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AWARD NUMBER: W81XWH-08-1-0276

TITLE: Ovarian Carcinoma Stem Cells

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REPORT DATE: May 2009

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 May 2009	Final	15 Apr 2008 – 14 Apr 2009

4. TITLE AND SUBTITLE

Ovarian Carcinoma Stem Cells

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0276

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Johns Hopkins University, School of Medicine
Baltimore, MD 21205

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Ovarian carcinoma is one of the most responsive solid tumors, with the majority of affected women now achieving complete remissions; unfortunately, most of these women eventually relapse and die from the disease. We hypothesized that the initial clinical responses represent therapeutic effectiveness against differentiated cancer cells making up the bulk of the tumor, while the high rate of relapses result from rare, biologically distinct resistant cancer stem cells (CSC). The limited understanding about the phenotype of normal ovarian epithelial stem cells is an obstacle to identifying ovarian CSC, if they exist. However, several characteristics that appear to be shared by normal stem cells from many tissues may serve as markers for CSC from many malignancies. We have developed one of the first true animal models of ovarian cancer, FNAR, a primary rat ovarian cancer that arose spontaneously (submitted for publication). FNAR parallels the human disease both biologically [expresses Her2/neu, estrogen receptors (ER) and androgen receptors (PR)] and clinically. The pan-stem cell marker ALDH expression appears to identify a CSC subpopulation from the FNAR cells, as the 2-4% of the cells expressing high levels of ALDH are enriched for both in vitro and in vivo clonogenic potential.

15. SUBJECT TERMS
No subject terms provided.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	28	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

Many cancers appear to arise from rare self-renewing cells that are biologically distinct from their more numerous differentiated progeny. Although the clinical significance of these so-called cancer stem cells (CSC) has been uncertain, recent data suggest that these cells are responsible for many of the relapses that follow anticancer therapy. Ovarian carcinoma is one of the most responsive solid tumors, with the majority of affected women now achieving complete remissions; unfortunately, most of these women eventually relapse and die from the disease. We hypothesized that the initial clinical responses in ovarian carcinoma represent therapeutic effectiveness against differentiated cancer cells making up the bulk of the tumor, while the high rate of relapses result from rare, biologically distinct CSC resistant to the therapies effective against the tumor bulk. The limited understanding about the phenotype of normal ovarian epithelial stem cells is an obstacle to identifying ovarian CSC, if they exist. However, several characteristics that appear to be shared by normal stem cells from many tissues, such as high expression of aldehyde dehydrogenase (ALDH) and the Hoechst side population (SP) phenotype, may serve as markers for CSC from many malignancies. The overall objective of this proposal is to further characterize and better understand the biology of CSC in ovarian carcinoma, with an eye toward improving therapeutic outcomes.

Body

Studies on the biology of ovarian cancer have been complicated by the absence of a good animal model for this disease. We have developed one of first true animal models of ovarian cancer, FNAR, that spontaneously arose in a Lewis rat (manuscript submitted, see Appendix). Upon intraperitoneal (IP) transplantation into rats, FNAR produces ascites and peritoneal implants; it can also be propagated *in vitro* as a cell line, that maintains the properties of the original tumor. The FNAR cells display striking similarities to human ovarian carcinoma biologically, as well as clinically. Histologically, the tumor is epithelial in origin, and expresses Her2/neu, estrogen receptors (ER), progesterone receptors (PR), and androgen receptors (AR). Gene expression profiling shows upregulation of a number of genes that are also upregulated in human ovarian carcinoma.

The development of this animal model has greatly facilitated our studies on ovarian CSC. Studies into identifying and characterizing CSC from hematologic malignancies have been possible because of a comprehensive understanding of cell surface antigen expression throughout lymphohematopoietic differentiation. In contrast, little is known about the cell surface phenotype associated with the growth and development of most non-lymphohematopoietic tissues. Thus, we have begun studying the ability of the pan-stem cell marker ALDH1 to identify ovarian CSC in the FNAR model. We found that 2-4% of the FNAR cells express high levels of ALDH. Moreover, the ALDH^{high} FNAR cells were significantly enriched for *in vitro* clonogenic potential (Fig 1A). IP injection of 50K FNAR cells leads to malignant ascites at 2-3 weeks, gross IP tumors between 6-8 weeks, and death of the rats between 12 -14 weeks. The cell populations (50K) separated by ALDH expression were injected IP into rats and preliminary results are available. The unfractionated cells generated malignant ascites at 2 weeks after IP transplantation with continued disease progression at 4 weeks (Fig 2B). The ALDH^{low} cells (>95% of the total cells) produced similar abdominal swelling and numbers of ascites tumor cells at 2 weeks, but the abdominal swelling and ascitic tumor cells disappeared. The ALDH^{high} cells produced no evidence of IP tumor growth at 2 weeks, but at 4 weeks produced more ascitic tumor cells than the ALDH^{low} cells. The ALDH^{high} cells produced large peritoneal tumors involving most of the peritoneal cavity by 2 months. The unfractionated cells produced

