Characterization of a preclinical model of simultaneous breast and ovarian cancer progression

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Introduction

Breast cancer chemoprevention trials are facilitated by minimally invasive techniques to sample breast tissue and the availability of a number of surrogate biomarkers to evaluate prevention drugs in phase II trials as well as the high incidence of this disease in phase III trials (1–3). In contrast, sampling of ovarian tissue is invasive and appropriate biomarkers for ovarian cancer prevention trials are controversial (4). Additionally, ovarian cancer is a relatively uncommon disease making testing of drugs for primary prevention difficult to justify. One possible solution is to develop breast cancer chemoprevention drugs that simultaneously prevent ovarian cancer. Indeed, successful human ovarian cancer chemoprevention has only been demonstrated incidentally during the course of breast cancer prevention trials (i.e. fenretinide) (5). An initial obstacle to the development of dual target breast and ovarian cancer prevention drugs is the absence of an appropriate animal model.

Rats are the most frequently used animal model for breast cancer chemoprevention, particularly chemical [7,12-dimethylbenzanthracene (DMBA) and 17β-estradiol (E2)] carcinogen models (6–8). In intact females, these carcinogens induce a high incidence of mammary adenocarcinomas (MACs) that express similar histology and biomarker expressions to the human disease within 2–5 months (6–8). No commonly used preclinical model of ovarian cancer has been used to test prevention drugs, but one promising method of inducing epithelial ovarian cancer (EOC) is the direct application of DMBA to the ovarian surface epithelium. This local ovarian DMBA treatment results in an ~30–40% occurrence of EOC and a greater incidence of preneoplastic changes in the ovary (9–11). In this study, these breast and ovarian cancer models were tested in combination in an effort to develop the first preclinical model of simultaneous breast and ovarian cancer progression.

Materials and methods

Animals and treatments
Female Fischer 344 rats (Harlan Breeding Laboratories, Indianapolis, IN, n = 6–8 per treatment × time group) weighing 50–55 g were housed three per cage in a climate and light (12L:12D) controlled environment, and received food and water ad libitum. All experimental protocols were approved by the University of Kansas Medical Center Animal Care and Use Committee. Within a week of arrival, rats were anesthetized using ketamine hydrochloride (80 mg/kg), atropine sulfate (0.2 mg/kg) and xylazine (8 mg/kg). Hemiovariectomy was performed aseptically in order to concentrate ovulation upon the treated ovary and hasten a senescent hormonal milieu (12,13). In addition to increasing ovulation rate on the remaining ovary, Anzalone et al. (12) have shown that hemiovariectomy mimics age-related alterations such as a lower incidence of regular cyclicity altered magnitude of the proestrous LH surge as well as reduced ovarian follicular reserve when
Rats were killed at 3 or 6 months post-treatment, and serum was collected.

Tissue preparation
Rats were killed at 3 or 6 months post-treatment, and serum was collected and stored at −80°C. Right thoracic mammary glands were excised, fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Right abdominal-inguinal mammary glands were spread out onto a glass slide, fixed in 4% PFA and infused with alum carmine following a whole mount preparation protocol (14). The ovary was bisected through the site of DMBA application. One half was fixed in 4% PFA and embedded in paraffin while the remainder was snap-frozen for future study.

Immunohistochemistry
Six micrometer sections of mammary glands and oocytes were deparaf- finized, rehydrated and stained with hematoxylin & eosin (H&E). H&E sections were evaluated for premalignant morphological changes associated with MAC and EOC progression (10,15) by an observer blinded to treatment groups. Additional sections were prepared for immunostaining by antigen retrieval (92.78°C, 10 mM citrate buffer, 25 min) and incubation with 0.3% hydrogen peroxide (Lab Vision, Fremont, CA). Non-immune serum or primary antibodies against estrogen receptor alpha (ER; 1:100; Clone SP1; rabbit monoclonal antibody; Lab Vision), cyclooxygenase-2 (COX-2; 1: 50; RB-9072; rabbit polyclonal antibody; Lab Vision) and Ki-67 (1: 25; Clone Ki-S5; mouse monoclonal antibody; Dako, Carpinteria, CA) were applied and visualized with DAB chromogen and biotinylated secondary antibodies. All incubations were carried using a Dako LV-1 autostainer.

