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CA125 expression in spontaneous ovarian adenocarcinomas from laying hens

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Abstract

Objective. Currently, there is not a fully characterized model for human ovarian cancer; however, 2- to 4-year-old laying hens spontaneously develop ovarian tumors. CA125 expression is a hallmark of ovarian cancer in women. The major objective of this study was to characterize the in vitro growth of avian ovarian tumor cells, and CA125 expression in avian ovarian tumors.

Methods. Immunohistochemistry was employed to evaluate CA125 expression in avian ovarian tumor tissue. A high temperature antigen retrieval step was an essential part of the CA125 staining procedure. In vitro growth curves were constructed for avian ovarian cancer cells. Western blotting was used to estimate the size of the CA125 reactive protein and to confirm CA125 expression.

Results. The growth of avian tumors in culture fits a sigmoidal curve for cell growth and suggests a cell cycle time of 28 h. The tumors taken from the chicken stained positive for CA125. Approximately 90% of cells isolated from avian ovarian tumors also stained positive for CA125. Western blots show a band of approximately 25 kDa when immunodetected with CA125.

Conclusions. Similar to human ovarian tumors, chicken ovarian tumors express CA125. Cultured chicken ovarian cancer cells express CA125 and CA125 expression does not appear to change with time in culture.

Keywords: Ovarian cancer; Chicken; Avian; Cancer markers; In vitro; Western blotting

Introduction

Ovarian cancer is the most lethal of all gynecological diseases, and it ranks fourth in cancer deaths among women [1,2]. The American Cancer Society predicted that in 2005 approximately 22,220 new cases of ovarian cancer will be diagnosed in the United States, and it is estimated that 16,210 of these women will succumb to the disease [3,4]. The reason for the alarmingly high mortality rate is late diagnosis at advanced stages of the disease, and after cancer has metastasized to other organs. Fewer than 30% of women can be successfully treated when the disease is diagnosed at late stage III compared to a 90% cure rate at stage I. However, less than 25% of all cases are diagnosed at stage I in the United States [3,5].

One factor hindering significant advancement in the study of ovarian cancer is the absence of a characterized animal model system. Ovarian tumors arise spontaneously with age in some strains of mice and in Wistar and Sprague–Dawley rats, but tumors are extremely rare [1,6]. Mice can also be inbred or manipulated by viral vectors to become tumorigenic. Tumor cells can be xenografted into SCID mice [6]. However, the majority of the mouse based animal models have genetically induced tumors that are inappropriate for large-scale chemoprevention studies. The mouse model may be inadequate because the tumors are not formed spontaneously and are biologically different from the naturally occurring tumors [1]. A better model system over the current mammalian systems is an animal that reproducibly spontaneously forms ovarian tumors.

The domestic hen [*Gallus domesticus*] may be a useful model for studying ovarian cancer because hens maintained under intensive egg-laying conditions spontaneously develop ovarian adenocarcinomas [6,7]. A typical hen will produce

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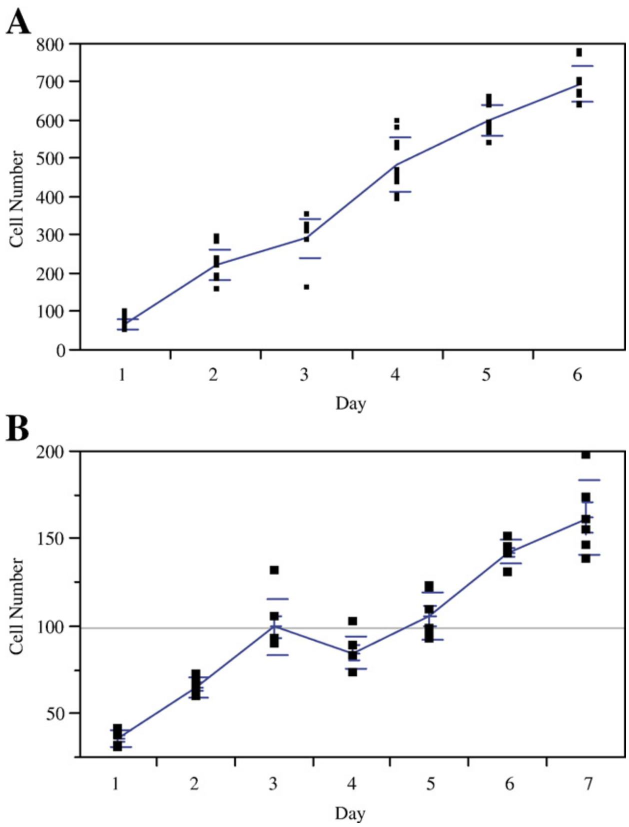

Fig. 1. Growth curve of chicken ovarian cancer cells grown for 7 days. Panels A and B show the results from two different experiments. The bars indicate standard errors.

