J Steroid Biochem. Molec. Biol. Vol. 43, No. 1–3, pp. 69–78, 1992
Printed in Great Britain.

0960-0760/92 $5.00 + 0.00

GROWTH FACTORS IN BREAST CANCER: MITOGENESIS TO TRANSFORMATION

ROBERT B. DICKSON,* MICHAEL D. JOHNSON, MOZEENA BANO, ERIC SHI, JUNICHI KUREBAYASHI, BARBARA ZIFF, ISABEL MARTINEZ-LACACI, LAUFEY T. AMUNDADOTTIR and MARC E. LIPPMAN
Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007, U.S.A.

Summary—While endocrine steroid hormones have been known for many years to regulate normal and malignant mammary epithelium, recent studies have led to an appreciation of polypeptide growth factors as locally-acting autocrine and paracrine effectors. In the current article we summarize what is known about growth factor regulation and action in the normal mammary gland and about perturbations of the steroid-growth factor interplay as cancer progresses. A major theme is that oncogenic activation modulates both regulation of production and function of growth factors in the mammary gland.

INTRODUCTION

Breast cancer arises in 1 in 9 women in North America. It is characterized by a nearly absolute dependence on intact ovaries for its onset and progression in women of all ages, but it is primarily a postmenopausal disease. The reason for this appears to be because prolonged exposure of the gland to ovarian hormones is required, but the disease is usually associated with a long latency of 20 years or more [1]. Excessive proliferation, dedifferentiation, genetic mutability, and metastases often characterize the disease when it is clinically manifest. It is not yet known what genetic defects occur early in the disease process, but ovarian hormones appear to be critical to the accumulation of genetic changes. The proliferation of the normal gland is highest in the luteal phase of the menstrual cycle, suggestive of an interaction of both estrogen and progesterone, which are present in this phase. In premenopausal disease, the ovarian hormone estrogen appears to be of primary importance in driving breast cancer proliferation. In postmenopausal breast cancer, when blood levels of ovarian-derived estrogen and progesterone are extremely low, breast tumors appear to arise under control of other factors, among which are estrogen synthesized peripherally by aromatase and sulfatase mechanisms. Candidates for additional factors regulating breast proliferation and tumorigenesis are locally acting growth factors, genetic alterations of protooncogenes, and aberrant interactions of stromal epithelial compartments [2]. Of note is the observation that of the most common oncogene activations in breast cancer (c-*erb*B2 and c-*myc* oncogene amplifications) c-*myc* amplification is associated with postmenopausal breast cancer. While c-*erb*B2 amplification is associated with particularly poor prognosis breast cancer, irregardless of menopausal status, c-*myc* does not appear to be of strong prognostic significance. This suggests that c-*myc* may function in premalignant changes and early in tumor progression and that c-*erb*B2 might be particularly important later in the progression. Alternatively, an unknown gene coamplified with c-*myc* might be of primary importance [3]. This article will focus on the potential mechanisms of action of c-*myc* in early stages of malignancy. We will address the hypothesis that c-*myc* may act, in part, by a synergistic transforming interaction with several growth factor receptor tyrosine kinase activities. This mechanism will be contrasted with that of c-*erb*B2 amplification which may act on its own, irrespective of c-*myc* amplification to contribute to other features of the cancer phenotype later in malignant progression.

Estrogen and progesterone are closely associated with breast epithelial proliferation, tumorigenesis, and malignant progression. Because of this, recent studies have begun to address the mechanisms of action of these two ovarian steroids. Numerous investigators are examining defective or overexpressed growth regulatory genes (oncogenes) and locally-acting

Proceedings of the Fourth International Congress on Hormones and Cancer, Amsterdam, The Netherlands, September 1991.

*To whom correspondence should be addressed.

polypeptide hormones (growth factors) as possible mediators and modulators of steroid action in breast cancer [4, 5]. One class of growth factors under investigation includes the transforming growth factors (TGFs). Undoubtedly a misnomer, they derive their names from their ability to reversibly induce the transformed phenotype (anchorage independent growth) in certain rodent fibroblasts. They were initially found to be synthesized and secreted by a variety of retrovirally, chemically, or oncogene-transformed human and rodent cell lines [4, 5]. They now are known to serve as locally-acting polypeptide hormones with widespread distribution and quite varied functions.

THE MANY ROLES OF TGFα AND EPIDERMAL GROWTH FACTOR (EGF) IN MAMMARY PROLIFERATION, CARCINOGENESIS AND TUMOR GROWTH

Milk, the natural secretory product of the mammary epithelial cell, is an extraordinarily rich source of growth factors including members of the TGFα, TGFβ, insulin, and mammary derived growth factor (MDGF) families. This observation suggests possible functional roles for newborn as well as for mammary gland physiology [6]. EGF and its closely related superfamily member TGFα appear to be important regulators both of the proliferation and differentiation of the mouse mammary gland *in vivo* and of mouse mammary explants *in vitro* [7, 8]. EGF or TGFα is also required as an *in vitro* supplement for the clonal anchorage dependent growth, of normal human mammary epithelial cells [9]. However, although human breast cancer cells do not require exogenous EGF for continuous growth, many retain receptors and growth stimulatory responses to exogenous TGFα and EGF [10, 11].

Biologically active, EGF/TGFα-like proteins have been extracted from mouse mammary glands undergoing lobuloaveolar growth of mid-pregnancy [7]. Studies by Vonderhaar and others have shown that TGFα can produce qualitatively the same biological effects as EGF in mouse mammary explants and cultured human and mouse mammary epithelial cell lines but TGFα appears more effective than EGF in explant cultures [7, 12], TGFα and EGF can both induce other morphogenic aspects of lobuloalveolar growth *in vitro* and *in vivo*. The localizations and potential functions of TGFα and EGF in mouse mammary development have been studied recently [13]. Different patterns of growth factor localization were observed, depending upon the individual growth factor and the stage of development ranging from virgin to mid-pregnant to mid-lactating. mRNA transcripts TGFα and EGF were both detected in virgin and mid-pregnant glands, but during lactation only EGF was expressed. TGFα was localized immunohistochemically in the proliferating, epithelial cap–cell layer of growing terminal endbuds and in stromal fibroblasts at the base of the terminal endbuds. This localization pattern strongly suggested a close association of TGFα with proliferation. In contrast to TGFα, EGF was localized in the inner layers of the terminal endbud and in luminal secretory ductal cells. This localization would strongly suggest a close association of EGF with secretion and lactation [2]. A functional role *in vivo* of EGF and TGFα has been demonstrated in the mouse with small pellet implants of the growth factors. EGF or TGFα pellet implants were able to stimulate endbud growth in regressed glands (following ovariectomy). This effect appeared to be specific, since insulin was ineffective [13, 14].