Hormone assays
Serum concentrations of E2 were determined by ELISA according to manufacturer’s protocol (DSL-10-4300, Diagnostic Systems Laboratories, Webster, TX). All samples were run within the same assay, and the intraassay CV was <10%.

Results

Histopathology
Mammary gland whole mounts. In the mammary gland, DMBA, MNU and E2 treatment increased ductal branching and area occupied by alveoli by 3 months, and the impact of E2 was the most extensive among these three carcinogens. These effects were further increased after 6 months of treatment (Figure 1B-D). Vehicle-treated rats (3 and 6 months) showed normal mammary morphology (Figure 1A).

Mammary histology. Vehicle-treated (3 and 6 months) and systemic DMBA-treated (3 months) rats displayed normal histology showing scattered acini throughout the mammary gland, each bearing a single layer of ductal epithelial cells surrounded by myoepithelial cells (Figure 1E). Increased dysplasia scores were observed in carcinogen-treated animals (Table I). Ductal hyperplasia was observed in some DMBA/DMBA- and MNU/DMBA-treated rats (3 and 6 months) and in all 3-month E2/DMBA-treated rats (Figure 1F and G). Six months of systemic E2 induced ductal hyperplasia (2/6), DCIS (3/6), and invasive adenocarcinoma (1/6) (Figure 1H).

Ovarian histology. Local ovarian DMBA application caused increased ovarian dysplasia and benign abnormalities such as local inflammation around suture materials, mild stromal hyperplasia and decreased follicle numbers (Figure 1J and K and Table I). Combined systemic E2 and ovarian DMBA treatment further induced ovarian preneoplastic changes of epithelial origin in 50% rats (i.e. epithelial hyperplasia and inclusion cyst) following 6 month treatment (Figure 1L and Table I). Vehicle-treated rats showed normal ovarian histology with mild inflammation induced by suture materials (Figure 1I).

Epithelial proliferation was stimulated by E2 treatment
Ki-67 expression was localized in the nucleus of ductal epithelial cells in the mammary gland and surface epithelial cells in the ovary. Following 6 month treatment, Ki-67-immunoreactive cells were increased in E2/DMBA mammary glands when compared with controls and other carcinogen-treated rats (Figure 2A–C; P ≤ 0.05). Ovarian epithelial staining for Ki-67 increased significantly from 3 to 6 months in rats treated with E2/DMBA (P ≤ 0.05) but did not differ significantly from controls and other carcinogen-treated rats.

Mammary COX-2 expression increased in E2/DMBA-treated rats
COX-2 expression was elevated in the mammary gland of E2/DMBA-treated rats when compared with controls and other carcinogen-treated rats (3 and 6 months) (Figure 2D and E; P ≤ 0.05). Although COX-2 expression in the ovarian surface epithelium did not increase after carcinogen treatments, preneoplastic changes of epithelial origin in the ovary of E2/DMBA-treated rats showed strong immunoreactivity to COX-2 (Figure 3).

ER expression decreased with carcinogen treatment
ER immunoreactivity in the mammary gland was decreased after 6 month systemic DMBA, MNU and E2 treatment (Figure 4A–C; P ≤ 0.05). Ovarian DMBA similarly decreased the ER expression in the ovarian surface epithelium by 6 months of treatment when compared with controls (Figure 4D–F; P ≤ 0.05).
Serum E₂ concentrations

The sustained release E₂ treatment provided a consistent and sustained increase in serum E₂ when compared with DMBA, MNU, or vehicle treatment (Table I: \( P \leq 0.05 \)). E₂-related side effects including pituitary and uterine hyperplasia were observed in two animals. Although ovarian granulosal proliferation was observed (in addition to epithelial and stromal ovarian hyperplasia) in all E₂/DMBA-treated rats, it was not considered a preneoplastic lesion for EOC when determining dysplasia scores.