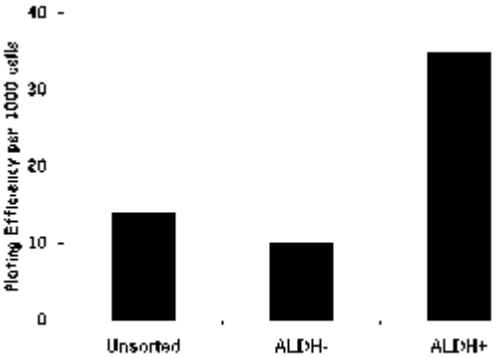

Fig 1A. Aldefluor enriches for clonogenic rat ovarian CA cells.

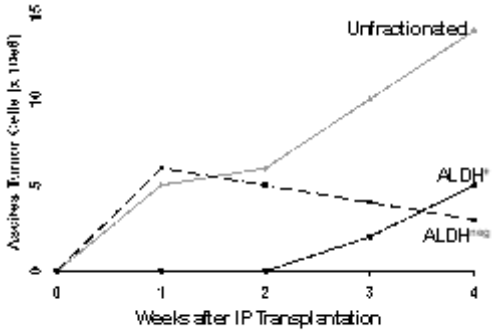

Fig 1B. ALDH expression distinguishes ovarian CA cells with short and long-term engraftment potential. Rat ovarian CA cells were separated by aldefluor and injected IP into rats.

only small peritoneal implants when the rats were sacrificed at 2 months, while rats receiving the ALDH^{low} cells showed no signs of ovarian cancer. Initial studies into characterizing the ALDH^{high} FNAR cells demonstrated that although ER, PR, and AR are expressed on the bulk FNAR cells, they are not expressed by the ALDH^{high} cells (Fig 2 a,b). Thus, ER, PR, and AR appear to be differentiation antigens for ovarian carcinoma. Conversely, Her2/neu is expressed equally on both populations (Fig 2c). Ovarian carcinoma cells from the malignant ascites of 2 patients

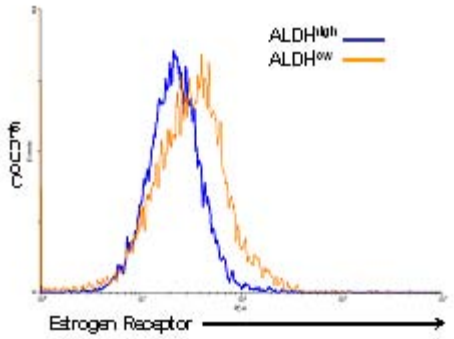

Fig 2A. Estrogen receptor expression in the FNAR rat ovarian carcinoma. The ALDH^{low} and unfractionated cells (not shown) express ER, while the ALDH^{high} cells do not.

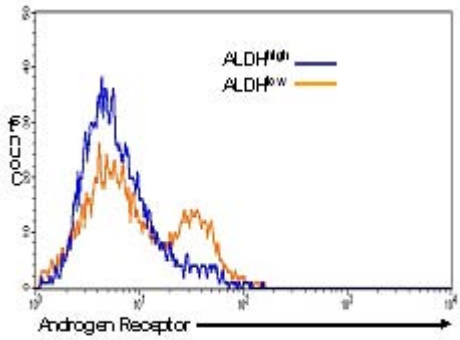

Fig 2B. Androgen receptor expression in FNAR rat ovarian carcinoma. The ALDH^{low} and unfractionated cells (not shown) exhibit a clear AR population that represents about 30% of the total cells, while the ALDH^{high} express no AR.

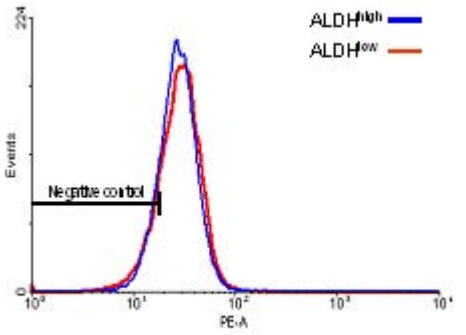

Fig 2C. Both stem cell (ALDH^{high}) and differentiated cell (ALDH^{low}) subsets from the FNAR rat ovarian carcinoma express Her2/neu.

have also been studied for the presence of ovarian CSC. In both patients, the ALDH expression pattern paralleled that seen in FNAR cells, with about 1% of cells showing high ALDH expression (Fig 3). Full characterization of the ALDH^{high} expressing ovarian carcinoma cells from patients is underway.