An additional study detected TGFα mRNA in mammary epithelium and some stromal cells by *in situ* hybridization. This study focused on the proliferative, lobuloalveolar development state of both rodent and human pregnancy [15]. TGFα mRNA and protein and EGF receptor are all detected *in vitro* in proliferating human mammary epithelium, but are expressed at very low levels in resting epithelial organoid remnants derived from reduction mammoplasty tissue [16, 17]. TGFα is known to act as an autocrine growth factor in normal human mammary epithelial cells in mass culture; an anti-EGF receptor antibody reversibly inhibits proliferation [17].

A new member of this growth factor superfamily, termed amphiregulin [18], has also been discovered in a breast cancer cell line treated with a tumor promoter. However, its exact physiological role in normal and malignant proliferation remains to be determined. Paradoxically, this growth factor appears to inhibit breast tumor cells, but not normal cells *in vitro* [19]. Two other members of this growth factor superfamily, a heparin-binding EGF-like macrophage derived factor [20] and a human embryonal carcinoma factor called cripto [21] have not yet been examined in breast tissue. Additionally, a 30 kDa factor which binds EGF

receptor and c-*erb* B2 has been described but not sequenced [22].

TGFα has been directly implicated as a mediator or modulator of mammary epithelial transformation in a number of studies. Overexpression of TGFα following transfection of a human TGFα DNA expression vector into the immortal, but non-tumorigenic, mouse mammary epithelial cell line NOG-8 led to its capacity for anchorage independent growth [23]. Another study utilized a newly described, spontaneously immortalized human breast ductal epithelial cell line, called MCF-10A as recipient for the TGFα gene. This cell line is negative for estrogen and progesterone receptors and contains a high level of EGF receptors. It was able to undergo morphologic transformation in response to transfection by TGFα [24]. In contrast, TGFα transfection of MCF-7 breast carcinoma cells, which have low levels of EGF receptor, does not confer a significant growth advantage *in vitro* or *in vivo* [25].

Studies of human breast cancer biopsies have established that TGFα mRNA and protein are detected in 70% or more of the specimens [26, 27] and in approx. 30% of benign breast lesions [28]. TGFα has been found by immunohistochemical techniques in fibroadenomas and 25–50% of primary human mammary carcinomas [29, 30] and an EGF related protein of 43 kDa has been recently isolated from breast cancer patient urine [31]. TGFα/EGF are subjects of future research in tumor biopsies serum or urine with the hope that will eventually be found useful in early detection, in determining prognosis, or in establishing tumor burden. While it is possible that TGFα is involved in tumor growth regulation, it is of note that TGFα itself can induce ascitic fluid [32] and contribute to angiogenesis, demoplasia, and immunosuppression [4–6].

In human breast cancer cell lines *in vitro*, unequivocal evidence of significant autocrine growth control by the TGFα–EGF receptor system has only been seen in the hormone independent MDA-MB-468 cell line, a line with high TGFα expression and an amplified EGF receptor [33]. If this observation were common in breast cancer cells, such studies would appear to have implications for developing novel therapeutic strategies. However, aside from the few percent of breast cancers overexpressing EGF receptor by such a gene amplification, this growth factor receptor system will probably not be of primary importance in autocrine growth regulation of malignant and metastatic breast cancer. For example, though hormone dependent MCF-7 cells both synthesize and respond to exogenous addition of TGFα *in vitro* and *in vivo* [34] no autocrine significance can be proven. In addition the CAMA-1 cell line responds to estrogen but does not possess EGF receptors [35]. Also, TGFα or EGF receptor gene transfection into MCF-7 or ZR 75-1 cells will not allow a significant growth advantage for the cells either *in vitro* or *in vivo* [36, 37]. It seems likely that the EGF receptor–TGFα system may be much more critical in normal gland growth and early stages of breast tumorigenesis than in the later stages of the disease. Therapeutic strategies employing EGF receptor ligands or antibodies coupled to toxins or therapeutic drugs are being actively pursued in the hope of therapeutic utility, since a large portion of hormone independent breast cancers express significant levels of this receptor even though a direct function in most breast cancer tumors of the receptor has not been proven [11, 38, 39].

EGF RECEPTOR SIGNAL TRANSDUCTION AND FUNCTION

The EGF receptor serves to regulate proliferation in multiple tissues during fetal development, adult life, and pregnancy [40]. The superfamily of this ligand–receptor system appears to be quite ancient because all 7 of the ligands described to date are structurally related to *Drosophila notch*, *delta*, and *slit* genes and to nematode *lin*-12 and *glp*-1 genes [41]. Members of this growth factor family are usually synthesized from transmembrane precursors and processed by proteolytic cleavage to yield the soluble final form. Though such processing usually occurs, it has been recently demonstrated that the uncleavaged precursor can act on the receptor of an adjacent cell in a mode of action termed "juxtacrine". Gycosylation and palmitoylation of this family of growth factors sometimes occurs. The characteristic three disulfide linkages in the secondary structure of all members of this growth factor superfamily are critical for growth factor action [42].

The EGF receptor and other members of its superfamily are transmembrane proteins with extensive external glycosylation. Signal transduction requires growth factor binding and is associated with receptor dimerization within the plane of the membrane. Signal transduction is mediated through the c-*src* oncogene-like kinase

domain, while kinase substrate specificity involves recognition by an additional region amino terminal to the c-*src* domain. Several receptor substrates of particular interest for signal transduction studies have been identified: phospholipase Cγ, phosphoinositol-3γ kinase, GAP, MAP kinase, *raf* kinase, and several growth factor receptors. Receptor function appears to be attenuated through a protein kinase C-mediated phosphorylation of a submembranous threonine residue [43]. In addition, receptors are internalized through a poorly understood mechanism involving yet another short segment intracytoplasmic region amino terminal to the kinase domain. Recognition of the receptor by coated pits results in internalization into endosomes and destruction in lysosomes [44].

It is not yet clear how genetic alterations acquired during the process of breast tumorigenesis might interact with the EGF receptor signal transduction mechanism. It seems possible that the receptor function could change as a result of modulation of receptor turnover kinetics, coupling of kinase to a particular substrate(s) or more modulation of function of a receptor substrate to alter gene control.