Discussion

Breast and ovarian cancer have interdependent risk factors, and women at increased risk for one of these cancers are often at risk for the other (17,18). While active clinical trials are common for the evaluation of breast cancer prevention drugs, ovarian cancer prevention trials are seldom attempted due to low incidence of the disease, relatively invasive procedures for tissue sampling and the lack of well-established serum or imaging-based biomarkers (4). A logical approach to ovarian cancer chemoprevention may be the development of breast cancer prevention drugs that simultaneously decrease the risk of ovarian cancer. Toward this end, the present study is intended to produce a preclinical model for the evaluation of simultaneous chemoprevention of breast and ovarian cancer.

Systemic E₂ and local ovarian DMBA induced preneoplastic changes in breast and ovary of the rat as demonstrated by elevated Ki-67 and COX-2 expression in addition to histological analysis. Unlike systemic DMBA and MNU, systemic E₂ appeared to contribute not only to mammary carcinogenesis but also to the initiation of ovarian neoplasia. This additive or synergistic effect of E₂ merits further exploration. One possible explanation is that the proliferative
effect of \( E_2 \) may increase the mutation rate of ovarian surface epithelial cells (19) and therefore accelerate the incidence of ovarian preneoplastic changes. Similarly, Stewart et al. (10) reported that when combined with gonadotropin hormones, local ovarian DMBA induced more ovarian preneoplastic lesions compared with DMBA treatment alone.

In the present study, putative ovarian preneoplastic changes such as inclusion cysts, epithelial hyperplasia, papilloma and stromal hyperplasia were used to evaluate progression toward ovarian cancer instead of actual cancer incidences. These criteria are the same as those of Stewart et al. (10) in DMBA-induced ovarian adenocarcinomas of the rat. While the presence of inclusion cysts in older women is common (20) and controversy concerning whether inclusion cysts are a preneoplastic lesion remains, many groups agree that ovarian inclusion cysts are a precursor for ovarian adenocarcinoma (21,22). Studies have shown that the number of inclusion cysts is increased in ovaries from patients with ovarian carcinoma, contralateral epithelial ovarian tumors or a family history of ovarian cancer compared with healthy subjects (23–26).

Elevated COX-2 expression has been observed in several tumors including ovarian neoplastic lesion (27–31). However, we found that overall ovarian epithelial COX-2 expression was not altered by \( E_2/DMBA \) treatment, while COX-2 was highly expressed by ovarian inclusion cysts. Recent studies have also revealed the relevance of COX-1 expression in ovarian tumor development (11,32,33), suggesting another target for ovarian cancer chemoprevention, and its role should be investigated using this combined breast and ovarian cancer model.

Although this study was intended primarily to develop a practical model for the evaluation of dual target chemoprevention drugs against breast and ovarian cancer, our results also emphasize the interaction of the etiologies of ovarian and mammary cancer. Previous studies showed that latencies of breast tumor formation induced by MNU, DMBA and \( E_2 \) in the rat are \(~3\), \(4\) and \(6\) months, respectively (34–37). However, systemic MNU and DMBA only caused precancerous mammary changes in the current experiment after \(6\) months of treatment. This is probably due to hemiovariectomy since the removal of both ovaries completely abolishes the ability of mammary carcinogens to induce mammary tumors (38). The intention of hemiovariectomy was to concentrate ovulation on the remaining treated ovary, which is a risk factor for human ovarian cancer (39,40). Approximately 50% of \( E_2/DMBA \)-treated rats developed preneoplastic changes in the ovary. One way to further increase ovarian cancer progression might be to prolong the treatment [rats develop EOC from ovarian DMBA at 10–12 months (11)], but this is difficult due to advanced mammary tumor formation by 6 months of treatment. Additionally, our intention was to parallel the approach of human cancer prevention trials that focus on reversible or preventable
preneoplasia and associated biomarkers rather than actual cancer incidence (3,41).

