Syngeneic mouse mammary carcinoma cell lines: Two closely related cell lines with divergent metastatic behavior

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Received 10 October 2004; accepted in revised form 24 February 2005

Key words: Akt, breast, breast carcinoma, cell line metastasis, comparative genomic hybridization, ERBB2, gene expression analysis, LY294002, mammary fat pad, mouse mammary tumor virus long terminal repeat, oncogene, orthotopic, phosphatidylinositol 3 kinase, polyoma middle T, pulmonary metastasis, Sept9, Spp1 osteopontin, Opn, syngeneic, transgenic, tumor transplant

Abstract
Two cell lines, Met-1fvb2 and DB-γfvb2, with different metastatic potential, were derived from mammary carcinomas in FVB/N-Tg(MMTV-PyVmT) and FVB/N-Tg(MMTV-PyVmTY315F/Y322F) mice, transplanted into syngeneic FVB/N hosts and characterized. The lines maintain a stable morphological and biological phenotype after multiple rounds of in vitro culture and in vivo transplantation. The Met-1fvb2 line derived from a FVB/N-Tg(MMTV-PyVmT) tumor exhibits invasive growth and 100% metastases when transplanted into the females FVB/N mammary fat pad. The DB-γfvb2 line derived from the FVB/N-Tg(MMTV-PyVmTY315F/Y322F) with a “double base” modification at Y315F/Y322F exhibits more rapid growth when transplanted into the mammary fat pad, but a lower rate of metastasis (17%). The Met1fvb2 cells show high activation of AKT, while DB-γfvb2 cells show very low levels of AKT activation. The DNA content and gene expression levels of both cell lines are stable over multiple generations. Therefore, these two cell lines provide a stable, reproducible resource for the study of metastasis modulators, AKT molecular pathway interactions, and gene target and marker discovery.

Introduction
Breast cancer is the second leading cause of cancer death in women [1]. The majority of breast cancer deaths occur as the result of distant metastasis, with few deaths due to locally advanced disease or local recurrence. Some progress in treatment of breast cancer has been made over the last decade. The majority of this progress is attributed to early detection and early treatment. Metastasis remains the single most important predictor of survival even in women with small primary breast cancers. An understanding of the biology and molecular mechanisms involved in metastasis is essential to improving survival. Ideally, animal models for these mechanisms can be used to test potential therapies.

A number of genetically engineered mouse (GEM) models of mammary carcinoma associated with metastatic disease have been developed [2]. Transgenic animals expressing the polyoma virus middle T (PyVmT) oncogene in the mammary epithelium under control of the MMTV-LTR promoter elements (FVB/N-Tg(PyVmT)) are one of the most robust models, developing palpable mammary carcinomas by 5–10 weeks of age with 100% penetrance [3]. Virtually 100% of animals develop pulmonary metastasis by 20 weeks of age. Since PyVmT is a surrogate ERB B2 molecule and metastatic tumors arise quickly, the model has become favored by many research groups [4–13].

Muller and colleagues [14] expanded the model by developing additional transgenic mice with targeted mutations of the transgene. The MMTV-LTR-
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PyVmT<sup>Y315F/Y322F</sup>, a double base (DB) mutant form of the transgene, was designed to abrogate the interaction of PyVmT protein with PI3K [14]. This DB mutant results in mammary tumorigenesis without activating PI3K. When compared to the FVB/N-Tg(PyVmT<sup>wt</sup>) wild type (<sup>wt</sup>), one founder line, the DB-<sup>4</sup>, has a decreased onset of mammary tumors and fewer tumor foci within the mammary gland. These rapidly growing tumors have histologic features of high grade, with frequent central necrosis and high proliferative and apoptotic rates [14, 15]. The DB tumors were similar to the Tg(PyVmT<sup>wt</sup>) tumors morphologically, but differed significantly in their rate of metastasis. In order to study the metastatic behaviors of these two tumor types, and to be able to rapidly reproduce them in cohorts of animals, the tumors were initially transplanted into the mammary fat pads of recipient nude mice [16].

