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BASEMENT MEMBRANE COMPONENTS (MATRIGEL) PROMOTE THE TUMORIGENICITY OF HUMAN BREAST ADENOCARCINOMA MCF7 CELLS AND PROVIDE AN *IN VIVO* MODEL TO ASSESS THE RESPONSIVENESS OF CELLS TO ESTROGEN

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Abstract—The ability to transplant human tumors into athymic nude mice allows studies of tumor cells *in vivo*. However, after s.c. injection the incidence of tumor and metastases in nude mice is frequently low. We have studied the tumorigenicity in nude mice of estradiol (E_2)-sensitive breast adenocarcinoma MCF7 cells. Matrigel, an extract of basement membrane proteins, induces rapid tumor development after s.c. injection of MCF7 cells. In the absence of this matrice, MCF7 cells failed to induce tumor growth. In this *in vivo* model, MCF7 cells were analysed for their E_2 sensitivity. Two weeks after inoculation in the presence of matrigel, cells formed growing tumors in intact mice supplemented with E_2. In ovariectomized or untreated mice, tumor appearance was delayed and the growth level was very low. Thus, MCF7 cells formed tumors in the absence of E_2 but retained *in vivo* their responsiveness to estrogen. Growing human tumors in nude mice provides a rapid and useful model for testing the sensitivity of cells to hormone.

The availability of athymic nude mice for transplanting human tumors [1, 2] has provided an interesting tool for studying different aspects of the biological behavior of human tumors *in vivo*. Tumor growth and the incidence of metastases increase when cells are transplanted into an orthotopic site, i.e. the tissue of origin, rather than into heterotopic sites [3, 4]. For example, the MDA-MB-435 human breast carcinoma cells metastasize in nude mice when implanted into the mammary fat pad, but not when implanted into the subcutis [2]. Tumor growth and metastases are thus influenced by specific interactions between tumor cells and the host tissue. Laminin, a major component of basement membrane, influences tumor cell adhesion [5, 6], collagenase type IV production [7], cell motility [8] and the formation of metastases [9–11]. We have shown previously the effect of a reconstituted basement membrane (matrigel) containing mostly laminin on human breast adenocarcinoma MCF7 cell adhesion and morphology *in vitro* [12]. Matrigel has been shown recently to influence the tumorigenicity of Small Cell Lung Cancer (SCLC) in athymic nude mice [13]. In this study, we present evidence that matrigel induces a rapid development of tumor after injection of estrogen-sensitive MCF7 cells into a heterotopic site (subcutis). In the absence of matrigel, these cells failed to induce tumors.

The MCF7 cells have been shown to require supplementation with estradiol (E_2†) in order to sustain progressive growth in athymic nude mice [14, 15]. We report that when MCF7 cells were injected with matrigel, tumor appearance was not fully dependent on E_2. However, despite the presence of basement membrane components, tumor growth remained sensitive to the hormone. Human tumor cells growing in nude mice in the presence of matrigel provide thus an interesting and rapid model for studying the hormone sensitivity of human breast cancer cells.

MATERIALS AND METHODS

Matrigel. The "reconstituted basement membrane" gel, matrigel was extracted from the Engelbreth–Holm–Swarm (EHS) tumor as described previously [16, 17].

Cell culture. MCF7 cells provided by G. Leclercq (Institut Bordet, Brussels, Belgium) were cultured in Dulbecco's modified Eagles medium (DMEM, Gibco, Paisley, U.K.) supplemented with 10% fetal calf serum, glutamine (292 mg/mL), penicillin–streptomycin (100 U/mL), sodium bicarbonate (2.1 mg/mL) and ascorbic acid (50 mg/mL).

In vivo *tumor studies.* Cells harvested by centrifugation (5 min at 1000 rpm) were resuspended in cold serum-free medium and mixed with an equal volume of cold liquid matrigel (10 mg/mL). A final volume of 0.5 mL was immediately injected s.c. into 6–8-week-old female athymic nu/nu mice (Iffa Credo). Mice were implanted s.c. with Silastic capsules filled with E_2. Silastic tubing (Dow Corning, Comesa, Belgium), 0.125 inch outer diameter × 0.078 inch inner diameter, was cut to 1 cm size. Capsules were filled with matrix consisting by weight of three parts Silastic medical-grade 382

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† Abbreviation: E_2, estradiol.