ADDITIONAL TYROSINE KINASE–RECEPTOR–GROWTH FACTOR SYSTEMS IN BREAST CANCER

Tyrosine kinase activity characterizes most of the cell surface receptors which appear to be important in positive regulation of mammary epithelial cells. As described above, the EGF receptor is probably the best understood. A second well known member of the EGF receptor superfamily, c-*erb*B2 also appears to be quite important, particularly in the context of its gene amplification and overexpression in malignancy [45, 46]. The structure of c-*erb*B2 is extremely similar to that of the EGF receptor. An external domain, transmembrane domain, and cytoplasmic domain with tyrosine kinase region are all present with high homology to the EGF receptor. Signal transduction through the receptor appears to occur in a manner quite similar to that of the EGF receptor. This has been documented in several studies demonstrating that in fibroblasts, hybrid receptors with EGF extracellular domain fused to c-*erb*B2 transmembrane and cytoplasmic domains can respond to EGF with production of a growth signal [47]. A new superfamily member, c-*erb*B3 has also been recently identified in breast cancer [48]. However, the implications of c-*erb*B3 for breast cancer biology or prognosis or for normal breast function are unknown at present.

Until recently, no ligands for c-*erb*B2 had been proposed. An approx. 30 kDa TGFα-related species has been isolated from the conditioned medium of the hormone independent MDA-MB-231 breast cancer cell line (and identified in some other hormone dependent and independent breast cancer cell lines [26, 49, 22, 50, 51]. When tested on cells containing EGF receptor, such as fibroblasts, normal mammary epithelial cells, and hormone dependent breast cancer cells, the purified growth factor has a stimulatory effect. However, on cells expressing high levels of c-*erb*B2 in addition to the EGF receptor, the growth factor derived from MDA-MB-231 cells appears to be biphasic: slightly stimulating and then inhibitory [22]. It is not known if inhibition can be obtained *in vivo* or if related growth factors from other breast cancer cell lines have this characteristic. The TGFα-like molecule from MDA-MB-231 cells is capable of displacing the monoclonal antibody 4D5 from its epitope on the extracellular domain of c-*erb*B2, and appears to be the first candidate ligand for c-*erb*B2 receptor [22]. The exact structure of the growth factor relationship between the gene for this protein and TGFα remains to be determined since sequencing has not been reported. An apparently similar factor has been isolated from transformed rat fibroblasts [52].

The fibroblast growth factors (FGFs) and insulin growth factors (IGFs) may also play an important role in mammary proliferation and cancer. Although FGF receptors are in normal mammary epithelial cells [52], they have not been extensively studied in breast cancer. Expression of IGF-I receptor has been correlated with good prognosis in hormone dependent breast cancer [54]. IGF-I, basic and acidic FGF as well as FGF-5 are also known to be produced by normal mammary stromal fibroblasts [53, 55]. Stroma of breast cancer patients produces IGF-II [81]. Normal mammary epithelial cells are stimulated by these growth factors. Breast cancer cell lines also have been shown to produce mRNA for most members of the FGF family as well as IGF-related activities and IGF-binding proteins [56–58] and platelet derived growth factor (PDGF) [49]. It is thought that IGF, PDGF, and FGF related growth factors may contribute

to stromal–epithelial communication in tumors. Conceivably, this communication could be bidirectional, with growth factors such as TGFα, PDGF, and FGF originating in the epithelium and stimulating the stroma. The stroma, in turn, could secrete factors such as IGF, FGF and TGFα-related factors to support early, or even later, more malignant lesions. Another major function of FGFs and other factors released by breast cancer may be in promoting tumor blood vesel infiltration, a process known as angiogenesis [59].

During development of the mammary gland, multiple roles have been suggested for some of these tyrosine kinase-related growth factors [7]. Functions for EGF, TGFα and their receptors have been previously described in both ductal growth of puberty and in lobuloalveolar growth of pregnancy/lactation. While IGF-I is important predominantly in the ductal growth, insulin is particularly important in lobuloalveolar growth. Insulin, IGF-I and IGF-II are all capable of mitogenic effects on human breast cancer as well [81–83]. Members of the FGF family are less well studied at present with respect to their effects on mammary growth and development. However, it is known that both basic and acidic FGF can stimulate normal human mammary epithelial cells in culture [52, 60]. In mouse mammary epithelial cells in culture, basic FGF has been shown to have both growth stimulatory as well as dedifferentiating effects (casein synthesis was inhibited) [61]. It has been shown that histologically normal human mammary epithelial cells are not as responsive to bFGF as breast carcinoma derived cells [60]. In addition, it is known that normal mammary epithelial cells and the milk-derived but immortalized HBL-100 all produce bFGF, but breast adenocarcinoma cell lines apparently do not. So far, only the carcinosarcoma Hs5789 was shown to produce bFGF [62].

The receptors for the insulin, PDGF and FGF families are all tyrosine kinases, but have different structures from the EGF receptor superfamily. The FGF and PDGF receptors are similar to the EGF receptor, but they have a split tyrosine kinase domain. Both of these receptors represent expanding superfamilies of receptors [62, 64]. Insulin and IGF-I receptors are tetrameric proteins; they contain two disulfide linked extracellular ligand-binding α subunits and two intracellular tyrosine kinase-containing β subunits. At present, it is not known which growth factor receptors are of primary importance in early proliferation of breast cancer versus later, in tumor progression to more malignant forms.

Another growth factor–receptor system has also been described in normal and malignant breast epithelium [65–67]. MDGF-1 is a 62 kDa glycosylated, disulfide independent growth factor purified from human milk and from primary human breast cancer. The growth factor stimulates proliferation of normal and some malignant human mammary epithelial cells. In addition, it stimulates fibroblasts to synthesize collagen but not proliferate. Its receptor is approximately 130 kDa, and receptor-binding is closely associated with tyrosine phosphorylation of a 180 kDa protein in the membrane.

INTERACTION OF *myc*, *fos*, AND *jun* NUCLEAR ONCOGENES WITH ESTROGEN AND GROWTH FACTORS

The nuclear protooncogenes, c-*myc*, c-*fos*, and c-*jun*, are of interest in human breast cancer. c-*fos* and c-*jun* form heterodimeric complexes termed AP-1, and they regulate multiple genes through a defined consensus sequence [68], c-*myc* also appears to form a heterodimeric complex with another protein called *max* (or *myn* in the murine species) to modulate a different set of genes through a different consensus sequence [69]. In many systems, c-*myc* acts to promote cell proliferation, and inhibit differentiation. On a molecular level, proliferation appears to be modulated by c-*myc* through regulation of initiation of DNA replication. The carboxyl end of the c-*myc* protein appears to be required for cell transformation and for autosuppression, one of the most clearly defined gene regulatory activities of the protein [70].