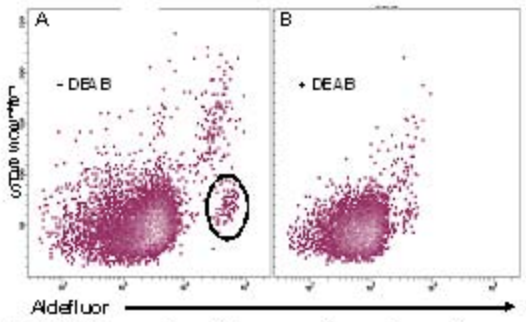

Fig 3. ALDH expression of human ovarian carcinoma from malignant ascites. (A) About 1% of total cells are ALDH^{high} and (B) their Aldefluorfluorescence is eliminated by the ALDH inhibitor DEAB.

Key Research Accomplishments

- Development of one of the first spontaneously-occurring animal models for ovarian carcinoma
- Identification of a population of cells within the rat ovarian carcinoma that has phenotypic and functional characteristics consistent with their being the CSC (i.e., cancer-initiating cells).

Reportable Outcomes

Manuscript: Sharrow AC, Ronnett BM, Thoburn CJ, Barber JP, Giuntoli RJ, Armstrong DK1, Jones RJ, Hess AD. Identification and characterization of a spontaneous ovarian carcinoma in Lewis rats. Submitted 2009.

Grant submitted and funded: DOD grant OC080269 - "Targeting Cancer Stem Cells" (W81XWH-09-1-0129; PERIOD OF PERFORMANCE: 1 May 2009 - 31May 2012 (Research Ends 30 April 2012)

Conclusion

Using a newly spontaneously-developing rat model of ovarian carcinoma, a small population of ALDH^{high} cells appear to be the cells responsible to the growth and development of the tumor both *in vitro* and *in vivo*. Like breast cancer stem cells, this cell population does not apppear to express sex hormone receptors. However, unlike breast cancer stem cells, the ovarian carcinoma CSC do appear to express Her2/neu. Preliminary data suggest that a similar subpopulation is present in primary explanted patient samples. Principles developed in this animal model will be used to perform similar studies in human ovarian carcinoma cell lines and then clinical ovarian carcinoma specimens.

Appendix

Publication:

Sharrow AC, Ronnett BM, Thoburn CJ, Barber JP, Giuntoli RJ, Armstrong DK1, Jones RJ, Hess AD. Identification and characterization of a spontaneous ovarian carcinoma in Lewis rats. Submitted 2009.

Identification and characterization of a spontaneous ovarian carcinoma in Lewis rats.

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Running Title: Rat Model of Ovarian Carcinoma

Keywords: rat, model, ovarian carcinoma, estrogen receptor α, progesterone receptor, androgen receptor, and her-2/neu

Funded in part by the Department of Defense grant number OC073136.

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ABSTRACT

Objective

Ovarian carcinoma is the fourth most common cause of death from cancer in women. Unfortunately, limited progress has been made toward improving the survival rate of patients with this disease. One obstacle to the development of new therapies for ovarian cancer has been the lack of a good animal model. We present here a model of spontaneous ovarian carcinoma arising in a normal Lewis rat.

Methods

The tumor was passages *in vivo* by intraperitoneal injection into immunocompetent Lewis rats. Tumors were sectioned for histological examination. A resulting cell line can be cultured *in vitro*. Cells were examined by flow cytometry for expression of estrogen receptor α, progesterone receptor, androgen receptor, her-2/neu, and Ep-CAM. RNA was harvested from cells for gene expression profiling and for studying the expression of cytokines.

Results

The tumor, designated FNAR, can be transplanted into Lewis rats and propagated as a cell line *in vitro*, maintaining the properties of the original tumor. The FNAR cells display striking similarities to human ovarian carcinoma, resembling the endometrioid carcinoma subtype of surface epithelial neoplasms. The cells express estrogen receptor α, progesterone receptor, androgen receptor, her-2/neu, and Ep-CAM. A gene expression profile shows upregulation of a number of genes that are also upregulated in human ovarian carcinoma.

Conclusion

This reliable model of ovarian carcinoma should be helpful in better understanding the biology of the disease as well as the development of novel treatment strategies.

INTRODUCTION

Ovarian cancer is the fifth most commonly diagnosed cancer in women and the fourth most common cause of death from cancer [1]. The high mortality can be attributed to the high percentage of affected women presenting at an advanced stage, with spread within the peritoneal cavity [2, 3]. With current therapies, including surgical debulking and platinum-based chemotherapy, patients in stage III or stage IV only have a 20% chance of long-term survival [2, 3]. Better understanding ovarian carcinoma biology, as well as the development of new therapies for the disease, has been hampered by the lack of suitable animal models.