In the present study, we have demonstrated that rats treated with systemic E2 and local ovarian DMBA develop preneoplastic and neoplastic changes in the breast and ovary simultaneously. This model benefits from apparent additive or synergistic effects of E2 and DMBA in early ovarian carcinogenesis unlike models addressing breast or ovarian cancer separately. This approach is intended to facilitate the identification of promising cancer prevention drugs that simultaneously decrease progression to breast and ovarian cancer [e.g. postmenopausal SERMs or retinoids (5)] or reveal drugs that might incidentally decrease the incidence of one cancer while predisposing to the other [e.g. progesterone (42–45)]. While all women would potentially benefit from a well-tolerated chemoprevention drug against breast and ovarian cancer, a more conservative estimate of benefit in the United States might be the ~10% of the female population considered at elevated risk for these diseases.

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References

1. Fabian,C.J., Kimler,B.F., Mayo,M.S. and Khan,S.A. (2005) Breast-tissue sampling for risk assessment and prevention. Endocr. Relat. Cancer, 12, 185–213.
2. Higgins,S.A., Matloff,E.T., Rimm,D.L., Dziura,J., Haafy,B.G. and King,B.L. (2005) Patterns of reduced nipple aspirate fluid production and ductal lavage cellularity in women at high risk for breast cancer. Breast Cancer Res., 7, R1017–R1022.
3. Khan,Q.J., Kimler,B.F., Clark,J., Metheny,T., Zalles,C.M. and Fabian,C.J. (2005) Ki-67 expression in benign breast ductal cells obtained by random periareolar fine needle aspiration. Cancer Epidemiol. Biomarkers Prev., 14, 786–789.
4. Gershenson,D.M., Tortolero-Luna,G., Malpica,A., Baker,V.V., Whittaker,L., Johnson,E. and Follen Mitchell,M. (1996) Ovarian intraepithelial neoplasia and ovarian cancer. Obstet. Gynecol. Clin. North Am., 23, 475–543.
5. De Palo,G., Mariani,L., Camerini,T., Marubini,E., Formelli,F., Pasini,B., Decensi,A. and Veronesi,U. (2002) Effect of ferretinide on ovarian carcinoma occurrence. Gynecol. Oncol., 86, 24–27.
6. Thompson,H.J., McGinley,J.N., Rothhammer,K. and Singh,M. (1995) Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. Carcinogenesis, 16, 2407–2411.
7. Swanson,S.M. and Unterman,T.G. (2002) The growth hormone-deficient spontaneous dwarf rat is resistant to chemically induced mammary carcinogenesis. Carcinogenesis, 23, 977–982.
8. Li,J.J., Papa,D., Davis,M.F., Weroha,S.J., Aldaz,C.M., El-Bayoumy,K., Ballenger,J., Tawfik,O. and Li,S.A. (2002) Ploidy differences between hormone- and chemical carcinogen-induced rat mammary neoplasms: comparison to invasive human ductal breast cancer. Mol. Carcinog., 33, 56–65.
9. Nishida,T., Sugiyama,T., Kataoka,A., Ushijima,K. and Yakushiji,M. (1998) Histologic characterization of rat ovarian carcinoma induced by intraovarian injection of a 7,12-dimethylbenz[a]anthracene-coated suture: common epithelial tumors of the ovary in rats. Cancer, 83, 965–970.
10. Stewart,S.L., Querec,T.D., Ochman,A.R. et al. (2004) Characterization of a carcinogenesis rat model of ovarian preneoplasia and neoplasia. Cancer Res., 64, 8177–1813.
11. Crist,K.A., Zhang,Z., You,M., Gunning,W.T., Conran,P.B., Steele,V.E. and Lubet,R.A. (2005) Characterization of rat ovarian adenocarcinomas developed in response to direct instillation of 7,12-dimethylbenz[a]-anthracene (DMBA) coated suture. Carcinogenesis, 26, 951–957.
12. Anzalone,C.R., Hong,L.S., Lu,J.K. and LaPolt,P.S. (2001) Influences of age and ovarian follicular reserve on estrous cycle patterns, ovulation, and hormone secretion in the Long-Evans rat. Biol. Reprod., 64, 1056–1062.
13. Chatterjee,A. and Greenwald,G.S. (1972) The long-term effects of unilateral ovariectomy of the cycling hamster and rat. Biol. Reprod., 7, 238–246.
14. Cotroneo,M.S., Wang,J., Fritz,W.A., Ettoum,I.E. and Lamartiniere,C.A. (2002) Genistein action in the prepubertal mammary gland in a chemoprevention model. Carcinogenesis, 23, 1467–1474.
15. Thompson,H.J. and Singh,M. (2000) Rat models of premalignant breast disease. J. Mammary Gland Biol. Neoplasia, 5, 409–420.
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16. Tawfik,O.W., Kimler,B.F., Davis,M. et al. (2006) Comparison of immunohistochemistry by automated cellular imaging system (ACIS) versus fluorescence in-situ hybridization in the evaluation of HER-2/neu expression in primary breast carcinoma. Histopathology, 48, 258–267.