The c-*myc* protooncogene can also confer immortality to fibroblasts [71] and alter fibroblast responsiveness to growth factors [72, 73]. In human primary breast cancer, c-*myc* amplification and overexpression have been reported in 15 to 40% of tumors depending upon the study [74–77]. c-*myc* amplification has not yet proven to be definitely associated with clinical staging or other known prognostic variables, it has been found to correlate with poor prognosis in only one study so far [100]. Recent reports have suggested that c-*myc* expression may also alter resistance to *cis*-platinum and other DNA strand-scission-inducing drugs [78] and suppress

differentiation in association with suppression of collagen gene transcription [79]. c-*myc* is also able to decrease transcription of rat *neu* oncogene (c-*erb*B2 homolog in murine species) expressed in an NIH 3T3 cell line [80]. The relevance of this observation for regulation of mammary tissue or for the human c-*erb*B2 homolog is not yet known, but the study supports a role of c-*myc* as transcription regulator. c-*myc* amplification also does not seem to be associated with c-*erb*B2 amplification in primary breast cancer [81]. It is also of interest that mutationally activated c-*myc* and v-*myc* genes can suppress endogenous c-*myc* transcriptional initiation when transfected into rat-1 fibroblasts [82]. c-*myc* and N-*myc* have been recently shown to bind the retinoblastoma gene product [83].

In vitro studies have also introduced the c-*myc* gene into immortalized human or mouse mammary epithelial cells using an amphotropic retroviral vector. In mammary epithelial cells immortalized and transfected either with c-*myc* or SV40T nuclear oncogene (but not v-*ras*^{H} or v-*mos*) it was observed that the cells could be stimulated to grow in soft agar by either bFGF, aFGF, EGF, or TGFα [53, 84]. Since normal human diploid human fibroblasts produce EGF- and FGF-related growth factors [53, 55] and their conditioned media can support transforming growth of nuclear oncogene transfected mammary epithelial cells there may be relevance *in vivo* for stromal–epithelial interactions. These data may suggest that c-*myc* might function in early breast cancer lesions to allow growth factors or hormones to act to drive aberrant, transformed growth.

The nuclear protooncogenes are also induced *in vitro* when human breast cancer cell lines are stimulated to proliferate in monolayer culture by estrogen and progesterone [85–88]. Within 1 h of treatment, estrogen induces c-*fos* and c-*jun* within 30 min and c-*myc* within 1 h of treatment. It is not yet clear how these nuclear protooncogenes contribute to estrogen action. However, a recent study has addressed c-*myc* regulation during tamoxifen-induced tumor regression in breast cancer patients. This study demonstrated that tamoxifen treatment of estrogen receptor positive breast tumors resulted in a substantial decrease in c-*myc* mRNA [89]. It is possible that estrogen and progesterone may modulate cell proliferation through simultaneous modulation of growth factor and receptor synthesis and through induction of nuclear protooncogenes to sensitize the tyrosine kinase-mediated growth controls.

c-*myc* protooncogene may play an important role in breast cancer in older women. Increased amplification of c-*myc* has been observed in human breast cancer tissue in postmenopausal patients compared to premenopausal patients. It is possible that this amplification reflects cumulative proliferation and/or contributes to aberrant mitogenic responses in postmenopausal breast cancer [74]. c-*myc* expression is also enhanced in many other tissues as aging progresses [90] for unknown reasons.

In vivo models of breast cancer: transgenic mice

Transgenic mice have been established which overexpress in the mammary glands c-*myc*, v-Ha-*ras*, and activated c-*neu* (c-*erb*B2) oncogenes, alone or in combination, to assess their transforming potential *in vivo*. Two different laboratories have succeeded in overexpressing the c-*myc* protooncogene in mammary tissue using either the MMTV long terminal repeat (LTR) [91] or the whey acidic protein (WAP) gene promoters [92] to drive expression. The WAP promoter is expressed primarily during lactation, while the MMTV LTR driven by glucocorticoid or progesterone will cause expression much earlier in pregnancy, and in a wider range of tissues including salivary glands, male accessory glands, and several secretory glands of the head. In these studies, multiparous females of 2/13 separate founder MMTV-*myc* transgenic lines developed moderately well-differentiated mammary adenocarcinomas. A much higher frequency (80%) of mammary tumors was observed in 3/3 founder lines of WAP-*myc* transgenic mice, where mammary tumors were observed only 2–3 months after the initial onset of lactation. The mammary tumors in WAP-*myc* mice appeared to be well-differentiated, continuing to express *myc* and acquiring constitutive expression and secretion of β-casein. These investigations clearly establish the likelihood of c-*myc* as a potent mammary tumorigenic agent. However, the requirement in these studies for initial overexpression to occur at pregnancy–lactation, and the emergence of extremely well-differentiated tumors may represent significant deviations from the human disease. The role of transgenic c-*myc* overexpression in a system allowing effects on virgin mammary tissue and of any resulting tumors have not yet been described.

A novel *ex vivo–in vivo* approach using c-*myc* expression directed by a retroviral vector has also been utilized in mice [93] in a system allowing construction of a "transgenic" gland. Edwards *et al.* [93] transduced primary cultures of mouse mammary epithelial cells with c-*myc* expressed in a retroviral vector and then retransplanted back to cleared mammary fat pads. This technique allows regrowth of the mammary ductal network and facilitates detection of abnormalities in growth or differentiation caused by the oncogene. These studies have demonstrated that the c-*myc* infectants grow as a hyperplastic ductal network with ducts more densely packed than normal. The impact of this hyperplastic growth on development of cancer has not yet been evaluated.

Another *ex vivo–in vivo* approach [94] has utilized the v-*myc* oncogene expressed by retroviral vector in chicken embryo fibroblasts (CEF). The fibroblast growth *in vitro* in anchorage independent soft agar conditions was enhanced by EGF. In addition, when the v-*myc*–CEF cells were implanted in the chorioallatic membrane of the chicken embryo to assess growth effects *in vivo*. Coimplantation of v-*myc*–CEF with irradiated rat-1 cells secreting recombinant EGF (but not growing) led to formation of vascular, proliferation and invasive tumors. Neither v-*myc*–CEF or irradiated rat-1–EGF cells implanted alone led to tumors. This study demonstrates that v-*myc* can sensitize cells *in vivo* to the paracrine tumorigenic effects of EGF.