Current ovarian cancer models fall into three broad categories: rare spontaneous carcinomas, induced tumors, and human xenografts [4]. These models have allowed researchers to gain valuable insight. However, as described in detail by Garson et. al. and Vanderhyden et. al., these models have deficiencies [4, 5]. Spontaneous ovarian cancer has been observed in mice, rats, and hens [6-8]. The drawback to these models is that the cancers tend to occur at an advanced age and at similar low frequencies as in humans. The low incidence and the length of time required for the development of these tumors render them of limited use for studying the biology and treatment of ovarian carcinoma. Induced tumor models circumvent these problems but create their own artificial systems, which may not accurately reflect the human disease. In one model of *in vitro* transformation, ovarian surface epithelium cells are subcloned until they exhibit the loss of contact inhibition, the capacity for substrate-independent growth, cytogenetic abnormalities, and the ability to form tumors when injected subcutaneously and/or intraperitoneally into athymic mice [9]. This model, though, fails to account for

critical interactions between the cancer cells and the host. Also, it is uncertain if these cells or their malignant transformation are representative of normal human cells or clinical disease. Animal models have been generated by expressing simian virus 40 large T antigen [10], by inactivating p53 and Rb1 [11], by inactivating p53 and activating an oncogene [12], and through hormone treatment [13-15]. The high rate of cancer development in these animals makes these models attractive, but they may not reliably represent human cancer because these genetic changes usually do not occur in patients. Xenografts of human cancers have undergone continuous improvement over the past twenty years [16-19]. These models allow for direct examination of the human cancer but do not allow the study of the early stages of the cancer. These models also rely on an immune-deficient host, which eliminates the interaction between the cancer and the immune system.

We present a new model of ovarian carcinoma, designated FNAR, that spontaneously developed in an untreated, previously normal Lewis rat. The ovarian tumor was harvested and transplanted into normal Lewis rats. In addition, an *in vitro* cell line and clones were generated from the tumor. Importantly, this model fully simulates human ovarian carcinoma by cell biology and growth characteristics.

MATERIALS AND METHODS

Animals. Female Lewis strain rats aged 4-6 weeks (purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA) were kept in sterile micro-isolator cages and fed food and water *ad libitum*. Institutional guidelines concerning the care and use of research animals were followed. The animals were challenged

intraperitoneally with graded numbers of FNAR cells and monitored daily for abdominal swelling. At various intervals after tumor challenge or when animals appeared moribund (pallor, lethargy, and marked abdominal distension) the animals were sacrificed by CO_2 asphyxiation and the cells within the peritoneal cavity harvested by flushing the abdomen with 35 milliliters of sterile phosphate buffered saline (PBS, Grand Island Biological Co., Gibco BRL, Grand Island, NY). At sacrifice, the animals were examined for tumor growth and tissues taken for histological examination. Slides were photographed at 200x with an Olympus DP70 digital camera.

***In vitro* propagation and growth curve.** A cell line (FNAR) that grows *in vitro* as an adherent monolayer was established by culture in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum in 30 ml tissue culture flasks (Corning Flask 3056, Corning Inc., Corning NY). Cells used for experiments were low passage and maintained in culture for one to three months. The doubling time of the cell line was measured by plating 10^4 cells into macrotiter wells then harvesting and counting at 19.5, 43.5, and 115.5 hours.

Flow Cytometric Analysis. Flow cytometry was utilized to assess *in vitro* FNAR cells for expression of known phenotypic markers. Briefly, 5 x 10^5 tumor cells were incubated in polystyrene tubes. Analysis of the intracellular antigens estrogen receptor α, progesterone receptor, and androgen receptor first required fixation in 2% formaldehyde (Polysciences, Warrington, PA) in phosphate buffered saline (PBS, Gibco Invitrogen, Carlsbad, CA) for 15 minutes at 4°C followed by permeabilization with 0.1%

Triton-X-100 (Sigma-Aldrich, St. Louis, MO) in PBS for 15 minutes at 4°C. The cells were then incubated for 30 minutes at 4°C with commercially purchased murine monoclonal antibodies. The concentrations of antibodies used are as follows: estrogen receptor (ER) α at 8 $\mu g/10^6$ cells (Abcam, Cambridge, MA), progesterone receptor (PR) at 16 $\mu g/10^6$ cells (Affinity Bioreagents, Golden, CO), or androgen receptor (AR) at 2 $\mu g/10^6$ cells (Pharmingen, San Diego, CA). The cells were washed and counterstained with phycoerythrin (PE) rat anti-mouse IgG_1 (Becton Dickinson, San Jose, CA) at 125 $ng/10^6$ cells for 30 minutes at 4°C. Commercially purchased murine monoclonal antibody to the rat c-neu oncogene product (Calbiochem, San Diego, CA) was used at 1 $\mu g/10^6$ cells and was counterstained with PE rat anti-mouse IgG_{2a+b} (Becton Dickinson, San Jose, CA) at 30 $ng/10^6$ cells for 30 minutes at 4°C. Tumor cells incubated with secondary antibody alone served as a negative control. Ep-CAM expression was analyzed using a PE-conjugated antibody (Santa Cruz, Santa Cruz, CA) at 1 $\mu g/10^6$ cells with mouse IgG_1-PE as a negative control (Becton Dickinson, San Jose, CA). The cells were analyzed on a Becton-Dickinson FACSCalibur flow cytometer and data was analyzed using FlowJo (Tree Star, Inc, Ashland, OR).