17. Martin,A.M. and Weber,B.L. (2000) Genetic and hormonal risk factors in breast cancer. J. Natl. Cancer Inst., 92, 1126–1135.

18. Antoniou,A.C., Gayther,S.A., Stratton,J.F., Ponder,B.A. and Easton,D.F. (2000) Risk models for familial ovarian and breast cancer. Genet. Epidemiol., 18, 173–190.

19. Syed,V., Ulniski,G., Mok,S.C., Yiu,G.K. and Ho,S.M. (2001) Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. Cancer Res., 61, 6768–6776.

20. Scully,R.E. (1995) Pathology of ovarian cancer precursors. J. Cell. Biochem. Suppl., 23, 208–218.

21. Feeley,K.M. and Wells,M. (2001) Precursor lesions of ovarian epithelial malignancy. Histopathology, 38, 87–95.

22. Brewer,M.A., Ranger-Moore,J., Greene,M.H., Alberts,D.S., Liu,Y., Bartels,H.G., Baruch,A.C. and Bartels,P.H. (2004) Preneoplastic lesions. Int. J. Oncol., 24, 62–66.

23. Mittal,K.R., Zeleniuch-Jacquotte,A., Cooper,J.L. and Demopoulos,R.I. (1993) Contralateral ovary in unilateral ovarian carcinoma: a search for preneoplastic lesions. Int. J. Gynecol. Pathol., 12, 59–63.

24. Resta,L., Russo,S., Colacci,G.A. and PålI. (1993) Morphologic precursors of ovarian epithelial tumors. Obstet. Gynecol., 82, 181–186.

25. Werness,B.A., Afferi,A.M., Bielat,K.L., Eltabbakh,G.H., Piver,M.S. and Paterson,J.M. (1999) Altered surface and cyst epithelium of ovaries removed prophylactically from women with a family history of ovarian cancer. Hum. Pathol., 30, 151–157.

26. Okamura,H. and Katabuchi,H. (2001) Detailed morphology of human ovarian surface epithelium focusing on its metastatic and neoplastic capability. Ital. J. Anat. Embryol., 106, 263–276.

27. Singhal,P.K., Spiegel,G., Driscoll,D., Odumsi,K., Lele,S. and Rodabaugh,K.J. (2005) Cyclooxygenase 2 expression in serous tumors of the ovary. Int. J. Gynecol. Pathol., 24, 62–66.

28. Matsumoto,Y., Ishikawa,O., Deguchi,M., Nakagawa,E. and Ogita,S. (2001) Cyclooxygenase-2 expression in normal ovaries and epithelial ovarian neoplasms. Int. J. Mol. Med., 8, 31–36.

29. Denkert,C., Kobel,M., Pest,S., Kohl,J., Berger,S., Schwabe,M., Siegert,A., Reles,A., Klosterhalfen,B. and Hauptmann,S. (2002) Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. Am. J. Pathol., 160, 893–903.

30. Shigemasa,K., Tian,X., Gu,L., Shiryo,Y., Nagai,N. and Ohama,K. (2003) Expression of cyclooxygenase-2 and its relationship to p53 accumulation in ovarian adenocarcinomas. Int. J. Oncol., 22, 99–105.

