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Influence of estradiol on mammary tumor collagen solubility in DMBA-induced rat mammary tumors

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Abstract

Estradiol plays a vital role in the growth and development of mammary glands. It is a potent stimulator of metabolic processes in normal and carcinoma breast. A critical factor in determining mammary glandular morphology is the stroma. Collagen is a predominant component of the extracellular matrix and cell–collagen interactions are essential carcinogenesis. The present investigation explored the influence of estradiol on collagen solubility and metabolism in mammary tumors during tumor progression and regression. A single injection of 20 mg of 9,10-dimethyl-1,2-benzanthracene was given to rats at 7 weeks of age. With the appearance of the first palpable mammary tumor, the rats were treated with 0.5 μg estradiol or 50 μg tamoxifen daily for 30 days. The rats were sacrificed 24 h after 30 days of treatment. Estradiol appears to stimulate the synthesis of new collagens and thus contributes to the enlargement of the mammary tumors. This might have created a potential microenvironment by increasing the synthesis of suitable matrix that sustains the growth of the mammary tumors. In short, the present findings emphasize a definite mediatory role for collagen in estradiol promoted mammary tumor growth.
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Keywords: Estradiol; Mammary tumor; Collagen; Extracellular matrix

1. Introduction

Many experimental approaches have been developed to study mammary tumorigenesis over the last several decades. A major advance was made by Huggins' group (Huggins et al., 1961) when they reported a simple method to induce mammary tumors in almost 100% of the animals using a chemical carcinogen. The mammary tumor induced by single administration of dimethylbenzanthracene (DMBA) is a widely accepted model of experimentally induced mammary tumors (Dauvois et al., 1989; Clarke, 1996; Russo and Russo, 1996). The most notable characteristic of DMBA-induced mammary tumors is their hormonal dependence. In this respect, this model system is more advantageous in understanding hormone dependent breast carcinoma (Jensen et al., 1975; McGuire et al., 1975). Normal development and function of the breast requires the coordinated action of various hormones. Abnormal hormonal environment is suggested to be responsible for the induction of mammary cancer and also for the clinical course of the disease (Deshpande, 1975; Nandi et al., 1995). Estradiol plays a vital role in the growth and development of mammary glands. It is a potent stimulator of metabolic processes in normal (Porter, 1974) and carcinoma breast (McGuire et al., 1975). Estradiol regulates growth and other activities thought to mediate mitogenic and metastatic events (Lippman et al., 1987; Van Slooten et al., 1995). Estradiol has a paradoxical effect on the growth of breast cancer. In low physiological doses, estradiol stimulates mammary cancer growth in rats (Hartmann, 1983), while administration of high doses of estradiol induces tumor regression, both in human breast cancers (Hawkins et al., 1980) and in the DMBA- and MNU-induced rat mammary tumor (Meites et al., 1971; Guzman et al., 1999; Rajkumar et al., 2001).

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Sakakura et al. (1976) have shown that the stroma is the critical factor in determining mammary glandular morphology. The extracellular matrix produced by mammary tumor cells has displayed different quantitative and qualitative characteristics compared with normal cells (David and Bernfield, 1982; David and Van Den Berghe, 1983; Castronovo et al., 1989). Collagen, one of the predominant components of the extracellular matrix provides the essential framework of connective tissue (Fleischmajer et al., 1990). Cell–collagen interactions are essential for cell movements in inflammation, wound healing, trophoblast implantation, fetal development and cancer (Anderson, 1993). Formation of collagen is characteristic of differentiated tumors such as those induced by DMBA (Bano et al., 1983). *cis*-Hydroxyproline blocks collagen synthesis 10 times more efficiently than total protein synthesis in cultured mammary epithelium and blocks the growth of DMBA- and MNU-induced mammary tumors in vivo (Lewko et al., 1981; Wicha et al., 1981). This clearly indicates that collagen is essential for the growth of mammary tumors. It has also been reported that collagen along with other extracellular matrix components undergoes cyclic changes during the menstrual cycle indicating the hormonal influence on collagen (Ferguson et al., 1992). From the foregoing literature it is clear that hormones and extracellular matrix are involved in the process of mammary tumor growth and development. Though numerous studies have demonstrated the influence of hormones on various aspects of mammary tumors, relatively not much work has been directed solely towards the hormonal influence on collagen solubility and metabolism in mammary tumors. Because, immature soluble collagen forms the matrix for tumor development and mature insoluble collagens are inhibitory for tumor progression and invasion, it would be of interest to study whether alterations in solubility and metabolism of collagen could be implicated in the progression and regression of hormone dependent mammary tumors. Hence the present investigation is aimed to study the influence of estradiol on collagen solubility and metabolism in mammary tumors during tumor progression and regression.