Transgenic mice have also been used by three laboratories to determine the effects of TGFα expression on mammary gland development and tumorigenesis. These transgenic studies complement those using *myc*, *ras* and c-*erb*B2 protooncogenes in that they test the effects of an agent thought to function primarily by inducing proliferation. Theoretically, *myc*, *ras* and c-*erb*B2 protooncogene activation and/or overexpression could be thought of as representing tumor initiation events, while TGFα overexpression could represent a tumor promotional event. Increased cell proliferation, such as that induced by a tumor promoter may function to increase cancer in the presence of background mutational events or carcinogenic initiators. In one study MMTV–TGFα transgenic mice exhibited terminal duct and alveolar hyperplasia in both virgin and pregnant animals in 3/10 founder lines. Lobular hyperplasia, cystic hyperplasia, adenoma and adenocarcinoma (with long latency) were observed [95]. Two other studies utilized the mouse metallothioneine promoter to drive TGFα expression [96,97]. In both of these studies mammary adenomas and adenocarcinomas developed in multiparous transgenic females after a long latency (6–14 months). Jhappan *et al.* [96] also reported a 2-fold increase in epithelial cell proliferation during glandular growth in addition to increased ductular branching. Furthermore, in this study a delayed epithelial penetration into the fat pad was observed in pubescent TGFα transgenic females, similar to earlier experiments in which EGF capsules were implanted near growing mammary epithelium [98].

SUMMARY

In summary, we have tried to present some of the evidence establishing that growth factors might be important local regulators of mammary growth and development. Nuclear oncogenes are emerging as important mediators and modulators of autocrine- and paracrine-acting growth factors, as well as endocrine-acting ovarian steroids. c-*myc* gene amplification, a frequent observation in breast cancer has the potential to derange these normal controls. Additional genetic changes leading to such events as amplification of c-*erb*B2 oncogene may help to complete the process of tumorigenesis.

REFERENCES

1. Pike M. D., Henderson B. E. and Casagrande J.T.: The epidemiology of breast cancer as it relates to menarche, pregnancy and menopause. In *Banbury Report 8 Hormone and Breast Cancer* (Edited by M. C. Pike, P. K. Siiteri and C. W. Welsch). Cold Spring Harbor Laboratory, NY (1981) pp. 3–21.
2. Dickson R. B. and Lippman M. E.: Molecular determinants of growth, engiogenesis, and metastases in breast cancer. *Sem. Oncol.* (1992) In press.
3. Sunderland M. C. and McGuire W. L.: Oncogenes as clinical prognostic indicators. In *Regulatory Mechanisms in Breast Cancer* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Academic Publishers, Boston (1991) pp. 3–22.
4. Paul D. and Schmidt G. H.: Immortalization and malignant transformation of differentiated cells by oncogenes *in vitro* and in transgenic mice. *Crit. Rev. Oncog.* **1** (1989) 307–321.
5. Goustin A. S., Leof E. B., Shipley G. D. and Moses H. L.: Growth factors and cancer. *Cancer Res.* **46** (1986) 1015–1029.
6. Salomon D. S. and Kidwell W. R.: Tumor associated growth factors in malignant rodent and human mammary epithelial cells. In *Breast Cancer: Cellular and Molecular Biology* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1988) pp. 363–390