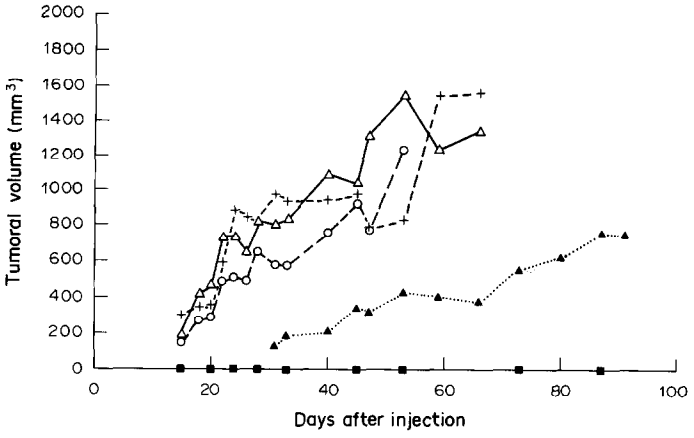

Fig. 1. Effect of matrigel on MCF7 cell tumor growth as a function of the number of cells injected. Different amounts of MCF7 cells were injected in the presence [(▲) 0.35×10^6, (○) 0.75×10^6, (+) 1.5×10^6, (△) 3×10^6] or absence (■) of matrigel. Administration of E_2 and estimation of the tumor volume were performed as described in Materials and Methods.

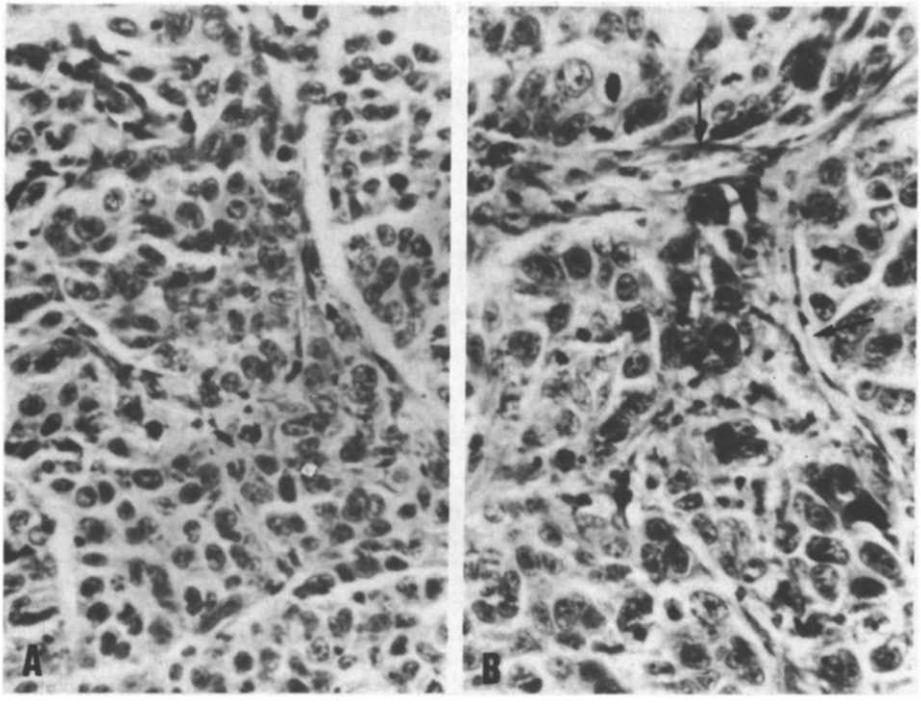

Fig. 2. Histology of tumor development after injection of MCF7 cells into athymic nude mice. Formalin-fixed tissue sections were embedded in paraffin and stained with hematoxylin and eosin. The MCF7 cells formed nodules (A). The arrows indicate the vascularization (B).

