenic founders and their descendants consisted of nine other transgenic founders containing a different construct which did not express in the mammary gland (see Materials and methods).

The number of litters and the mean litter interval of these

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Table I Incidence of mammary tumours

| Transgenic line | Total no. of tumours | Age (months) when first detected | Number of new tumours found in a particular age interval (months) |
|----------------|----------------------|---------------------------------|---------------------------------------------------------------|
| Laura          | 3                    | 5, 5, 16                         | 0.17 0.17 2.13 0 5 ND                                      |
| Leroy          | 6                    | 4, 7, 8, 8, 9                    | 0.49 1.35 0.24 4.17 ND                                    |
| Lesley         | 6                    | 6, 8, 9, 10, 11, 12              | 0.37 0.37 1.27 1.6 4.5                                    |
| Lorna          | 2                    | 6, 11                            | 0.31 0.31 1.27 0.10 1.9                                    |

The number of new tumours found in a particular age interval is expressed as a ratio of the number of new tumours detected in that interval over the total number of transgenic females at that age. ND means that observations on transgenic mice without tumours did not continue up to that age and therefore a tumour incidence ratio can not be given. No mammary tumours were found in control transgenic lines which contained different constructs.
transgenic controls was similar to the experimental (3.3 weeks), and the mean age was 10 months at the end of detailed observations of these controls.

Tumour incidence was scored as the number of new tumours which were detected in the transgenic females over a particular time interval, divided by the number of age-matched transgenic females which did not develop tumours (Table I). In this table, the total number of mice decreases with age because many were used for other experiments.

Transgene expression and tumour histology

There were high levels of the transgene Igf-2 transcripts in the primary tumours found in non-lactating females (Figure 2). These levels were similar to those found in the normal lactating mammary gland of these transgenic lines (day 10-12 of lactation) and they were maintained in the transplanted tumours, as judged by the reference mGAP and endogenous Igf-2 transcripts (not shown).

Igf-2 gene expression is subject to extensive post-transcriptional control, and there is no simple relationship between mRNA and protein abundance in cell culture, human tumours or the normal mouse embryo (e.g. Haselbacher et al., 1987; Nielsen 1992; Newell et al., 1994). The expression of Igf-2 mRNA in the tumours increased the circulating levels of Igf-II protein in plasma when compared with normal virgin females or virgin transgenic females of the same lines (Fisher et al., unpublished). The extent of the increase was very variable. Three tumour-bearing mice of the Laura line were studied, and the Igf-II levels were 185, 454, and 3920 ng ml⁻¹. One tumour bearing Lorna mouse had 185 ng ml⁻¹ and a similar Lesley mouse had 386 ng ml⁻¹. Virgin transgenic mice had mean levels of 33 ng ml⁻¹, slightly higher than those found in normal mice (DaCosta et al., 1994).

The tumours were classified as mammary carcinoma type B (Squartini and Pingitore, 1994). The primary tumours contained epithelial and stromal elements but the stromal elements were reduced on transplantation.

Tumour distribution and transplantation

The tumours were first detected as single lumps but they were found in several glands at autopsy. When the initial tumours were surgically removed, tumours subsequently developed in other glands. Such a multigland distribution is also common in mice expressing extra cyclin D1 in the gland and it contrasts with most spontaneous mammary adenocarcinomas which develop in single glands (Wang et al., 1994).

It is important to establish that growths with a neoplastic appearance can grow progressively after transplantation. Three lines with tumours were tested and at least one tumour was transplanted to at least four normal female and one normal male recipient. The Laura and Lesley tumours grew progressively in each host, while the single Lorna tumour was not transplantable and the Leroy tumour was not tested.

The primary tumours had first been detected in females which had carried several litters. It was decided to find out if the hormonal changes of successive pregnancies had any effect on tumour growth. Equal volumes of one primary tumour line were transplanted to mating and virgin females (Laura line). Four months after transfer the animals were killed. The final mean tumour weight was a quarter higher in the mating females, which all carried litters (Table II). This weight increase was not significant because of the high standard deviation in both sets of recipients.

Discussion

Local IGF-II action in mammary carcinoma formation

The pattern of tumour occurrence strongly supports the conclusion that these lesions are an effect of the transgene. With each of the four separate lines suffering tumours, the tumorigenic effect is clearly integration site independent and must be a consequence of IGF-II expression. A survey of the mammary gland wet weight, lipid content and histology in these four lines has shown that there are no gross changes in the virgin female gland at around 3 months old (Fisher et al., unpublished). A detailed analysis of tumour incidence awaits further work but it is already clear that mammary carcinoma development is slow when compared with some transgenic lines which overexpress oncogene products in the mammary gland (see Table I: reviewed in Webster and Muller, 1994). However, the tumours appear much faster than those which develop after extra expression of cyclin D1 in this site (Wang et al., 1994). Some other transgenic mouse lines with excess IGF-II in adults also develop tumours: hepatocellular and other carcinomas form in the second year of life, when extra IGF-II is expressed from a major urinary protein (MUP) promoter in the liver (Rogler et al., 1994).