7. Vonderhaar B K.· Regulation of development of the normal mammary gland by hormones and growth factors. In *Breast Cancer: Cellular and Molecular Biology* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1988) pp. 251–266.
8. Oka T., Tsutsumi O., Kurachi H. and Okamoto S.: The role of epidermal growth factor in normal and neoplastic growth of mouse mammary epithelial cells. In *Breast Cancer. Cellular and Molecular Biology* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1988) pp. 343–362.
9. Stampfer M. R.: Isolation and growth of human mammary epithelial cells. *J. Tiss. Cult. Meth.* **9** (1985) 107–115.
10. Osborne C. K., Hamilton B., Titus G. and Livingston R. B.: Epidermal growth factor stimulation of human breast cancer cells in culture. *Cancer Res.* **40** (1980) 2361–2366
11. Davidson N. E., Gelmann E. P., Lippman M. E. and Dickson R. B.: Epidermal growth factor receptor gene expression in estrogen receptor-positive and negative human breast cancer cell lines. *Molec. Endocr.* **1** (1987) 216–223.
12. Salomon D. S., Perroteau I., Kidwell W R., Tam J. and Derynck R.. Loss of growth responsiveness to epidermal growth factor and enhanced production of alpha-transforming growth factors in *ras*-transformed mouse mammary epithelial cells. *J. Cell. Physiol.* **130** (1987) 397–409.
13. Snedeker S. M., Brown C. F. and DiAugustine R. P · Expression and functional properties of transforming growth factor alpha and epidermal growth factor during mouse mammary gland doctoral morphogeneses. *Proc Natn Acad. Sci. U.S.A.* **88** (1991) 276–280.
14. Daniel C. W. and Silberstein G. B.: Local effects of growth factors. In *Regulating Mechanisms in Breast Cancer* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1991) pp. 77–92.
15. Liscia D. S., Merlo G., Ciardiello F., Kim N., Smith G. H., Callahan R. H. and Salomon D. S.: Transforming growth factor-α messenger RNA localization in the developing adult rat and human mammary gland by *in situ* hybridization. *Dev. Biol.* **140** (1990) 123–131
16. Valverius E. M., Bates S. E., Stampfer M. R., Clarke R., McCormick F., Salomon D. S., Lippman M E. and Dickson R. B : Transforming growth factor alpha production and EGF receptor expression in normal and oncogene transformed human mammary epithelial cells *Molec. Endocr* **3** (1989) 203–214.
17. Bates S. E., Valverius E. M., Ennis B. W., Bronzert D. A., Sheridan J. P., Stampfer M. R., Mendelsohn S., Lippman M. E. and Dickson R. B.: Expression of the transforming growth factor α/Epidermal growth factor receptor pathway in normal human breast epithelial cells. *Endocrinology* **126** (1990) 596–607.
18. Shoyab M., Plowman G. D., McDonald V L., Bradley J. G. and Todaro G. J.: Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science* **243** (1989) 1074–1076.
19. Plowman G. D., Green J. M., McDonald V. C., Neubauer M. G., Disteche C. M., Todaro G. J. and Shoyab M.: The amphiregulin gene encodes a novel epidermal growth factor-related protein with tumor inhibitory activity. *Molec. Cell. Biol.* **10** (1990) 1969–1981.
20. Higashigama S., Abraham J. A., Miller J., Fiddes J. C and Klagsbren M.: A heparic-binding growth factor secreted by macrophage-like cells that are related to EGF. *Science* **251** (1991) 936–9349.
21. Cioccodioola A., Dono R., Obici S., Simeone A., Zolo M. and Persico M. G.: Molecular characterization of a gene of the EGF family expressed in undifferentiated human NTERA teratocarcinoma cells. *EMBO Jl* **8** (1989) 1987–1991.
22. Lupu R., Colomer R., Zugmaier G., Slamon D. and Lippman M. E.: A ligand for the $erbB_2$ oncogene product interacts directly with both the EGF receptor and $erbB_2$. *Science* **249** (1990) 1552–1555.
23 Shankar V., Ciardiello F., Kim N., Derynck R., Liscia D. S., Merlo G., Langton B. C., Sheer D., Callahan R., Bassin R. H., Lippman M. E., Hynes N. and Salomon D. S.. Transformation of normal mouse mammary epithelial cells following transfection with a human transforming growth factor alpha cDNA. *Molec Carcinog.* **2** (1989) 1–11.
24 Ciardiello F., McGready M., Kim N., Basalo F., Hynes N., Langton B. C., Yokozaki H., Sucki T., Elliot J. W., Masui H., Mendelsohn J., Soule H., Russo J. and Salomon D.· TGFα expression is enhanced in human mammary epithelial cells transformed by an activated c-Ha-ras but not by the c-neu proto-oncogene and overexpression of the TGFα cDNA leads to transformation *Cell Growth Diff* **1** (1990) 407–420.
25. Clarke R , Brunner N., Katz D., Glenz P., Dickson R. B., Lippman M. E. and Kern F.· The effects of a constitutive production of TGFα on the growth of MCF-7 human breast cancer cells *in vitro* and *in vivo*. *Molec. Endocr.* **3** (1989) 372–380.
26 Bates S. E., Davidson N E., Valverius E. M., Dickson R. B , Freter C E., Tam J. P., Kudlow J. E., Lippman M. E and Salomon D. S.: Expression of transforming growth factor alpha and mRNA in human breast cancer: its regulation by estrogen and its possible functional significance. *Molec. Endocr.* **2** (1988) 543–555.
27 Gregory H., Thomas C. E , Willshire I R., Young J. A., Anderson H., Baildan A. and Howell A.. Epidermal and transforming growth factor α in patients with breast tumors *Br. J. Cancer* **59** (1989) 605–609
28. Travers M. R., Barrett-Lee P. J., Berger U., Luqmani Y A., Gazet J.-C , Powles T. J. and Coombes R C.· Growth factor expression in normal, benign, and malignant breast tissue. *Br. Med. J.* **296** (1988) 1621–1630
29. Perroteau I., Salomon D., DeBortoli M., Kidwell W., Hazarika P , Pardue R , Dedman J and Tam J.· Immunological detection and quantitation of alpha transforming growth factors in human breast carcinoma cells *Breast Cancer Res. Treat* 7 (1986) 201–210.
30. Macias A., Perez R., Hagerstrom T and Skoog L.· Identification of transforming growth factor alpha in human primary breast carcinomas. *Anticancer Res.* **7** (1987) 1271–1280.
31. Eckert K , Granetzny A., Fischer J., Nexo E. and Grosse R.: An Mr 43,000 epidermal growth-factor related protein purified from the urine of breast cancer patients. *Cancer Res.* **50** (1990) 642–647
32. Ohmura E., Tsushima T., Kamiya M , Onada N., Shizume K., and Demura H.: Epidermal growth factor and transforming growth factor alpha induce ascitic fluid in mice. *Cancer Res.* **50** (1990) 4915–4917
33. Ennis B. W., Valverius E. M., Lippman M. E., Bellot F., Kris R., Schlessinger J., Masui H., Goldberg A., Mendelsohn J. and Dickson R. B.: Anti EGF receptor antibodies inhibit the autocrine stimulated growth of MDA-MB-468 breast cancer cells. *Molec. Endocr.* **3** (1989) 1830–1838.
34. Dickson R. B., McManaway M. E. and Lippman M. E.: Estrogen-induced factors of breast cancer cells partially replace estrogen to promote tumor growth. *Science* **232** (1986) 1540–1543.
35. Leung B. S., Stout L., Zhou L., Ji H. J., Zhang Q. Q. and Leung H. T.: Evidence of an EGF/TGFα-independent pathway for estrogen-regulated cell proliferation. *J. Cell. Biochem.* **46** (1991) 125–133.