Gene Expression Analysis by cDNA Microarrays. RNA was extracted and purified from cell lysates of 1-5 x 10^5 *in vitro* FNAR tumor cells and the REH cell line of normal rat endothelial cells with 500 µl Trizol reagent (Invitrogen, Carlsbad, CA). Tissue samples were frozen in liquid nitrogen and pulverized with a mortar and pestle. The powder was dissolved in Trizol and centrifuged. Purified RNA was dissolved in 20µl diethyl-pyrocarbonate-treated distilled water. The resulting RNA was analyzed at the

Johns Hopkins microarray core. RNA from control and experimental samples was processed using the RNA amplification protocol described by Affymetrix (Affymetrix Expression Manual). Briefly, 5 μg of total RNA was used to synthesize first strand cDNA using the SuperScript Choice System (Invitrogen, Carlsbad, California) and oligonucleotide primers with 24 oligo-dT plus the T7 promoter (Proligo LLC, Boulder, Colorado). Following the double stranded cDNA synthesis, the product was purified by phenol-chloroform extraction and biotinilated anti-sense cRNA was generated through *in vitro* transcription using the BioArray RNA High Yield Transcript Labeling kit (ENZO Life Sciences Inc., Farmingdale, New York). Fifteen μg of the biotinilated cRNA was fragmented at 94°C for 35 minutes in buffer (100mM Tris-acetate, pH 8.2, 500mM potassium acetate, and 150mM magnesium acetate), and 10μg of total fragmented cRNA was hybridized to the Affymetrix GeneChip rat 230 2.0 array (Santa Clara, CA) for 16 hours at 45ºC with constant rotation (60 rpm). Affymetrix Fluidics Station 450 was then used to wash and stain the chips with a streptavidin-phycoerythrin conjugate. The staining was then amplified as follows: blocking was performed using goat IgG, then a biotinilated anti-streptavidin antibody (goat) was bound to the initial staining, and amplification was completed by the addition of a streptavidin-phycoerythrin conjugate. Fluorescence was detected using the Affymetrix 3000 7G GeneArray Scanner and image analysis of each GeneChip was done through the GeneChip Operating System 1.4.0 (GCOS) software from Affymetrix using the standard default settings. For comparison between different chips, global scaling was used to scale all probesets to a user defined target intensity (TGT) of 150.

Quantitative RT-PCR for Cytokine Expression. Quantitative RT-PCR (Taqman, Applied Biosystems, ABI, Foster City, CA) was utilized to assess levels of cytokine mRNA transcripts of *in vitro* FNAR cells as previously described [20]. The oligonucleotide primers and fluoresceinated probes for the cytokine genes (IL-6, IL-12, and IL-18) were purchased from ABI. Data were analyzed in real-time with Sequencer Detection version 1.6 software, with the results normalized against mRNA transcripts for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GADPH).

RESULTS

Description of proband. Examination of a normal female Lewis rat sacrificed for harvesting normal splenic T cells showed a spontaneously occurring tumor (approximately 0.5 cm^3) derived from the left ovary and attached to and invading the abdominal wall (Figure 1A). In addition, tumor studding was observed at several sites on the wall of the peritoneum, and ascites was present. Histologically, the tumor is an epithelial neoplasm with features most consistent with an adenocarcinoma (Figure 1B). It is composed of nests displaying admixed cribriform and solid architecture. The tumor cells have modest amounts of amphophilic / eosinophilic cytoplasm and relatively uniform oval nuclei that are predominantly vesicular to modestly hyperchromatic and have small nucleoli. Occasional mitotic figures and apoptotic bodies are noted, as is focal necrosis. Based on analogy to human ovarian epithelial tumors, this most closely resembles an endometrioid carcinoma (a cribriform variant of that subtype, with cells being less columnar than the classical human endometrioid carcinoma). Lymphocyte infiltration into the tumor mass was minimal at best, although numerous lymphocytes

were present in the peritoneal fluid. The tumor was excised and pushed through a 100 micron wire mesh screen to obtain a single cell suspension.