The initial studies demonstrated that the two lines maintained the metastatic phenotype of the primary tumors through numerous passages in cell culture and as transplants. The lines were used to compare pathological features such as histological grade and angiogenesis [16]. Subtraction hybridization demonstrated several molecular differences, including the relative overexpression of osteopontin (Spp1) in the metastatic tumor types [9]. However, these studies were carried out in immunodeficient nude mice (CbyJ.Cg-Foxn<sup>nu</sup> homozygotes). In the current study, we describe the biology of these tumor cells, designated Met-1<sup>16<sup>502</sup></sup> and DB-7<sup>16<sup>50</sup></sup>, in immunologically intact syngeneic FVB/N mice. We report here the in vitro and in vivo characterization of these two tumor cell lines, performed on a standardized early passage pool of expanded cells. These lines have been distributed to numerous colleagues interested in the metastatic phenotype.

Materials and methods

Transgenic and tumor transplant mice

FVB/N mice used for tumor transplantation and tail vein injection experiments were purchased from Jackson Laboratory (Bar Harbor, ME) and Charles River (Wilmington, MA), respectively. The origin of the cells and transplants used in this study were primary mammary tumors from FVB/N-Tg(MMTV-PyVmT<sup>634Mul</sup>) founder 7 mouse (not bred) derived by the Muller lab [3, 14]. Palpable tumors were excised, and minced to roughly 0.5 mm<sup>2</sup>. The 0.5-mm<sup>2</sup> tumor pieces were minced in fresh trypsin–EDTA solution removing any necrotic or fibrous areas. The harvested tumor pieces were embedded in the adipose tissue. The skin was re-apposed and closed with surgical staples. Subcutaneous transplants were performed by placement between the third and fourth fat pads with care not to disrupt the thin fascia sheath covering the pads.

In vitro culture

Cells were cultured in DMEM complete medium (Dulbecco modified Eagle medium, supplemented with 10% fetal calf serum, penicillin–streptomycin, l-glutamine and sodium pyruvate) at 37 °C in humified 5% CO<sub>2</sub>. Tumors were surgically removed from the host animal and washed in PBS with penicillin-streptomycin and gentamicin on a Petri dish. Solid pieces of the tumors were harvested, removing any necrotic or fibrous areas. The harvested tumor pieces were minced in fresh trypsin–EDTA solution until the cells were dissociated. Dissociation was stopped by adding DMEM complete medium. The cells were washed twice before plating in DMEM culture media. Several rounds of trypsin treatment were performed to remove trypsin sensitive non-epithelial cell contaminants, as previously described [17]. Cells were maintained in DMEM complete media. Cells were removed from the plates by trypsin digestion, and split 1:2 to 1:4.

Cryopreservation

Dissociated tumor cells and finely minced tumor pieces were frozen in freezing medium (complete DMEM with 10% DMSO, 20% FBS, penicillin–streptomycin, sodium pyruvate, l-glutamine).

Mammary fat pad and tail vein injection of cells

With 0.25% trypsin 70–80% confluent cells were harvested and washed with PBS. Vital cells were counted using trypan blue and suspended to a final concentration in PBS. For mammary fat pad injection, bilateral inguinal (4) mammary fat pads of FVB/N female mice were exposed and 10<sup>6</sup> cells/20 µl per fat pad were injected using a 30-gauge needle. For tail vein injection, 10<sup>4</sup> or 10<sup>6</sup> cells/50 µl per animal were injected using a 25-gauge needle.

Histopathology and whole mounts

Lung whole mount preparations were prepared using anesthetic overdose. The trachea was cannulated with mouse hepatitis virus (MHV) positivity (a pathogen which should not pass through the microisolator filter).

Serial transplantation

Fresh or frozen tumor samples were transplanted by exposing the inguinal (4) mammary gland fat pad. A small incision was made in the thin fascia surface of the mammary gland, and a small void was generated with the tip of a fine forceps. The 0.5-mm<sup>2</sup> tumor sample was inserted in the void with care to ensure that the transplanted tissue was totally embedded in the adipose tissue. The skin was re-aposed and closed with surgical staples. Subcutaneous transplants were performed by placement between the third and fourth fat pads with care not to disrupt the thin fascia sheath covering the pads.