Molecular and Cellular Endocrinology 329 (2010) 37–46

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Review

Chronology and complexities of ovarian tumorigenesis in FORKO mice: Age-dependent gene alterations and progressive dysregulation of Major Histocompatibility Complex (MHC) Class I and II profiles

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ARTICLE INFO

Article history:
Received 5 January 2010
Received in revised form 26 May 2010
Accepted 26 May 2010

Keywords:
FORKO
Microarray
Ovary
Aging
Tumors

ABSTRACT

Among gynecologic malignancies ovarian cancer is the deadliest and most difficult to detect at early stages. As ovarian tumors have long latency and are relatively more frequent in postmenopausal women, revealing chronological changes in model systems might help in the discovery of novel molecular targets and diagnostic biomarkers for disease detection and management. Follitropin receptor knockout (FORKO) mice with early and sustained sex steroid hormone disharmony develop various age-dependent ovarian abnormalities including increased incidence ovarian tumors in complete absence of ovulation. These mutants show various tumor cell types including those related to ovarian surface epithelium around 12–15 months of age. To explore why the FORKO mice develop ovarian tumors later in life, we assessed global gene expression changes during the pre-tumor period (at 8 months). Age-matched wild-type and FORKO mice were compared to gain a comprehensive view of genes that are misregulated, even before overt tumors appear in mutants. Applying a conservative 2-fold change to detect changes, our study identified 476 genes (338 upregulated and 138 downregulated) to be altered between 8-month-old FORKO and wild-type ovaries. Using Ingenuity Pathway Analysis (IPA), we found highly significant alterations in five functional networks in pre-tumor stage FORKO ovaries. Notably, the top network to change in 8-month-old FORKO ovaries was associated with functions implicated in immune system development and function. We selected 9 immune related genes that are reportedly altered in Epithelial Ovarian Cancer (EOC) in women and confirmed their expression and chronology of changes in FORKO ovaries before and after tumor development. Our data indicate that immune surveillance mechanisms are compromised with in a 4-month window of tumorigenic alterations. In addition, expression of previously unrecognized genes misregulated in the dysfunctional FORKO ovaries suggests mechanisms not yet appreciated to date. We propose that a better understanding of genes that change before overt tumors develop could provide useful insights into ovarian carcinogenesis and open the door to additional new targets for treating ovarian cancers.

Contents

Abbreviations: EOC, Epithelial Ovarian Cancer; FORKO, follitropin receptor knockout; FSH, follicle stimulating hormone or follitropin; GDNF, glial cell derived neurotropic factor; HLA, human leukocyte antigens; IPA, Ingenuity Pathway Analysis; MHC, Major Histocompatibility Complex; OC, ovarian cancer; OSE, ovarian surface epithelium; TAP, transporter associated antigen processing.

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doi:10.1016/j.mce.2010.05.015

1. Introduction

Ovarian cancer (OC) described as a silent killer is the leading cause of death from a gynecologic malignancy and currently the 5th major cause of cancer among North American women, with a mean age of incidence of 58 years (Jemal et al., 2008). Despite being a 10th as common as breast cancer, OC is apparently 3× more lethal because the disease is usually detected at late stages due to lack of reliable early detection methods. Although only 20% of OCs is detected early, combining serum CA125 levels and transvaginal ultrasound could be useful (Menon et al., 2009) in this regard. More recent studies suggest a cautious approach to the use of symptom patterns for medical evaluation of early OC (Rossing et al., 2010). The biology of Epithelial Ovarian Cancer (EOC) constituting majority of ovarian malignancies in older women is quite complex as the disease is heterogeneous with different subtypes and recognition of molecular features are mostly based on analysis of tumor samples at advanced stages or cell lines derived from them (Bast et al., 2009).