***In vivo* and *in vitro* growth characteristics.** Normal Lewis rats were given either intraperitoneal (IP) or subcutaneous injection of graded numbers (5×10^4, 1×10^5, 5×10^5, or 1×10^6) of tumor cells. The animals were monitored daily for overall general health as well as degree of abdominal extension. The tumor repeatedly failed to grow subcutaneously, even with the administration of systemic immunosuppression (Cyclosporine, 10 mg/kg/d) or passage into thymectomized animals. However, all rats became moribund at 150-160 days after IP injection with 5×10^5 or 1×10^6 cells (Table 1). Rats injected with 1×10^5 cells became moribund around 175 days. Rats receiving IP injections of 5×10^4 cells generally did not appear ill by 6 months, but tumor cells were detected in the peritoneal cavity when sacrificed on day 175. Tumor growth recapitulated that seen in the initial rat with IP tumoral masses adhering to all of the visceral organs and the abdominal wall. Histologically, the tumors appeared to be of epithelial origin. This was confirmed by expression of Ep-CAM using flow cytometry (Figure 3E). Affected rats also showed enlargement of the ovaries and fallopian tubes, with a marked increase in vascularization. Successful serial passage was conducted by IP challenge with $1 \text{ X } 10^5$ tumor cells harvested by flushing of the peritoneal cavity.

The doubling time of the FNAR cell line was measured by plating 10^4 cells into macrotiter wells then harvesting and counting at 19.5, 43.5, and 115.5 hours (Figure 2). The slope of the line of log number of tumor cells versus hours estimates a doubling time of 22.9 hours.

Biological characterization of FNAR. Like the clinical presentation, the phenotype of this tumor most resembled epithelial ovarian carcinoma. ER is detected in 60-90% of ovarian carcinomas [21-25], 25-50% express PR [21, 23-26], and 45% expressed both [23, 25]. AR is expressed in 50-70% of ovarian carcinomas [24, 26]. Accordingly, in the appropriate clinical setting, sex hormone receptor expression is diagnostic of ovarian carcinoma [25, 27]. The tumor expressed ER, PR, and AR by flow cytometry (Figure 3A-C), with ER and PR confirmed by PCR (data not shown). The tumor also expressed her-2/neu (Figure 3D), which is expressed in 25-35% of ovarian carcinomas [28, 29]

Gene expression profiling demonstrated that FNAR gene expression was similar to that reported for human ovarian carcinoma (Table 2). Metallothioneins are generally not found at immunohistochemically detectable levels in normal cells, but their expression increases in ovarian carcinoma with increasing grade [30-32]. Metallothionein I was overexpressed 11.38-fold in FNAR cells when compared to endothelial cells, and metallothionein II showed 3.56-fold increased expression. Thioredoxin expression correlates with cis-diaminedichloroplatinum resistance [33] and is expressed in FNAR cells 3.07-fold higher than in endothelial cells. Stathmin regulates microtubules during the formation of the mitotic spindle and is not expressed at detectable levels in normal cells; however, high-level expression is generally seen in ovarian carcinoma [34-36]. Accordingly, stathmin expression was 3.23-fold higher in FNAR cells than in endothelial cells. A nuclear factor that it is involved in cell cycle

progression, b-myb, is also highly expressed in both FNAR cells (3.33-fold) and human ovarian carcinoma [37].

High levels of interleukin-6 (IL-6), a proinflammatory cytokine and hematopoietic growth factor, are found in both normal ovarian epithelium and human ovarian carcinoma [38, 39]. Interleukin-18 (IL-18) is a proinflammatory cytokine that stimulates interferon-γ production. Ovarian carcinoma expresses IL-18, but it is predominantly the pro-IL-18 form [40]. Interleukin-12 (IL-12) is a cytokine that encourages a T_h1 immune response. IL-12 has been detected in ascites fluid and serum of ovarian cancer patients [41], although no reports have examined the expression of IL-12 by the ovarian carcinoma cells themselves. Expression of all three cytokines by FNAR cells was detected by real time RT-PCR (Figure 4).

DISCUSSION

We present here a model of ovarian carcinoma, designated FNAR, that arose spontaneously in a normal Lewis rat. IP transplantation into rats produces malignant ascites and peritoneal carcinomatosis, leading to death at 5-6 months. Cells from the tumor can be easily passaged *in vitro,* and the cell line shows similar growth characteristics when returned to rats. FNAR's biology closely parallels the human disease. It appears to be epithelial in origin by histology and expression of Ep-CAM. Like human ovarian carcinoma, it expresses her-2/neu, sex hormone receptors, and characteristic cytokines. It displays a similar gene expression pattern to the human disease. The tumor only develops in the peritoneal cavity, suggesting the tumor microenvironment is intact during formation.