36. Valverius E. M., Velu T., Shankar V., Ciardiello F., Kim N. and Salomon D. S.: Overexpression of epidermal growth factor receptor in human breast cancer cells fails to induce an estrogen-independent phenotype. *Int. J. Cancer* **46** (1990) 712–718.
37. Clarke R., Brunner N., Katz D., Glanz P., Dickson R. B., Lippman M. E. and Kern F. G.: The effects of a constitutive expression of transforming growth factor alpha on the growth of MCF-7 human breast cancer cells *in vitro* and *in vivo*. *Molec. Endocr.* **3** (1989) 372–380
38. Sainsbury J. R., Farndon J. R., Needham G. K., Malcolm A. J. and Harris A. L.: Epidermal-growth-factor receptor status as predictor of early recurrence of and death from breast cancer. *Lancet* **i** (1987) 1398–1402.
39. Perez R., Pascual M., Macias A. and Lage A.: Epidermal growth factor receptors in human breast cancer. *Breast Cancer Res. Treat.* **4** (1984) 189–193.
40. Fisher D. A. and Lakshmanan J.: Metabolism and the effects of epidermal growth factor and related growth factors in mammals. *Endocrine Rev.* **11** (1990) 418–431.
41. Todaro G. J., Rose T. M., Spooner C. E., Shoyab M. and Plowman G. D.: Cellular viral ligands that interact with the EGF receptor. *Sem. Cancer Biol.* **1** (1990) 257–264.
42. Luetteke N. C. and Lee D. C.: Transforming growth factor alpha expression, regulation and biological action of its integral membrane precursor. *Sem. Cancer Biol.* **1** (1990) 265–276.
43. Nishibe S. and Carpenter G.: Tyrosine phosphorylation and the regulation of cell growth: growth factor-stimulated tyrosine phosphorylation of phospholipase C. *Sem. Cancer Biol.* **1** (1990) 285–292.
44. Segatto O., Leonardo F., Wexler D., Fazioli F., Pierce J. H., BoHaro D. P., White M. F. and Difiore P. P.: The Juxtamembrane regions of the EGF receptor and gp185erbB2 determine the specificity of signal transduction. *Molec. Cell. Biol.* **11** (1991) 3191–3202.
45. Slamon D. J., Godulphin W., Jones L. A., Holt J. A., Wong S. G., Keith D. E., Levin W. J, Stuart S. G., Udove J., Ullrich A. and Press M. J.: Studies of the HER-2/neu protooncogene in human breast and ovarian cancer. *Science* **244** (1989) 621–624.
46. Paik S., Hazan R., Fisher E. R., Sass R. E., Fisher B., Redmond C., Schlessinger J., Lippman M. E. and King C. R.: Pathologic findings from the National Surgical Adjuvant breast and bowel project: prognostic significance of erbB_2 protein overexpression in primary breast cancer. *J. Clin. Oncol.* **8** (1990) 103–112.
47. Lehvaslaiho H., Lehotola L., Sistonen L. and Alitalo K.: A chimeric EGFR/neu proto-oncogene allows EGF to regulate new tyrosine kinase and cell transformation *EMBO Jl* **8** (1989) 159–166.
48. Kraus M. H., Issing W., Miki T., Popescu N. C. and Aaronson S. A.: Isolation and characterization of *ERBB3*, a third member of the ERB/epidermal growth factor receptor family: evidence for overexpression in a subset of human mammary tumors. *Proc. Natn Acad. Sci. U.S.A.* **86** (1989) 9193–9197.
49. King R. J. B., Wang D. Y., Daley R. J. and Darbre P. D.: Approaches to studying the role of growth factors in the progression of breast tumors from the steroid sensitive to insensitive state. *J. Steroid Biochem.* **34** (1989) 133–138.
50. Dickson R. B., Kasid A., Huff K. K., Bates S., Knabbe C., Bronzert D., Gelmann E. P. and Lippman M. E.: Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17-β-estradiol or v-*ras*^{H} oncogene. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 837–841.
51. Lupu R., Dickson R. B. and Lippman M. E.: Biologically active glycosylated TGFα released by estrogen receptor negative human breast cancer cell line. *UCLA Symp. Growth Regul. Cancer* (1989) (Abstr.).
52. Yarden Y. and Peles E.: Biochemical analysis of the ligand for the new oncogenic receptor. *Biochemistry* **30** (1991) 3543–3550.
53. Valverius E. M., Ciardiello F., Heldin N. E., Blondel B., Merlo G., Smith G., McGready M., Stampfer M. R., Lippman M. E., Dickson R. B. and Salomon D. S.: Stromal influences on transformation of human mammary epithelial cells expressing c-*myc* and SV40T. *J Cell. Physiol.* **145** (1990) 207–216.
54. Foekens J. A., Portengen M., Janssen M. and Klijn J. G. M.: Insulin-like growth factor I receptors and insulin-like growth factor I activity in primary human breast cancer. *Cancer* **63** (1989) 2139–2147.
55. Cullen K. J., Hill S., Paik S., Smith H. S., Lippman M. E. and Rosen N.: Growth factor mRNA expession by human breast fibroblasts from benign and malignant lesions. *Proc. Am. Assoc. Cancer Res.* (1990) (Abstr.).
56. Yee D., Cullen K. J., Paik S., Perdue J. F., Hampton B., Schwartz A., Lippman M. E. and Rosen N.: Insulin-like growth factor II mRNA expression in human breast cancer. *Cancer Res.* **48** (1988) 6691–6696.
57. Huff K. K, Lippman M. E., Spencer E. M. and Dickson R. B.: Secretion of an insulin-like growth factor I-related polypeptide by human breast cancer cells. *Cancer Res.* **46** (1986) 4613–4619.
58. Yee D., Rosen N., Favoni R. and Cullen K. J.: The insulin-like growth factors, their receptors and their binding proteins in breast cancer. In *Regulation of Breast Cancer* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1990) pp. 93–106.
59. Folkman J. and Klagsbrun M.: Angiogenic Factors. *Science* **235** (1987) 442–447.
60. Tukahashi K., Suzuki S, Kawahara S. and Ono T.: Growth stimulation of human breast cancer epithelial cells by basic fibroblast growth factor in serum free medoim. *Int. J. Cancer* **43** (1989) 870–874.
61. Levay-Young B. K., Imagawa W., Wallace D. R. and Nandi S.: Basic fibroblast growth stimulates the growth and inhibits casein accumulation in mouse mammary epithelial cells *in vitro*. *Molec. Cell. Endocr.* **62** (1989) 327–336.
62. Li S. and Shipley G. D.: Expression of multiple species of basic fibroblast growth factor mRNA and protein in normal and tumor-derived mammary epithelial cells in culture. *Cell Growth Diff.* **2** (1991) 195–202.
63. Robinson C. J.: Multiple receptors found for growing FGF family. *Trends Pharmac. Sci.* **12** (1991) 123–125.
64. Thomas K. A.: Transforming potential of the fibroblast growth factor genes. *Trends Biochem. Sci.* **13** (1988) 327–328.
65. Bano M., Kidwell W. R., Lippman M. E. and Dickson R. B.: Characterization of MDGF-1 receptor in human mammary epithelial cell liver. *J. Biol. Chem.* **265** (1990) 1874–1880.
66. Bano M., Solomon D. S. and Kidwell W. R.: Purification of mammary derived growth factor 1 (MDGF 1) from human milk and mammary tumors. *J. Biol. Chem.* **260** (1985) 5745–5752.
67. Bano M., Lupu R., Kidwell W. R., Lippman M. E. and Dickson R. B.: Characterization of MDGF1 and its receptor in human breast cancer cells. *Proc. Am. Assoc. Cancer Res.*, Washington, D.C. (1991) (Abstr.)
68. Jones N. Structure and function of transcription factors. *Sem. Cancer Biol.* **1** (1990) 5–17.
69. Prendergast G. C., Lawe D. and Ziff E. B.: Association of *myn*, the murine homologue of max with c-myc stimulating methylation-sensitive DNA binding and ras cotransformation. *Cell* **65** (1991) 395–407.
70. Penn L. J. Z., Laufer E. M. and Land H.: C-myc: evidence for multiple regulating functions. *Sem. Cancer Biol.* **1** (1990) 69–80.

