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Establishment of a mammary carcinoma cell line from Syrian hamsters treated with N-methyl-N-nitrosourea

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ABSTRACT

Clearly new breast cancer models are necessary in developing novel therapies. To address this challenge, we examined mammary tumor formation in the Syrian hamster using the chemical carcinogen N-methyl-N-nitrosourea (MNU). A single 50 mg/kg intraperitoneal dose of MNU resulted in a 60% incidence of premalignant mammary lesions, and a 20% incidence of mammary adenocarcinomas. Two cell lines, HMAM4A and HMAM4B, were derived from one of the primary mammary tumors induced by MNU. The morphology of the primary tumor was similar to a high-grade poorly differentiated adenocarcinoma in human breast cancer. The primary tumor stained positively for both HER-2/neu and pan-cytokeratin, and negatively for both cytokeratin 5/6 and p63. When the HMAM4B cell line was implanted subcutaneously into syngeneic female hamsters, tumors grew at a take rate of 50%. A tumor derived from HMAM4B cells implanted into a syngeneic hamster was further propagated *in vitro* as a stable cell line HMAM5. The HMAM5 cells grew in female syngeneic hamsters with a 70% take rate of tumor formation. These cells proliferate *in vitro*, form colonies in soft agar, and are aneuploid with a modal chromosomal number of 74 (the normal chromosome number for Syrian hamster is 44). To determine responsiveness to the estrogen receptor (ER), a cell proliferation assay was examined using increasing concentrations of tamoxifen. Both HMAM5 and human MCF-7 (ER positive) cells showed a similar decrease at 24 h. However, MDA-MB-231 (ER negative) cells were relatively insensitive to any decrease in proliferation from tamoxifen treatment. These results suggest that the HMAM5 cell line was likely derived from a luminal B subtype of mammary tumor. These

Abbreviations: MNU, N-methyl-N-nitrosourea; DMBA, 7,12-dimethylbenz[a]anthracene; DES, diethyl-stilbestrol; BOP, N-nitrosobis(2-oxopropyl)amine; IACUC, Institutional Animal Care and Use Committee; i.p., intraperitoneal; PET, Positron Emission Tomography; ^{18}F-FDG, 2-deoxy-2-[^{18}F]fluoro-D-g1ucose; LSO, lutetium oxyorthosilicate crystal; DMEM, Dulbecco's Modified Essential Medium; FBS, fetal bovine serum; s.c., subcutaneous; ADH, atypical ductal hyperplasia; DCIS, ductal carcinoma *in situ*; MGMT, O^6-methylguanine-DNA methyltransferase.

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results also represent characterization of the first mammary tumor cell line available from the Syrian hamster. The HMAM5 cell line is likely to be useful as an immunocompetent model for human breast cancer in developing novel therapies.

1. Introduction

Well characterized cancer cell lines are powerful research resources for studying cancer biology and new therapeutic strategies against this disease. Many of these cell lines represent the genetic alterations and features of breast cancer progression observed in human breast cancer. These cell lines allow for *in vivo* examination of complex multicellular interactions involved in the initiation and progression of breast cancer, using human xenograft cells implanted into immunocompromised animals and syngeneic rodent cells implanted into immunocompetent animals [1]. However, because breast cancer represents a heterogeneous molecular disease [2], no individual model system can adequately reflect its nature. Thus, additional breast cancer model systems are clearly necessary.

The Syrian hamster has been used for a number of different carcinogenesis and cancer chemoprevention models, but historically these have not included mammary carcinogenesis. These models have included the hamster cheek pouch model [3], in which polycyclic aromatic hydrocarbons such as 7,12-dimethylbenz[a]anthracene (DMBA) are painted on the cheek pouch either as a complete carcinogen or as the initiating agent followed by a tumor promoter such as benzoyl peroxide. The tumors that develop are typically squamous cell carcinomas that contain a mutation in codon 61 of the Ha-ras gene. In addition, Syrian hamsters are susceptible to diethyl-stilbestrol (DES) induced renal carcinomas, in which the kidneys are estrogen sensitive [4] and may behave more like breast cancer in this species; this was the basis for establishing a hamster renal carcinoma cell line [5]. Syrian hamsters also develop cholangiocarcinomas, bile duct hyperplasia, and pancreatic cancer when exposed to aromatic amines such as N-nitrosobis(2-oxopropyl)amine (BOP), which can be partially prevented by certain phytochemicals [6,7]. Inducing pancreatic cancer, however, requires augmentation pressure, which includes feeding the hamsters a choline-deficient diet and subjecting the animals to two to three rounds of ethionine–methionine–BOP injections [8].

The chief objective of this project was to establish a mammary cancer model in the Syrian hamster for investigating the potency of novel therapies alone and in combination with standard chemotherapy agents and radiotherapy. For example, the Syrian hamster has been identified as an animal model for oncolytic adenovirus vectors with promising results [9,10]. The adenovirus is able to infect, replicate, and spread from cell-to-cell in cancer cell lines of this animal. Adenovirus replicates in the lungs, liver, and other organs [11]. However, hamsters are resistant to mammary cancer, and few hamster studies have been reported [12,13]. Therefore, our initial effort was to establish mammary tumor models in the Syrian hamster using the chemical carcinogen N-methyl-N-nitrosourea (MNU). MNU-induced mammary carcinoma in rats is a well-established animal model for breast cancer, exhibiting similarities to human ER + breast tumors [14]. This design was modeled after the rat mammary MNU model [14], in which rats were treated with a single dose of MNU (50 mg/kg body weight) at 50 days of age. In rats, this protocol leads to mammary tumor latency of approximately 4 weeks, with an incidence of 85–100% and a multiplicity of approximately 3.5 mammary tumors per animal. Most mammary tumors in rats resulting from this protocol were adenocarcinomas; other tumor types were rare [15].

In the current study, a 50 mg/kg dose of MNU resulted in a 60% incidence of premalignant mammary lesions, and a 20% incidence of mammary adenocarcinomas. One mammary tumor induced by MNU was used for the initiation and characterization of a new mammary carcinoma cell line HMAM4B. One tumor derived from HMAM4B cells implanted into a syngeneic hamster was further propagated as a stable cell line HMAM5; these cells grew in female syngeneic hamsters with a 70% take rate of tumor formation. These cell lines are likely to be useful as a model for human breast cancer in developing novel therapies.

2. Materials and methods

2.1. Syrian hamsters

Female inbred Syrian (Golden) hamsters (*Mesocricetus auratus*) at 4 weeks of age were obtained from Harlan (Prattville, AL animal colony) and housed in a temperature- and humidity-controlled AAALAC approved facilities with a 12 h light/dark cycle. All procedures were approved by the LSUHSC Institutional Animal Care and Use Committee (IACUC) or Southern Research Institute IACUC in accordance with NIH and USDA guidelines. The hamsters were housed one or two per cage, provided environmental enrichment, and allowed access to food and water *ad libitum*. The hamsters were acclimated to the laboratory conditions and handled at least 2 days/week to become accustomed to the human touch.

2.2. MNU experimental design

In the current experiment, female Syrian hamsters were acclimated for 2 weeks and at 6 weeks of age, were dosed once with MNU (50 or 100 mg/kg body weight) by intraperitoneal (i.p.) administration. MNU (Sigma; St. Louis, MO) was dissolved in sterile acidified 0.9% saline immediately before use. Control hamsters received saline only. The hamsters were monitored by daily observation and palpation and weekly body weight measurement, and any tumors observed were recorded. Hamsters appearing to be