Although most OCs occur during postmenopausal years after a long latency, very few experimental paradigms in animals have duplicated these conditions to effectively address issues related to hormonal aberrations and their potential relationship to the induction of ovarian carcinogenic events. FSH as the primary endocrine signal is essential for normal folliculogenesis in the ovary and acts by binding to its receptor expressed on granulosa cells. Targeted disruption of the mouse FSH-R gene results in female sterility (Dierich et al., 1998; Danilovich et al., 2000). The null mutants with early hormonal imbalances as well as the heterozygous females that develop early reproductive senescence and obesity, show increased tumor incidences (Danilovich et al., 2001; Chen et al., 2005, 2006,2007; Aravindakshan et al., 2006a). We have also reported that in our follitropin receptor knockout (FORKO) mice, pituitary gonadotropins (FSH and LH) and ovarian androgen levels are significantly increased, while progesterone is reduced and estrogen levels remain very low (Danilovich et al., 2000)—an endocrine profile that is similar, in many respects to postmenopausal conditions and other hormone-related disorders in women (Janssen et al., 2008). Furthermore, by 12–15 months, >90% of FORKO mice developed different kinds of ovarian tumors. The complex ovarian pathology included granulosa cell tumor; Sertoli-Leydig cell tumor and serous papillary epithelial adenoma (Danilovich et al., 2001; Chen et al., 2007). Thus the occurrence of various types of ovarian pathology in majority of the FORKO mice including those of the EOC type prompted the current investigation to first determine the global pattern of gene changes. To better understand this process, we profiled mutant ovaries at different ages with particular focus at 8 months of age. This age is considered a 'pre-tumor stage' because we have shown that significant histological changes occur in the mutant ovary at this period (Chen et al., 2007) and long before the appearance of visible tumors in aged animals at 12–15 months (Danilovich et al., 2001). Therefore, we have hypothesized that examination of molecular changes in the ovary at an age before they develop tumors could provide useful clues necessary to define the chronology and biology of ovarian tumorigenesis with special focus on identifying molecular changes that could be attributable to epithelial tumor types.

Microarray studies provide a useful approach for exploring clues to disease mechanisms and generating novel hypothesis (Dmitrovsky, 2004). In complex diseases like cancer, it is unlikely that individual genes rather than the interaction among many genes could be responsible for triggering carcinogenic events. In addition, epigenetic modifications resulting from their interaction with the environment could also contribute to the tumorigenic process. Therefore, dozens of suspect genes included in an identified signature are insufficient for understanding the underlying mechanisms behind a specific disease phenotype (Dmitrovsky, 2004). In order to gain deeper understanding of complex diseases from a set of differentially expressed genes, one common practice is to convert the information from gene space to structured pathway space via enrichment test of the differential expressed genes in predefined pathways. In this communication, we propose a general framework to analyze the effects of FSH-R deletion on ovarian tumorigenesis via networking pathways that have also been recently applied to study changes in metastatic OC (Barnett et al., 2010). Herein we have identified a number of pathways and gene sets with known and unknown functions that might be responsible for the disease phenotype. More importantly, by networking, we have uncovered a set of pathways and their interactions that could be implicated in the development of tumors in FORKO mice. Applying these considerations to analyze gene expression data in FORKO ovaries has revealed for the first time that immune surveillance mechanisms (genes) that were not expressed in the very young mutant ovary are highly activated in pre-tumor stages of ovarian pathology and then severely downregulated again in tumor-bearing ovaries in mutant mice.

2. Materials and methods

2.1. Animals and tissue collection for mRNA expression analyses

The studies described herein were performed according to accepted and approved guidelines of the institutional animal care committee. FORKO mice were established as previously described (Dierich et al., 1998; Danilovich et al., 2000) and housed under controlled temperature and constant light (12 h of light, 12 h of darkness), with food and water provided ad libitum. The SV129 female mice were derived by breeding heterozygotes and genotyped by PCR (Danilovich et al., 2000). Age-matched animals were used for comparisons. Sex hormone profiling was performed in different age-matched mice using standard methods (Danilovich et al., 2000).

For RNA extraction both ovaries from 1- and 8-month-old wild-type and mutant mice were carefully dissected and pooled into respective groups. Tissues were frozen in liquid nitrogen and homogenized in TRIZOL (Invitrogen, Burlington, Ontario) and processed in accordance with the manufacturer's protocol. As the integrity of RNA samples is essential for gene expression analysis via microarray technology, RNA quality and quantity were analyzed using Lab-on-a-Chip technology using RNA 6000 Nano LabChip kit (Agilent technologies, Mississauga, ON). The system automatically calculates the ratio of ribosomal bands in total RNA samples and shows the percentage of ribosomal impurities in mRNA samples. Only RNA samples of the highest quality were used for microarray analysis.