2. Materials and methods

2.1. Animals

Virgin Wistar rats were maintained in a temperature-controlled room with 12 h light and 12 h dark schedule, in well-ventilated cages. They were fed with standard rat pellet diet (Gold Mohur, Hindustan Lever, India), and drinking water was made available ad libitum.

2.2. Carcinogen treatment

A single injection of 9,10-dimethyl-1,2-benzanthracene (Sigma) was given at a dose of 20 mg/animal into the air pouch to all rats at 7 weeks of age (Arun et al., 1984). DMBA was dissolved in sesame oil.

2.3. Hormone treatment

All hormone treatments were given by intramuscular injection. Estradiol was dissolved in propylene glycol, and Tamoxifen was dissolved in saline. Control rats received the vehicle treatments. With the appearance of the first palpable mammary tumor, the rats were treated with 0.5 μg estradiol or 50 μg tamoxifen daily for 30 days. Each group consisted of 5 rats. The rats were sacrificed by decapitation after 30 days of treatment.

2.4. Biochemical analysis

Mammary tumors were excised after the different treatments, and the tissues were used for further biochemical analysis. The neutral salt soluble collagen was extracted with 0.5 M Tris–HCl buffer (pH 7.2) containing 1.0 M NaCl. The residue left after NaCl extraction was again extracted with 0.5 M citrate buffer (pH 3.6) to remove acid soluble collagen. The insoluble collagen was calculated by subtracting the sum of soluble collagen from that of total collagen. Tissues were also used for measuring the concentrations of ascorbic acid and lactate using standard spectrophotometric methods.

2.5. Hormone assay

Blood samples were collected from the different groups of animals on the day when the animals were sacrificed. Serum was separated, frozen and assayed for 17β estradiol and progesterone by using the solid phase RIA kit purchased from Diagnostic Products Corporation (Los Angeles, USA). Prolactin was measured by radioimmunoassay using reagents obtained from NIDDK.

2.6. Mammary carcinogenesis

Rats were palpated twice every week beginning 1 month after carcinogen exposure until the end of the experiment. Histopathological examination was performed to confirm the carcinomatous nature of the palpable tumors.

2.7. Statistics

The effects of the different treatments were analyzed by using one way ANOVA followed by the multiple range test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Serum hormone

The data on serum estradiol or progesterone or prolactin show that DMBA-induced tumor bearing rats did not show any significant change compared to control rats. The increase in the levels of prolactin after estradiol administration is in agreement with earlier findings (Chen and Meites, 1970; Meites, 1972), as estradiol is a well-known stimulator of prolactin secretion. A significant increase in serum estradiol levels of tumor bearing rats was observed after estradiol treatment when compared with untreated tumor bearing rats (Table 1). There was no significant fall in serum estradiol levels in the tumor bearing rats treated with tamoxifen when compared with untreated tumor bearing rats. Administration of

Table 1
Effect of estradiol treatment on serum levels of estradiol, progesterone and prolactin in DMBA-induced tumor bearing rats (*$p < 0.05$)

Group/hormone	Estradiol (pg/ml), mean ± SEM	Progesterone (ng/ml), mean ± SEM	Prolactin (ng/ml), mean ± SEM
Intact control	18.1 ± 3.9	10.4 ± 2.1	22.1 ± 6.2
DMBA control	20.5 ± 4.6	11.5 ± 1.9	24.1 ± 5.3
DMBA + estradiol	84.2 ± 10.2*	16.7 ± 3.6	93.2 ± 10.4*
DMBA + tamoxifen	17.8 ± 4.2	11.3 ± 3.2	13.1 ± 4.1*