The FNAR model overcomes many of the limitation of current model systems for ovarian carcinoma. Rats transplanted with FNAR consistently become moribund by 5-6 months, overcoming the low frequency and long latency of spontaneous animal models. Xenografts of primary human tumors in immunodeficient mice are perhaps the most attractive current model [16-19]. Although spontaneous human cancers can be studied and used to test treatments in these mice, the study of immunotherapeutic approaches is problematic. Conversely, FNAR develops in immunocompetent rats, allowing the study of immunotherapeutic approaches. The expression of all three sex hormone receptors and her-2/neu also allows for manipulations of these pathways using this model. However, the relevance of this model to the treatment of human disease still remains to be established.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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TABLE and FIGURE LEGENDS

Table 1. The survival time of rats corresponds to the number of FNAR cells injected intraperitoneally. The animals were monitored daily for overall general health as well as degree of abdominal extension and were sacrificed when appearing moribund.

Table 2. Gene chip analysis of FNAR shows similarities to human ovarian carcinoma. RNA was harvested from FNAR and REH endothelial cell lines and analyzed by GeneChip at a Johns Hopkins core facility. Data are presented as the relative expression of the gene in FNAR compared to expression in endothelial cells.

Figure 1. Proband shows tumor of the left ovary and intraperitoneal tumor studding (A). The tumor is an adenocarcinoma resembling human ovarian endometrioid carcinoma (B).

Figure 2. *In vitro* doubling time was measured by plating 10^4 cells into large flat bottom macrotiter wells. At the designated intervals, cells were harvested and counted. Data is presented as log number of tumor cells versus growth time. The slope of the line represents an estimate of the doubling time.

Figure 3. Flow cytometric evaluation of expression of ER (A), PR (B), AR (C), her-2/neu (D), and Ep-CAM (E). In all five graphs, isotypic control is shown with a solid line and the antibody of interest is shown with a shaded area.

Figure 4. FNAR tumor cells express IL-6, IL-12, and IL-18. Expression was assessed by qPCR. Data are standardized against GAPDH.

TABLE 1

Survival Following Tumor Challenge

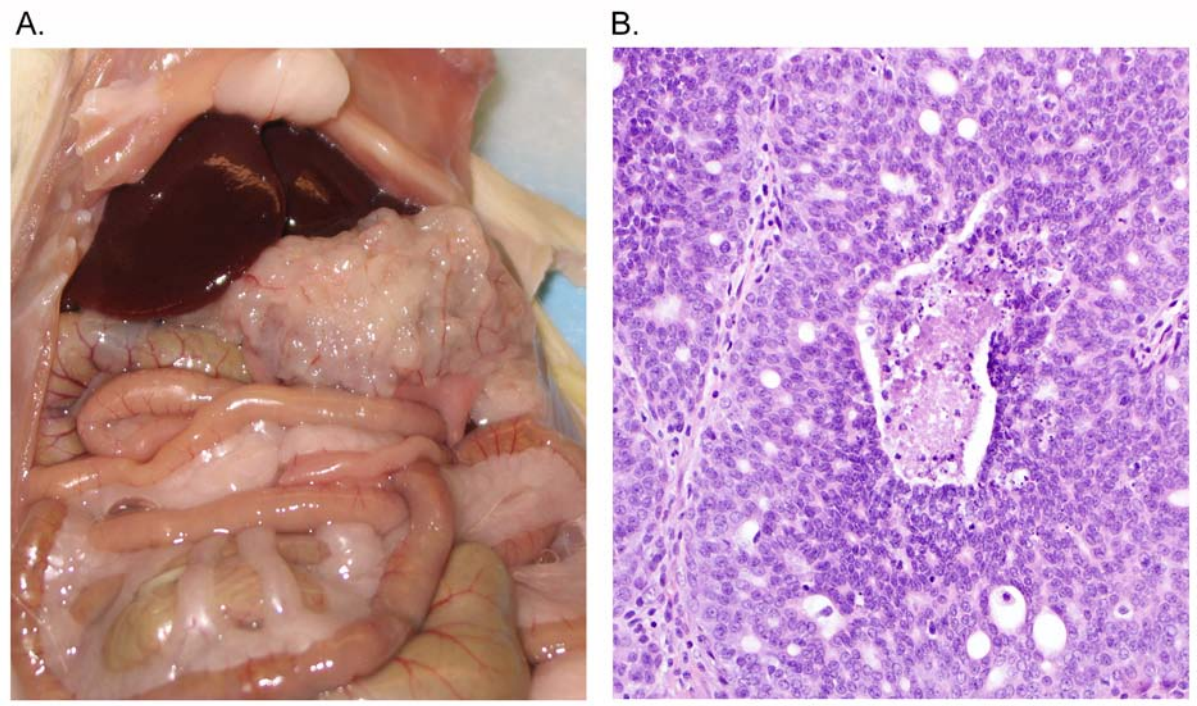
No. of Cells Injected	No. of Animals	Survival - Days[A] (No. of Animals)
5×10^4	N = 6	175 (6)
1×10^5	N = 8	150 (4) 155 (3), 160 (1)
5×10^5	N = 6	155 (2), 160 (4)
1×10^6	N = 6	150 (5), 152 (1)