All four lines of transgenic mice used in the present study had more IGF-II protein in the plasma than normal mice (Fisher et al., unpublished), and the circulating growth factor might increase the incidence of mammary tumours. However, high circulating IGF-II levels are unlikely to be the immediate cause of the tumours observed in the present study because much higher levels are found in other transgenes (Rogler et al., 1994), and these do not increase the incidence of mammary tumours in the first year of life. It is therefore probable that it is the local high expression of IGF-II in the mammary gland which accelerates tumour formation.

![Figure 2 Transgene expression in tumours. A probe protection assay of IGF-II transgene expression in mammary carcinomas from each of the three transgenic lines which developed tumours. The position of the transgene transcript is marked by Endo. The position of the endogenous Igf-2 mRNA is shown (Endo). The use of a probe to the cellular RNA which codes for mouse glyceraldehyde 3-phosphate dehydrogenase (mGAP) demonstrates that Igf-2 mRNA expression is excessive in the tumours when compared with NIH 3T3 cells in culture. The probes do not react with any sequences in transfer RNA (tRNA). The mobility of probes before RNase treatment is shown in the left track (Probes).](image-url)
Tumour histology
The tumours were classified as mammary carcinoma type B (Squarantine and Pintge, 1994). The 3-dimensional architecture of the mouse mammary gland is regulated by the mesenchyme during development, while the type of cytodifferentiation of the epithelium is determined by its embryonic origin (e.g. Sakakura et al., 1976). It follows that the disorganised architecture of a tumour could be caused by a change of either partner in this interaction. Although all primary tumours displayed excess growth of both the stromal and epithelial parts of the gland, it was the epithelial elements which predominated after transplantation. For this reason, we believe that the important change was in the epithelial cells.

Transgene expression and host type
Although most of the lines first developed tumours after multiple pregnancies, the tumours could be readily transplanted into a variety of hosts which were not exposed to the hormonal changes of pregnancy. The tumours continued to express high levels of the transgene Igf-2 transcripts after transplantation to non-pregnant hosts (not shown).

Excess IGF-II is not sufficient to cause tumours in all organs
In the present work, excess IGF-II expression is shown to contribute to tumour formation in the mouse mammary gland; it is the experimental "cause" of these tumours. In contrast, excess IGF-II does not cause tumours in several other organs. In adult mice, the cell numbers of the skin and the uterus greatly increase when IGF-II is expressed in these organs, but malignant tumours do not develop from these disproportionate overgrowths (Ward et al., 1994). Further, the extent of normal fetal tissue growth depends directly or indirectly on the normal action of the endogenous Igf-2 genes (DeChiara et al., 1990; Baker et al., 1993; Lee et al., 1993). It is therefore unlikely that a single genetic change in IGF-II expression is sufficient in itself to make cells malignant, and IGF-II presumably has this tumorigenic action in the mammary gland because it increases the probability that other genetic changes will occur in this organ.

Action of IGF-II in tumour formation
The IGFs have long been known to maintain the health of cells in culture and there is now a plausible mechanism for this action (Conover et al., 1993; 1994). It is certainly possible that IGF-II's main function in tumour formation is to increase cell survival (e.g. Biddle et al., 1988; Harrington et al., 1994), and render cells competent to respond to other growth factor signals: twin actions which are emphasised by IGF-II expression in mouse pancreatic tumours (Christofori et al., 1994; Christofori and Hanahan, 1994). In mammary tumorigenesis, an effect of IGF-II on cell survival might first show up as inhibition of the apoptosis which accompanies mammary gland regression during the 4 days after weaning the young (Guenette et al., 1994). We have not yet measured this feature.

It is also possible that the mammary gland is particularly susceptible to IGF-II driven tumorigenesis because the gland is a major organ of fat metabolism (Williamson, 1991), and high carcass fat is often associated with frequent tumour development in mice (e.g. Wolff et al., 1986; Wolff 1987). IGF-II certainly has the capacity to alter lipid metabolism, with a relatively high fat content retained in organs expressing high levels of IGF-II (DaCosta et al., 1994). Although the mechanism by which high fat promotes tumour development is not understood, it might be the metabolic actions of IGF-II which account for its particular tumour promoting effects in the mammary gland.

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References

ALL S AND CLARK AJ (1988). Characterisation of the gene encoding ovine beta-lactoglobulin: similarity to the genes for the retino binding proteins. J. Mol. Biol., 199, 415-426.

ALLEN ND, BARTON SC, SURANI MAH AND REIK W (1987). Production of transgenic mice. In Mammalian Development, a Practical Approach, Monk M. (ed.) pp. 217-234. IRL Press: Oxford.

BAKER J, LIU J-P, ROBERTSON EJ AND ESFRAT LAIDIS A. (1993). Role of insulin-like growth factors in embryonic and postnatal growth. Cell, 75, 73-82.

BERNS EMU, KLUN JGM, VAN STAVERTEN IL, PORTENGHN H AND FOEKES KA. (1992). Sporicidal amplification of the insulin-like growth factor I receptor gene in human breast tumours. Cancer Res., 52, 1036-1039.