Table 2
Effect of estradiol treatment on collagen solubility in DMBA-induced mammary tumors (*$p < 0.05$)

Treatment	Neutral salt soluble collagen (mg/g wet tissue), mean ± SEM	Acid soluble collagen (mg/g wet tissue), mean ± SEM	Insoluble collagen (mg/g wet tissue), mean ± SEM	Total collagen (mg/g wet tissue), mean ± SEM
DMBA control	12.5 ± 0.9	16.3 ± 1.2	29.3 ± 2.2	58.1 ± 4.9
DMBA + estradiol	21.6 ± 1.6*	24.7 ± 1.8*	34.4 ± 2.8	80.7 ± 6.8*
DMBA + tamoxifen	7.2 ± 0.6*	9.1 ± 1.0*	21.6 ± 1.9*	39.1 ± 3.8*

estradiol or tamoxifen registered no significant change in the levels of serum progesterone. Administration of tamoxifen in tumor bearing rats markedly reduced serum prolactin levels compared to untreated tumor bearing rats (Table 1).

3.2. Mammary tumor collagen

Extracellular matrix creates a suitable microenvironment and the natural substrata upon which cells migrate, proliferate and differentiate in vivo (Gospodarowicz et al., 1978; Reid and Jefferson, 1984). The concentrations of neutral salt soluble and acid soluble collagens markedly increased in the mammary tumors of rats treated with estradiol compared to the untreated tumor bearing rats (Table 2). Tamoxifen treatment to tumor bearing rats drastically decreased the concentrations of neutral salt soluble collagen and acid soluble collagens. Estradiol treatment registered no significant change in insoluble collagen concentration compared to untreated tumor bearing control rats. Administration of tamoxifen was able to decrease the insoluble collagen concentration compared to the control untreated tumor bearing rats. Overall, the total collagen concentration in tumor bearing rats was increased by estradiol and decreased by tamoxifen compared to untreated tumor bearing rats (Table 2).

3.3. Mammary ascorbic acid and lactate

Ascorbic acid deficiency results in partial hydroxylation of newly synthesized collagen, which is degraded at a faster rate (Barnes and Kodicek, 1972) and also reduced collagen accumulation (Chapvil, 1982). In our present investigation ascorbic acid concentration was remarkably elevated in mammary tumors of rats treated with estradiol, while tamoxifen was effective in reversing this effect (Table 3). This elevation in ascorbic acid could have resulted in increased activities of the collagen synthesizing enzymes thus leading to increased concentrations of soluble collagens. Another factor, which is considered vital for collagen synthesis, is lactate. It has been well established that lactate increases the activity of prolylhydroxylase, a key enzyme involved in collagen synthesis (Cerbon-Ambriz et al., 1991). Lactate concentration increases with the administration of estradiol and tamoxifen decreased its concentration (Table 3). The increase in lactate and ascorbic acid concentrations may be responsible for the increase in soluble and total collagen concentrations of the mammary tumors treated with estradiol.

Table 3
Effect of estradiol treatment on ascorbic acid and lactate concentrations in DMBA-induced mammary tumors (*$p < 0.05$)

Treatment	Ascorbic acid (mg/g wet tissue), mean ± SEM	Lactate (μg/g wet tissue), mean ± SEM
DMBA control	5.9 ± 0.6	40.2 ± 3.4
DMBA + estradiol	10.3 ± 0.9*	55.7 ± 4.8*
DMBA + tamoxifen	3.1 ± 0.4*	26.4 ± 2.0*