Elastomer (Dow Corning) and one part E_2 [18]. Mean E_2 sample weight was 1.5 mg/capsule according to a previous study [19].

In order to test the requirement of E_2 for tumorigenicity of MCF7 cells in the presence of matrigel, some of the experiments were performed with athymic nude mice that had been ovariectomized when 5 weeks old (Iffa Credo) or with athymic nude mice not implanted with Silastic capsule (intact mice).

Each experiment using five mice per group was repeated two or three times. Injected mice were examined weekly. Calculation of tumor volumes was made from measurements of the larger (a) and smaller (b) diameters according to $a \times b^2 \times 0.4$ [20]. We have determined the latency period defined as

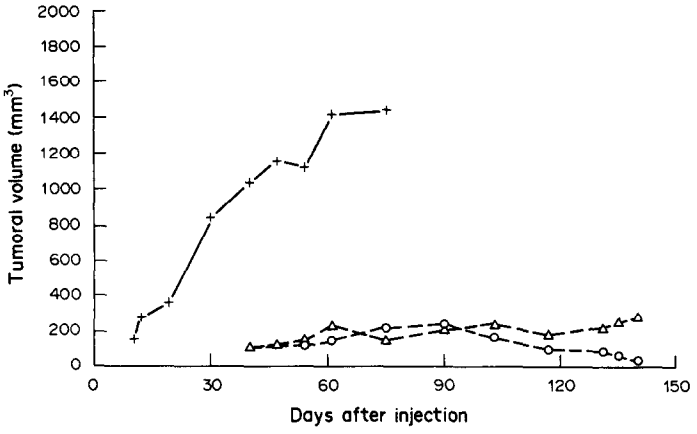

Fig. 3. Effect of matrigel on MCF7 cell tumor growth after injection into ovariectomized, untreated or estrogen-supplemented nude mice. Tumoral MCF7 cells (1.5×10^6) were injected s.c. in the presence of matrigel into ovariectomized (○), untreated (△) or estrogen-supplemented (+) nude mice. The tumor volume was estimated periodically as described in Materials and Methods.

Table 1. Tumorigenicity of cells after s.c. injection of MCF7 cells into ovariectomized, untreated or estrogen-supplemented nude mice

Treatment	Tumorigenicity (N/N)*	Tumor volume (mm^3)†	Latency period (days)
Ovariectomy	4/10	220 ± 40	54
Intact ovaries	6/10	160 ± 40	50
Intact ovaries + E_2	8/10	1500 ± 200	12

* Number of mice bearing tumor larger than 150 mm^3/total number of injected mice.
† Tumor volume calculated as described in Materials and Methods at the end of the experiment (time of observation = 135 days).

the time between injection and appearance of a 150 mm^3 nodule with progressive growth.

Autopsy was done on all mice. Primary tumors, axillary lymph nodes, lung liver and spleen were fixed for routine histology. In order to distinguish between host tissue and MCF7 cells in tumors and to search for metastases, sections were stained with a murine monoclonal IgG antibody against MCF7 cells [21].

RESULTS

Effect of matrigel on MCF7 cells tumorigenicity

In order to investigate the effect of matrigel on tumor growth, various amounts of MCF7 cells (0.35, 0.75, 1.5, 3×10^6 cells) were injected s.c. into nude mice supplemented with E_2, in the presence of matrigel or medium. In the absence of matrigel, cells failed to induce tumor appearance, regardless of the number of cells injected. In the presence of matrigel, the injection of MCF7 cells resulted rapidly in the development of growing tumors (Fig. 1). Injection of 0.75, 1.5 or 3×10^6 MCF7 cells caused the appearance of tumoral nodules about 2 weeks after inoculation (Fig. 1). The latency period was 15 ± 3 days. The tumoral volume increased progressively reaching 1500 mm^3 after 1.5 months. The development of tumors was delayed when 0.35×10^6 MCF7 cells were injected. The latency period under these conditions was 30 ± 4 days. These tumors grew more slowly reaching a volume of 1800 mm^3 after 3 months.