BIDDLE C, LI CH, SCHOFIELD PN, TATE VE, HOPKINS B, ENG STROM W, HUSKISSON N AND GRAHAM CF. (1988). Insulin-like growth factors and the multiplication of Tera-2, a human teratoma-derived cell line. J. Cell. Sci., 98, 475-484.

BREIER BH, MELSON SR, BLUM WF, SCHWANDER J, GALLAHER BW AND GLUCKMAN PD. (1993). Insulin-like growth factors and their binding proteins in plasma and milk after growth hormone-stimulated galactopoiesis in normally lactating woman. Acta Endocrinol., 129, 427-435.

BRUNNER R, MOSE C, CLARKE R AND CULLEN K. (1992). IGF-I and IGF-II expression in human breast cancer xenografts: relationship to hormone independence. Breast Cancer Res. Treat., 22, 39-45.

CALLAHAN R AND SALOMON DS. (1993). Oncogenes, tumour suppressor genes and growth factors in breast cancer: novel targets for diagnosis, prognosis and therapy. In Breast Cancer, Fentiman IS and Taylor-Papadimitriou J. (eds.) pp. 35-56. Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York.

CHRISTOFORI G, NAIO P AND HANAHAN D. (1994). A second signal supplied by insulin-like growth factor II in oncogene-induced tumorigenesis. Nature, 369, 414-417.

CHRISTOFORI G AND HANAHAN D. (1994). Molecular dissection of multi-stage tumorigenesis in transgenic mice. Seminars in Cancer Biology, 5, 3-12.

CONOVER CA, KIEFFER MC AND ZAPF J. (1993). Post-translational regulation of insulin-like growth factor-4 in normal and transformed human fibroblasts. J. Clin. Invest., 91, 1129-1137.

CONOVER CA, CLARKSON JT AND BALE LK. (1994). Insulin-like growth factor-II enhancement of human fibroblast growth via a non-receptor mediated mechanism. Endocrinology, 135, 76-82.

DACOSTA TM, WILLIAMSON DH, WARD A, BATES P, FISHER R, RICHARDSON L, HILL D, ROBINSON IGF AND GRAHAM CF. (1994). High plasma insulin-like growth factor-II (IGF-II) and low lipid content in transgenic mice. Measurements of lipid metabolism. J. Endocrinol., 143, 433-439.

DECHIARA TM, ESFRATIADIS A AND ROBERTSON EJ. (1990). A growth deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeted disruption. Nature, 345, 78-80.

DONOVAN SM, HINTZ RL AND ROSENFIELD RG. (1991a). Insulin-like IGF-II expression in fibrinogen. Ia IGF-1 in human milk: effect of heat treatment on IGF and IGF binding protein stability. J. Ped. Gastroent. Nutr., 13, 242-253.

DONOVAN SM, HINTZ RL, WILSON DM AND ROSENFIELD RG. (1991b). Insulin-like growth factors I and II and their binding proteins in rat milk. Pediatr. Res., 29, 50-55.

DULBECCO R AND VOGT M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med., 99, 167-182.
EDWARDS PAW, WARD JL AND BRADBURY JM (1988). Alterations of morphogenesis by the v-myc oncogene in transplants of the mammary gland. Oncogene, 2, 407–412.

FLETCHER L, RIDER C, TAYLOR CB, ADAMSON ED, LUKE BM AND-graff CF (1994). Discriminating translation of Insulin-like growth factor-II (IGF-II) during mouse embryogenesis. Mol. Reprod. Dev., 39, 249–258.

PEYRAT JP AND BONNETTERRE J (1992). Type 1 IGF receptor in human breast diseases. Breast Cancer Res. Treat., 22, 59–67.

RATHJEN PD, NICHOLS J, TOTH S, EDWARDS JR, HEATH JK AND SMITH AG (1990). Developmentally programmed induction of differentiation inhibiting activity in the control of stem cell populations. Genes Dev., 4, 2308–2318.

ROGLER CE, YANG D, ROSSETTI L, DONOHUE J, ALT E, CHANG CJ, ROSENFELD N, NEELY K AND HINTZ R (1994). Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. J. Biol. Chem., 269, 13779–13784.

ROTWEIN P AND HALL LJ (1990). Evolution of insulin-like growth factor II: characterization of the mouse IGF-II gene and identification of two pseudo-exons. DNA Cell Biol., 9, 725–735.

SAKAKURA T, NISHIZUKA Y AND DAWE CI (1976). Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. Science, 194, 1439–1441.

SQUARTINI F AND PINGITORE R (1994). Tumours of the mammary gland. In Pathology of Tumours in Laboratory Animals, vol 2: Tumours of the Mouse, VS Turusov and U: Mohr, (eds.) pp 47–100. International Agency for Research on Cancer (WHO): Lyon.

WANG TC, CARDIFF RD, ZUKERBERG L, LEES E, ARNOLD A AND SANCHEZ J (1994). Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. Nature, 369, 669–671.

WEBSTER MA AND MULLER WJ (1994). Mammary tumorigenesis and metastasis in transgenic mice. Seminars in Cancer Biology, 5, 69–76.