4. Discussion

Tissue architecture depends on proper cell–cell and cell–extracellular matrix interactions involving a reciprocal exchange of mechanical and biochemical information to maintain homeostasis (Grobstein, 1967). There are both quantitative and qualitative modifications in ECM components in breast cancer (Kosmehl et al., 1996). Cell–collagen interaction plays an important role in growth, differentiation and tumorigenesis of the mammary gland (Anderson, 1993). Although breast cancer cells originate mainly in the epithelium, evidence suggests that the stroma is an active participant in cancer progression and constitutes the majority of the tumor mass (Dvorak, 1986; Thomasset et al., 1998). Compared with normal mammary gland stroma, which mainly consists of fibroblasts and adipocytes, tumor stroma contains changes in the cellular composition and in the amounts of certain protein constituents, often referred to as reactive stroma or desmoplasia (Hansen and Bissell, 2000). Most of the matrix within tumor is newly synthesized. A new matrix may be either of host or of neoplastic origin. De novo synthesis of extracellular matrix occurs at the tumor site either by host or tumor cells. Synthesis of extracellular matrix by host cells can be induced by specific growth factors secreted by neoplastic cells or by products released from necrotic neoplastic cells. Although both the host and neoplastic cells are capable of secreting extracellular matrix components, the relative contribution of each has been difficult to define (Martinez-Hernandez, 1988). Human fibroblasts cocultured with neoplastic MCF7 cells produce increased amounts of collagen (Noel et al., 1992). The data on collagen (total as well as soluble collagens) indicate that DMBA-induced tumorigenesis is associated with an increment in soluble (salt and acid) and total collagens. The present findings are in agreement with earlier reports wherein the importance of collagen in tumor growth is well emphasized (Lewko et al., 1981; Wicha et al., 1981; Bano et al., 1983). The increase in soluble collagen indicates new collagen synthesis which in turn could have contributed to a higher cell–matrix interaction and cell–cell contact thus leading to the loss of cell death and

enhanced cell gain (Pullan et al., 1996). It has been reported earlier that newly synthesized collagens favor the growth of mammary tumor cells (Lewko et al., 1981; Wicha et al., 1981; Bano et al., 1983), while the maturation of collagen by cross-link formation encased the tumors and prevents proliferation of tumor cells (Kimoto et al., 1988; Vaage and Harlos, 1991). The significance of collagen in tumor development is further emphasized by the fact that administration of *cis*-hydroxyproline, the collagen synthesis inhibitor, blocked the development of DMBA- and MNU-induced mammary tumors (Lewko et al., 1981; Wicha et al., 1981).

Thus, these studies clearly demonstrate the essential role played by collagen in the growth of mammary tumors. In this regard, evidence for a causal role of extracellular matrix as a survival factor in vivo comes from studies with transgenic mice that express ectopic stromelysin-I (Sympson et al., 1994). In these mice, active stromylesin-I was approximately expressed which extensively degraded the basement membrane and thus leading to apoptosis. Further, it is also reasonable to point out here the identification of collagen synthesis stimulating activity observed in the extracts of DMBA-induced mammary tumors (Bano et al., 1983). In short, an increment in the collagens could have mediated the DMBA-induced mammary tumor growth by estradiol. Earlier studies on hepatoma have suggested that an increase in the concentration of lactate leads to increased prolyl-hydroxylase activity, which in turn increases collagen synthesis (Tanner et al., 1981). The significant increment in the levels of ascorbic acid and lactate in the mammary tumor tissues by estradiol observed in the present study further lends support to the concept of mammary tumor progression through enhanced collagen production. Because ascorbic acid and lactate serve as factors involved both in synthesis and posttranslational modifications of collagen proteins, an increment in these factors by estradiol could have favored the accumulation of collagen in the mammary tumor tissues. Further, all these changes appear to be estradiol specific effects as treatment with the antiestrogen tamoxifen reversed these changes. A further elevation in collagen is comparable to the increase in mammary tumor size and weight in these groups of rats. Estradiol appears to stimulate the synthesis of new collagens and thus contribute to the enlargement of the mammary tumors. This might have created a potential microenvironment by increasing the synthesis of suitable matrix that sustains the growth of the mammary tumors. The slight increase in insoluble collagen after estradiol treatment in mammary tumor indicates that in addition to stimulatory effect on new collagen synthesis estradiol is also capable of stimulating maturation of collagen. At present it is not clear whether an increase in insoluble collagen may inhibit tumor metastasis or not. In short, the present findings emphasize a definite mediatory role for collagen in estradiol promoted mammary tumor growth.

References

Anderson JC. Biochemical basis of connective tissue disease. In: Gardner DL, editor. Pathological basis of the connective tissue disease. London: Edward Arnold; 1993. p. 173–226.

Arun B, Udayachander M, Meenakshi A. 7,12-Dimethylbenzanthracene induced mammary tumours in Wistar rats by 'air pouch' technique – a new approach. Cancer Lett 1984;25:187–94.