71. Kelekar A. and Cole M. D.: Immortalization by c-*myc*, H-*ras*, and Ela oncogenes induces differential cellular gene expression and growth factor responses. *Molec. Cell Biol.* **7** (1987) 3899–3907.
72. Leof E. B., Proper J. A. and Moses H. L.: Modulation of transforming growth factor type β action by activated *ras* and c-*myc*. *Molec. Cell Biol.* **7** (1987) 2649–2652.
73. Stern D. F., Roberts A. B., Roche N. S., Sporn M. B. and Weinberg R. A.: Differential responsiveness of *myc*- and *ras*-transfected cells by epidermal growth factor *Molec. Cell. Biol.* **6** (1986) 870–877.
74. Escot C., Theillet C., Lidereau R, Spyratos F., Champeme M. H., Gest J. and Callahan R.: Genetic alteration of the c-*myc* proto-oncogene in human primary breast carcinomas. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 4834–4838.
75. Bonilla M., Ramirez M., Lopez-Cuento J. and Garıglıo P.: *In vivo* amplification and rearrangements of c-*myc* oncogene in human breast tumors. *J. Natn. Cancer Inst* **80** (1988) 665–671.
76. Cline M., Battifora H. and Yokota J. J.· Protooncogene abnormalities in human breast cancer: Correlations with anatomic features and clinical course of diagnosis *J. Clin. Oncol.* **5** (1987) 999–1006.
77. Varlay J. M., Swallow J. E., Brammer V. J., Wittaker J. L. and Waekor R. A.: Alterations to either c-*erb*B_2 (*neu*) short term prognosis. *Oncogene* **1** (1987) 423–430
78. Sklar M. D. and Prochownik E. V.: Modulation of *cis*-platinum resistance in friend erythroleukemia cells by c-*myc*. *Cancer Res.* **51** (1991) 2118–2123.
79. Yang B. S., Geddes T. J., Pogulis R. J, de Crombrugghe B. and Freytag S. O.: Transcriptional suppression of cellular gene expression by c-*myc* *Molec Cell. Biol.* **11** (1991) 2291–2295.
80. Suen T. C. and Hung M -C.: c-*myc* reverse neu-induced transformed morphology by transcriptional repression *Molec. Cell. Biol.* **11** (1991) 354–362.
81. Gullick W. J., Tuz N. C., Kumar S., Paterson H, Quirke P. and Vanter D. J : c-*erb*B_2 and c-*myc* genes and their expression in normal tissues and in human breast cancer. *Cancer Cells Cold Spring Harbor* **7** (1989) 393–398.
82. Penn L. J. Z., Brooks M. W., Laufer E M. and Land H.: Negative autoregulation of c-*myc* transcription. *EMBO Jl* **9** (1990) 1113–1121.
83. Rusty A. K., Dyson N. and Bernands R.: Amino terminal domains of c-*myc* and N-*myc* proteins mediate binding to the retinoblastoma gene product. *Nature* **352** (1991) 541–544.
84. Telang N. T., Osborne M. P., Sweterlitsch L. A. and Tarayanan R.: Neoplastic transformation of mouse mammary epithelial cells by deregulation *myc* expression. *Cell Regul.* **1** (1990) 863–872
85. Weisz A., Cicatiello L., Perisco E., Scalona M. and Bresciani F.: Estrogen stimulates transcription of c-*jun* protooncogene. *Molec. Endocr.* **4** (1990) 1041–1050.
86. Musgrove E. A. and Sutherland R. L : Steroids, growth factors and cell cycle controls in breast cancer. In *Regulation of Breast Cancer* (Edited by M. E. Lippman and R. B. Dickson). Kluwer Press, MA (1990) pp. 305–331.
87. Dubik D., Dembinski T C. and Shiu R. P. C.: Stimulation of c-*myc* oncogene expression associated with estrogen-induced proliferation of human breast cancer cells. *Cancer Res.* **47** (1987) 6517–6521.
88. Wong M. S. J and Murphy L. C.: Differential regulation of c-*myc* by progestins and antiestrogens in T47D human breast cancer cells. *J. Steroid Biochem. Molec. Biol.* **39** (1991) 39–44
89. LeRoy X., Escot C., Brouillet J. P., Theillet C., Maudelonde T., Simony-Lafontaine J., Pujol H. and Rochefort H.: Decrease of c-*erb*B2 and c-*myc* RNA levels in tamoxifen-treated breast cancer. *Oncogene* **6** (1991) 431–437
90. Semsei I., Ma S. and Cutler R. G.· Tissue and age specific expression of the *myc* proto-oncogene family throughout the lifespan of the C57BL/6J mouse strain. *Oncogene* **4** (1989) 465–470.
91. Stewart T. A., Pattengale P. K. and Leder P.: Spontaneous mammary adenocarcinoma in transgenic mice that carry and express MTV/myc fusion genes. *Cell* **38** (1984) 627–637.
92. Schonenberger C. A., Andres A. C., Groner B., van der Valk M., LeMeur M. and Gerlinger P.. Targeted c-myc gene expression in mammary glands of transgenic mice induces mammary tumors with constitutive milk protein gene transcription. *EMBO Jl* **7** (1988) 169–175
93. Edwards P. A. W., Ward J. L. and Bradbury J. M.: Alteration of morphogeneses by the v-*myc* oncogene in transplants of mammary gland. *Oncogene* **3** (1988) 407–412.
94. Khazaie K., Panayotou G., Aguzzi A., Samarat J, Gazzolo L. and Jurdie P· EGF promotes *in vivo* tumorigenic growth of primary chicken embryo fibroblasts expressing v-*myc* and enhances *in vitro* transformation by the v-*erb*A oncogene. *Oncogene* **6** (1991) 21–28
95. Matsui Y., Halter S. A., Holt J T., Hogan B. L. and Coffey R. J.. Development of mammary hyperplasia and neoplasia in MMTV-TGF alpha transgenic mice. *Cell* **61** (1990) 1147–1155.
96 Jhappan C., Stahle C., Harkins R. N., Fausto N., Smith G. H. and Merlino G. T.: TGF alpha overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* **61** (1990) 1137–1146.
97. Sandgren E. P., Luetteke N. C., Palmiter R. D., Brinster R. L. and Lee D. C.: Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* **61** (1990) 1121–1135.
98. Coleman S. and Daniel C W.: Inhibition of mouse mammary ductal morphogenesis and down regulation of the EGF receptor by epidermal growth factor. *Dev Biol.* **137** (1990) 425–433