Figure 2 shows a representative MCF7 tumor growing s.c., with evidence of vascularization (Fig. 2B). In these tumors, cells were associated into nodules (Fig. 2A) and they all showed features characteristic of MCF7 cells (rounded cells with well-defined nucleoli in the nucleus). All primary tumors were positively stained with anti-MCF7 antibodies. No micrometastases were found in the organs examined, including lymph nodes, spleen, liver, kidneys and lungs.

Effect of E_2 on MCF7 cell tumorigenicity induced by matrigel

We investigated whether the MCF7 cells require E_2 for tumor formation in the presence of matrigel. Cells were injected into ovariectomized mice, intact mice or intact mice supplemented with E_2. As described above (Fig. 1), treatment of mice with E_2

in addition to physiological levels resulted in the rapid appearance of eight tumors in 10 mice, after about 12 days (Fig. 3 and Table 1). In the absence of E_2 supplementation, in either intact mice or ovariectomized mice, the development of tumors was delayed. The latency period under these conditions was about 50 days (Table 1). In these two experimental groups, in the absence of E_2 supplementation, tumors grew very slowly and their volumes reached about 300 mm^3 at the end of the experiment versus 1500 mm^3 in the presence of E_2 (Table 1 and Fig. 3). On the other hand, in ovariectomized mice, the tumor incidence was lower (Table 1) and tumors regressed 4 months after injection (Fig. 3).

DISCUSSION

Heterotransplantation of human breast cancer cells into athymic nude mide has a relatively low success rate and a long latency period. In this study, we demonstrate that basement membrane proteins influence the tumorigenicity of human MCF7 cells. Although in the absence of matrigel cells failed to induce tumor growth, this basement membrane gel caused a rapid development of tumors. These appeared 2 weeks after cell injection. Tumors that developed from MCF7 cell subclones in the presence of added matrigel retained the histological features of MCF7 cells [19]. Even after inoculation of 0.35×10^6 cells, mice developed growing tumors. While matrigel promotes tumor growth, it does not induce expression of the metastatic phenotype of the cells tested. Our observation that tumor growth is identical when 0.75, 1.5 or 3×10^6 MCF7 cells were injected also indicates that local host factors probably limit the rate of tumor development. These data emphasize the importance of tumor–host interactions and particularly the effect of basement membrane components on cell tumorigenicity [11, 13, 22]. The various mechanisms which may be involved in this process have been described previously [13, 22]. This effect could be ascribed at least partly to specific cell–matrix interactions. Indeed, we have shown that matrigel affects the cell culture organization by promoting the tridimensional clustering of MCF7 cells [12].

Usually, the hormone responsiveness of E_2 receptor-positive human breast carcinoma cells is maintained in nude mice [2, 14, 15]. Mice received supplementary E_2 because their serum levels of the hormone are much lower than those in premenopausal women [19]. In the absence of matrigel, no tumors appeared even when MCF7 cells were exposed *in vivo* to E_2. In the presence of matrigel, MCF7 cells formed tumors in ovariectomized mice. However, the incidence was lower than in intact mice supplemented with E_2 or not. In intact mice, cells induced the development of slowly growing tumors which retained their responsiveness to estrogen since, in the presence of the hormone, the tumor volume was much higher. Furthermore, in ovariectomized mice, tumors regressed 4 months after injection. Our results indicate that after injection of MCF7 cells in the presence of matrigel, the tumor take is hormone-independent while tumor growth is hormone-responsive. Several sublines of cells presenting similar features have been isolated by Clarke *et al.* [23] after selection for growth *in vivo* and *in vitro* in the absence of estrogen. Our observation that matrigel alone but not E_2 elicits tumor formation indicates the close dependence of this MCF7 cell clone on an adequate environment for tumorigenicity. The use of matrigel for growing human breast adenocarcinoma cells in athymic nude mice allows rapid tumor development and studies on hormone responsiveness. This *in vivo* model may thus be useful for many studies in cancer research.

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