Bano M, Zwiebel JA, Salomon DS, Kidwell WR. Detection and partial characterization of collagen synthesis stimulating activities in rat mammary adenocarcinomas. J Biol Chem 1983;258:2729–35.

Barnes MJ, Kodicek E. Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Vitam Horm 1972;30:1–43.

Castronovo V, Taraboletti G, Liotta LA, Sobel ME. Modulation of laminin receptor expression by estrogen and progestins in human breast cancer cell lines. J Natl Cancer Inst 1989;81:781–8.

Cerbon-Ambriz J, Cerbon-Solorzano J, Rojkind M. Regulation of collagen production in freshly isolated cell populations from normal and cirrhotic rat liver: effect of lactate. Hepatology 1991;13:551–6.

Chapvil M. Experimental modifications of collagen synthesis and degradation and their therapeutic applications. In: Jayson MIV, Weiss JB, editors. Collagen in health and diseases. Edinburgh: Churchill Livingstone; 1982. p. 206–17.

Chen CL, Meites J. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Endocrinology 1970;86:503–5.

Clarke R. Animal models of breast cancer: their diversity and role in biomedical research. Breast Cancer Res Treat 1996;39:1–6.

Dauvois S, Spinola PG, Labrie F. Additive inhibitory effects of bromocryptine (CB-154) and medroxyprogesterone acetate (MPA) on dimethylbenz[*a*] anthracene (DMBA)-induced mammary tumors in the rat. Eur J Cancer Clin Oncol 1989;25:891–7.

David G, Bernfield M. Defective basal lamina formation by transformed mammary epithelial cells: a reduced effect of collagen on basal lamina (heparan sulfate-rich) proteoglycan degradation. J Cell Physiol 1982;110:56–62.

David G, Van Den Berghe H. Transformed mouse mammary epithelial cells synthesize undersulfated basement membrane proteoglycan. J Biol Chem 1983;258:7338–44.

Deshpande N. Hormonal imbalance in breast cancer. J Steroid Biochem 1975;6:735–41.

Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–9.

Ferguson JE, Schor AM, Howell A, Ferguson MW. Changes in the extracellular matrix of the normal human breast during the menstrual cycle. Cell Tissue Res 1992;268:167–77.

Fleischmajer R, Olsen BR, Kuhn K. Structure moleculer biology and pathology of collagen. Ann N Y Acad Sci 1990;580:7–19.

Gospodarowicz D, Greenburg G, Birdwell CR. Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. Cancer Res 1978;38:4155–71.

Grobstein C. Mechanisms of organogenetic tissue interaction. Natl Cancer Inst Monogr 1967;26:279–99.

Guzman RC, Yang J, Rajkumar L, Thordarson G, Chen X, Nandi S. Hormonal prevention of breast cancer: mimicking the protective effect of pregnancy. Proc Natl Acad Sci U S A 1999;96:2520–5.

Hansen RK, Bissell MJ. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. Endocr Relat Cancer 2000;7:95–113.

Hartmann RW. Tumor growth stimulating and inhibiting effects of antiestrogens on the DMBA-induced mammary carcinoma of the ovariectomized. Eur J Cancer Clin Oncol 1983;19:959–64.

Hawkins RA, Roberts MM, Forrest AP. Oestrogen receptors and breast cancer: current status. Br J Surg 1980;67:153–69.

Huggins C, Morii S, Grand LC. Mammary cancer induced by a single dose of polynuclear hydrocarbons: routes of administration. Ann Surg 1961;154(Suppl.):315–8.

Jensen EV, Polley TZ, Smith S, Block GE, Ferguson DJ, Desombre ER. Prediction of hormone dependency in human breast cancer. In: McGuire WL, Carbone PD, Vollmer EE, editors. Estrogen receptors in human breast cancer. New York: Raven Press; 1975. p. 37–53.

Kimoto T, Watanabe S, Hyodoh F, Saito T. Collagen fiber formation and proliferation as a mechanism of cancer prevention and regression induced by extract from *Mycobacterium tuberculosis*: correlation between clinical observation and animal experiments. Cancer Detect Prev 1988;11:173–89.