31. Roland,I.H., Yang,W.L., Yang,D.H., Daly,M.B., Ozols,R.F., Hamilton,T.C., Lynch,H.T., Godwin,A.K. and Xu,X.X. (2003) Loss of surface and cyst epithelial basement membranes and preneoplastic morphologic changes in prophylactic oophorectomies. Cancer, 98, 2607–2623.

32. Daikoku,T., Tranguel,S., Trofimova,I.N., Dinulescu,D.M., Jacks,T., Nikitin,A.Y., Connolly,D.C. and Dey,S.K. (2006) Cyclooxygenase-1 is overexpressed in multiple genetically engineered mouse models of epithelial ovarian cancer. Cancer Res., 66, 2527–2531.

33. Li,S., Minner,K., Funnin,R., Carl Barrett,J. and Davis,B.J. (2004) Cyclooxygenase-1 and 2 in normal and malignant human ovarian epithelium. Gynecol. Oncol., 92, 622–627.

34. Turan,F.K., Sanchez,R.I., Li,J.J., Li,S.A., Reuhl,K.R., Thomas,P.E., Conney,A.H., Gallo,M.A., Kauffman,F.C. and Mesia-Vela,S. (2004) The effects of steroidal estrogens in ACI rat mammary carcinogenesis: 17beta-estradiol, 2-hydroxyestradiol, 4-hydroxyestradiol, 16alpha-hydroxyestradiol, and 4-hydroxyestrone. J. Endocrinol., 183, 91–99.

35. Li,S.A., Weroha,S.J., Tawfik,O. and Li,J.J. (2002) Prevention of solely estrogen-induced mammary tumors in female aci rats by tamoxifen: evidence for estrogen receptor mediation. J. Endocrinol., 175, 297–305.

36. Thompson,H.J. and Adlakha,H. (1991) Dose-responsive induction of mammary gland carcinomas by the intraperitoneal injection of 1-methyl-1-nitrosourea. Cancer Res., 51, 3411–3415.

37. Jang,T.J., Jung,H.G., Jung,K.H. and O.M.K. (2002) Chemopreventive effect of celecoxib and expression of cyclooxygenase-1 and cyclooxygenase-2 on chemically-induced rat mammary tumours. Int. J. Exp. Pathol., 83, 173–182.

38. Diao,T.L. (1962) The role of ovarian hormones in initiating the induction of mammary cancer in rats by polynuclear hydrocarbons. Cancer Res., 22, 973–981.

39. Murdoch,W.J. and McDonnell,A.C. (2002) Roles of the ovarian surface epithelium in ovulation and carcinogenesis. Reproduction, 123, 743–750.

40. Dietl,J. and Marzusch,K. (1993) Ovarian surface epithelium and human ovarian cancer. Gynecol. Obstet. Invest., 35, 129–135.

41. Fabian,C.J., Kimler,B.F., Anderson,J. et al. (2004) Breast cancer chemoprevention phase I evaluation of biomarker modulation by arzoxifene, a third generation selective estrogen receptor modulator. Clin. Cancer Res., 10, 5403–5417.

42. Liang,Y. and Hyder,S.M. (2005) Proliferation of endothelial and tumor epithelial cells by progestin-induced vascular endothelial growth factor from human breast cancer cells: paracrine and autocrine effects. Endocrinology, 146, 3632–3641.

43. Bu,S.Z., Yin,D.L., Ren,X.H., Jiang,L.Z., Wu,Z.J., Gao,Q.R. and Pei,G. (1997) Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines. Cancer, 79, 1944–1950.

44. Ivarsson,K., Sundfeldt,C., Brannstrom,M. and Janson,P.O. (2001) Production of steroids by human ovarian surface epithelial cells in culture: possible role of progesterone as growth inhibitor. Gynecol. Oncol., 82, 116–121.

45. Jabara,A.G. and Harcourt,A.G. (1971) Effects of progesterone, ovariotomy and adrenalectomy on mammary tumours induced by 7,12-dimethylbenz(a)anthracene in Sprague-Dawley rats. Pathology, 3, 209–214.

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