280 or more eggs in 50 weeks [8]. Egg production drastically decreases after 2 years of age when the hens begin to be considered unproductive for agricultural purposes. Likewise, fertility decreases with age as the period between clutches of eggs increases [7]. Currently, the incessant ovulation theory of ovarian cancer formation suggests that the risk of developing ovarian cancer is directly related to the number of ovulations a woman experiences in her lifetime. Women who start menstruating at an early age, women with no children, and women who experience menopause after age 50 are at an increased risk [3]. The observed spontaneous tumor formation in two-year-old laying hens led to its consideration as a model for human ovarian cancer. The presence or absence of the human tumor marker CA125 in the domestic fowl would support the utility of the avian model to study ovarian cancer.

The current method of screening for ovarian cancer involves a blood test to detect the cancer antigen CA125, which is a glycoprotein expressed on the cell membrane of ovarian tumors that has a molecular weight above 200,000 [9–11]. CA125's use in the clinical setting makes it an important marker to verify the avian model. The presence or absence of the human tumor marker CA125 in the domestic hen would support the utility of the avian model to study ovarian cancer. The objective of the study was to confirm the presence of CA125 in avian ovarian tumors and cells isolated from the tumors.

Materials and methods

Tissue collection

Hens employed in this study were commercial Single Comb White Leghorn [*G. domesticus*] that had been maintained for 2 years at the Piedmont Research Station in Salisbury, NC. Each hen ovulated approximately 450 times prior to death, and they had never been subjected to a synchronized molt. Hens ranged in age from 86 weeks to 105 weeks of age. In total, 450 hens were necropsied for tumors, and 15 ovarian tumors were discovered for an ovarian cancer incidence rate of 3%. Necropsies were performed at North Carolina State University. All procedures involving animals were approved by the North Carolina State University Institutional Animal Care and Use committee. When an ovarian adenocarcinoma was identified, it was aseptically removed and cut into several pieces, which were placed either in 4% paraformaldehyde overnight for immunohistochemistry or into 5 ml of Hank's Balanced Salt Solution for primary cell culture.

For cell culture, tumor tissue was mechanically and enzymatically digested using 0.17% Trypsin/0.085% collagenase for 30 min at 37°C. Tissue was washed with media containing Dulbecco Modified Eagles Medium (DMEM), 10% Fetal Bovine Serum (FBS), 1% Gentamicin, and 1% Non-Essential Amino Acids. The pellet was passed through a 100 μm Nitex filter to separate cells from debris. The cells were cultured with DMEM, 1% Gentamicin, 1% Non-Essential Amino Acids, and 10% FBS.

Growth data and immunocytochemistry

Isolated cells were plated into ten wells of ten twenty-four well plates at a density of 11,202 cells per well. Cells were also seeded onto three two-well chamber slides (Fisher Scientific, Chicago, IL) at a density of 22,404 cells per chamber. A plate and slide were fixed at different time intervals to assess different stages of growth. The cells in plates were fixed at 24 h, 72 h, 96 h, 110 h, 122 h, and 134 h after initial plating. At 134 h, the cells were 100% confluent in all wells. The cells in chamber slides were fixed at 24 h, 110 h and 158 h, at which time the cells were 100% confluent. The cells were fixed using 4% paraformaldehyde and stored at 4°C until processed for immunohistochemistry.

The remaining 4 plates were allowed to grow to confluency, trypsinized using 0.25% Trypsin:EDTA, and re-inoculated onto seven six-well plates (Fisher Scientific, Chicago, IL) at a density of 375,000 cells per well. Three chamber slides were inoculated at a density of 562,500 cells per chamber. A higher cell number than the initial seeding was employed because it appeared qualitatively that cell growth was beginning to slow. More cells were plated to ensure cells reached a confluent state. Plates were fixed with 4% paraformaldehyde at 24 h, 48 h, 72 h, 96 h, 144 h, 192 h, and finally 240 h. Slides were fixed at 24 h, 144 h, and 528 h.

To assess growth, the cells on the plates were stained with propidium iodide (PI) and examined under a Leica fluorescence microscope using a 40× objective. PI stains the nucleus of the cell, making it possible to visualize each viable cell on the plate. Images of ten fields per well were obtained from each six well plate using a Spot-RT CCD (Diagnostic Instruments, Sterling Heights MI) camera. The images were collected and used to quantify growth. The cells per image were enumerated using Image Pro Plus 6.0 imaging software (Media Cybernetics Inc., Silver Springs MD, USA).

The chamber slides were used for immunocytochemistry. The slides were washed three times for 2 min each with distilled H_2O placed into boiling 0.01 M citrate buffer pH 6.0 for 15 min and then cooled at room temperature for 20 min. Citrate buffer was removed using two rinses with PBS for 2 min.

Table 1
Mean and standard error values for the proportion of cells stained with CA125 primary antibody at different time intervals in primary culture

	Proportion of cells stained CA125 positive per slide
Time 0	0.93 ± 0.04
Time 96 (50% confluency)	0.93 ± 0.03
Time 144 (100% confluency)	0.89 ± 0.03