Kosmehl H, Berndt A, Katenkamp D. Molecular variants of fibronectin and laminin: structure, physiological occurrence and histopathological aspects. Virchows Arch 1996;429:311–22.

Lewko WM, Liotta LA, Wicha MS, Vonderhaar BK, Kidwell WR. Sensitivity of *N*-nitrosomethylurea-induced rat mammary tumors to *cis*-hydroxyproline, an inhibitor of collagen production. Cancer Res 1981;41:2855–62.

Lippman ME, Dickson RB, Gelmann EP, Rosen N, Knabbe C, Bates S, et al. Growth regulation of human breast carcinoma occurs through regulated growth factor secretion. J Cell Biochem 1987;35:1–8.

Martinez-Hernandez A. The extracellular matrix and neoplasia. Lab Invest 1988;58:609–12.

McGuire WL, Carbone PD, Sears ME, Escher GC. Estrogen receptors in human breast cancer! An overview. In: McGuire WL, Carbone PD, Vollmer EE, editors. Estrogen receptors in humanbreast cancer. New York: Raven Press; 1975. p. 1–7.

Meites J. Relation of prolactin and estrogen to mammary tumorigenesis in the rat. J Natl Cancer Inst 1972;48:1217–24.

Meites J, Cassell E, Clark J. Estrogen inhibition of mammary tumor growth in rats; counteraction by prolactin. Proc Soc Exp Biol Med 1971;137: 1225–7.

Nandi S, Guzman RC, Yang J. Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. Proc Natl Acad Sci U S A 1995;92:3650–7.

Noel A, Munaut C, Nusgens B, Foidart JM, Lapiere CM. The stimulation of fibroblasts' collagen synthesis by neoplastic cells is modulated by the extracellular matrix. Matrix 1992;12:213–20.

Porter JC. Proceedings: hormonal regulation of breast development and activity. J Invest Dermatol 1974;63:85–92.

Pullan S, Wilson J, Metcalfe A, Edwards GM, Goberdhan N, Tilly J, et al. Requirement of basement membrane for the suppression of programmed cell death in mammary epithelium. J Cell Sci 1996;109:631–42.

Rajkumar L, Guzman RC, Yang J, Thordarson G, Talamantes F, Nandi S. Short-term exposure to pregnancy levels of estrogen prevents mammary carcinogenesis. Proc Natl Acad Sci U S A 2001;98:11755–9.

Reid LK, Jefferson DM. Cell culture studies using extracts of ECM to study growth and differentiation in mammalian cells. In: Mather JP, editor. Mammalian cell culture. New York: Plenum; 1984. p. 239–80.

Russo J, Russo IH. Experimentally induced mammary tumors in rats. Breast Cancer Res Treat 1996;39:7–20.

Sakakura T, Nishizuka Y, Dawe CJ. Mesenchyme-dependent morphogenesis and epithelium-specific cytodifferentiation in mouse mammary gland. Science 1976;194:1439–41.

Sympson CJ, Talhouk RS, Alexander CM, Chin JR, Clift SM, Bissell MJ, et al. Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. J Cell Biol 1994;125:681–93.

Tanner MS, Jackson D, Mowat AP. Hepatic collagen synthesis in a rat model of cirrhosis, and its modification by colchicine. J Pathol 1981;135:179–87.

Thomasset N, Lochter A, Sympson CJ, Lund LR, Williams DR, Behrendtsen O, et al. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. Am J Pathol 1998;153:457–67.

Vaage J, Harlos JP. Collagen production by macrophages in tumour encapsulation and dormancy. Br J Cancer 1991;63:758–62.

Van Slooten HJ, Bonsing BA, Hiller AJ, Colbem GT, Van-Dierendonck JH, Cornelisse CJ, et al. Outgrowth of BT-474 human breast cancer cells in immune-deficient mice: a new in vivo model for hormone-dependent breast cancer. Br J Cancer 1995;72:22–30.

Wicha MS, Liotta LA, Lewko WM, Kidwell WR. Blocking basement membrane collagen deposition inhibits the growth of 7,12-dimethylbenzanthracene-induced rat mammary tumors. Cancer Lett 1981;12:9–21.