[A]Animals were observed daily for general health and abdominal extension. The animals were sacrificed upon becoming moribund that was characterized by extreme lethargy, paleness and abdominal extension. The abdominal cavity was examined histologically for the presence of tumor cells in the peritoneal fluid and for tumor masses attached to the visceral organs and the abdominal wall.

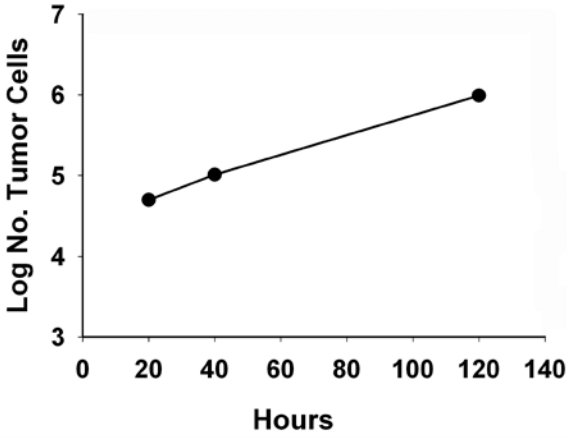
TABLE 2

Gene Expression Profiling of FNAR Cells

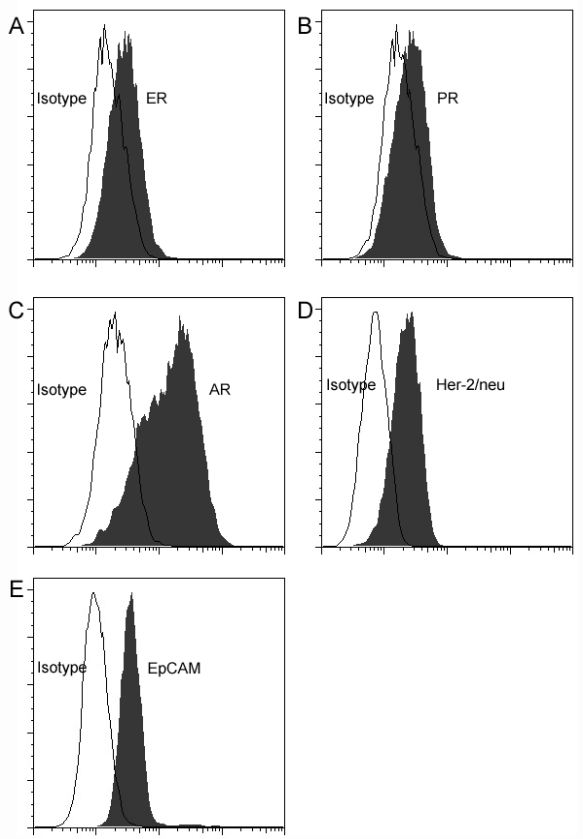
Gene Description	EST Accession #	Relative Expression
Metallothionein I	AW141679	11.38
Metallothionein II	AW916991	3.56
Thioredoxin	AW140607	3.07
Stathmin	BF281472	3.23
b-myb	RGIAC37	3.33



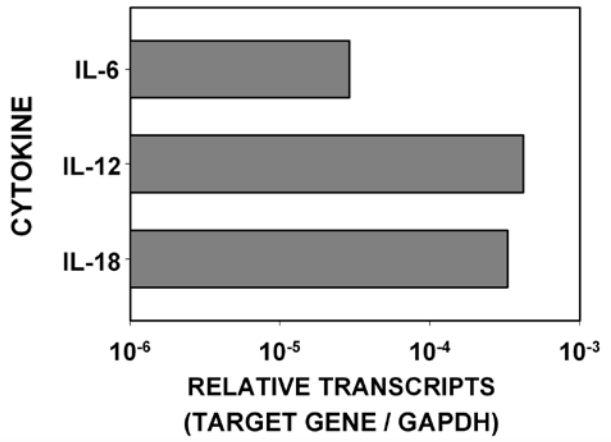
Sharrow Figure 1.



Sharrow Figure 2.



Sharrow Figure 3



Sharrow Figure 4.

ARTICLE PRÉCIS

This article describes a spontaneous rat model of ovarian carcinoma that resembles the human endometrioid carcinoma subtype of surface epithelial neoplasms.