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Original Article

Involvements of Estrogen Receptor, Proliferating Cell Nuclear Antigen and p53 in Endometrial Adenocarcinoma Development in Donryu Rats

Midori Yoshida[1*], Shin-ichi Katsuda[2], and Akihiko Maekawa[3]

[1] Division of Pathology, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
[2] Department of Biological Safety Research, Japan Food Research Laboratories, 2-3 Bunkyo, Chitose-shi, Hokkaido 066-0052, Japan
[3] Chemical Management Center, National Institute of Technology and Evaluation, 2-24-9 Nishihara, Shibuya-ku, Tokyo 151-0066, Japan

Abstract: Involvements of estrogen receptor (ER)α, proliferating cell nuclear antigen (PCNA) and p53 in the uterine carcinogenesis process in Donryu rats, a high yield strain of the uterine cancer were investigated immunohistochemically. ERα was expressed in atypical endometrial hyperplasia, accepted as a precancerous lesion of the uterine tumors, as well as well- and in moderately-differentiated endometrial adenocarcinomas, and the intensities of expression were similar to those in the luminal epithelial cells of the atrophic uterus at 15 months of age. The expression, however, was negative in the tumor cells of poorly differentiated type. Good growth of implanted grafts of the poorly-differentiated adenocarcinomas in both sexes with or without gonadectomy supported the estrogen independency of tumor progression to malignancy. PCNA labeling indices were increased with tumor development from atypical hyperplasia to adenocarcinoma. The tumor cells in poorly-differentiated adenocarcinomas were positive for p53 positive but negative for p21 expression, suggesting accumulation of mutated p53. These results indicate that the consistent ERα expression is involved in initiation and promotion steps of uterine carcinogenesis, but not progression. In addition, PCNA is related to tumor development and the expression of mutated p53 might be a late event during endometrial carcinogenesis. (DOI: 10.1293/tox.25.241; J Toxicol Pathol 2012; 25: 241–247)

Key words: Endometrial adenocarcinoma Donryu, rat, ERα, PCNA, p53

Introduction

Naturally occurring endometrial adenocarcinomas are rare in rats, whereas inductions of uterine adenocarcinomas have been reported in some safety evaluation studies[1–3]. The mechanisms of rat uterine cancer development are not fully determined; however, estrogens are accepted to play a crucial role in the development in rodents[4–10] as well as endometriod adenocarcinomas in humans[11,12]. Maekawa and co-workers[13] found a high-occurrence of spontaneous uterine endometrial adenocarcinomas in aged Donryu rats, which are similar to human cases as follows: 1) multistep development of uterine lesions from atypical hyperplasias to adenocarcinomas 2) ovarian hormonal imbalance especially elevation of the serum 17β-estradiol (E2) level relative to progesterone, which manifests as atrophic ovaries with cystic follicles and lack of a corpus luteum and 3) morphologic similarities to endometrioid adenocarcinomas of humans[11,12]. The high yield of endometrial adenocarcinomas in Donryu rats is considered to be closely linked to earlier occurrence of the ovarian imbalance detected as persistent estrus by vaginal smear[4,14].

Based on these characteristics, a 2-stage uterine carcinogenesis model was established with intrauterine treatment of *N*-ethyl-*N'*-nitro-*N*-nistrosoguanidine (ENNG) using this rat strain[15] to detect promoting or preventive effects of test chemicals[16–18].

In humans, endometrial adenocarcinoma is the most common malignant tumor of the female genitals in developed countries. The tumors are fundamentally sub-classified into two types, type I, endometrioid adenocarcinoma and type II, serous carcinoma based on epidemiological, clinico-pathological and molecular findings[11,12,19,21]. The former, the most common type, is considered to be related excess estrogen exposure, developing from endometrial glandular hyperplasia (AH). In contrast, the latter, accounting for a minority of endometrial carcinomas, does not seem to be related to estrogenic risk factors or elevated serum hormone levels. The estrogen receptor (ER) can usually be

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*Corresponding author: M Yoshida (e-mail: midoriy@nihs.go.jp)

identified in endometrioid adenocarcinomas, whereas serous lesions are generally negative[11,22]. In addition, mutations in the p53 tumor suppressor gene and accumulation of p53 protein have been detected in approximately 90% of serous carcinomas[12,23,24], whereas they are not common in endometrioid adenocarcinomas and AH[12,25]. In the endometrial adenocarcinomas in Donryu rats, however, the role of ERα and other molecular pathology in uterine carcinogenesis has hitherto been obscure in this model except for K-*ras* point mutaions[26].

The present study was conducted to determine the expression profile of ERα as well as the mutation of p53 during development of endometrial adenocarcinomas in Donryu rats and its link to immunohistochemically determined cellular proliferative activity and p53 protein expression. Furthermore, to confirm hormone-independency in poorly differentiated endometrial adenocarcinomas in Donryu rats, some tumor grafts were implanted into the back subcutis of female and male rats, with or without gonadectomy, and growth of the grafts was assessed.

Materials and Methods

Samples obtained

The numbers of normal tissue samples and proliferative lesions in the endometrium of the uteri examined are shown in Table 1. Uterine samples were obtained from aged Donryu rats with or without various proliferative lesions (97 rats, 12 to 15 months of age) used in uterine carcinogenicity studies previously. The aged normal uterus and proliferative lesions including well- or poorly differentiated adenocarcinomas were treated with intrauterine administrations of ENNG (Nakalai Tesque, Kyoto, Japan) at 10 or 11 weeks of age using assays of uterine carcinogenicity. The tissues were fixed in 10% neutral buffered formaldehyde solution and embedded in paraffin. The specimens were routinely processed and stained with hematoxylin-eosin (HE) for histopathological classification.

After fixation, the upper, middle and lower parts of each uterine horn and the cervix of all uteri were dissected into 3 pieces in cross section to classify uterine proliferative lesions into three degrees of atypical hyperplasia (slight, moderate or severe) and adenocarcinomas, according to the criteria described previously[4,14]. Briefly, atypical hyperplasia is defined as irregular proliferation of atypical glands in the endometrium, and adenocarcinomas were diagnosed on the basis of invasion of the atypical glands into the muscularis. The adenocarcinomas were divided into well-, moderately- and poorly-differentiated by morphological malignancy and degree of invasion: limited to the uterus, invading into the serosa and/or surrounding adnexae, and with distant metastasis, in accordance with the simplified FIGO histopathological grades for human uterine cancers[27]. Specimens from other reproductive tracts or representative organs were examined microscopically.

Immunohistochemistry

Serial sections of the uterus and various neoplastic lesions were incubated with the following antibodies: ERα (catalog No. NCL-ER-LH2; dilution at ×50; NovoCastra Laboratories Ltd, Newcastle, UK), proliferating cell nuclear antigen (PCNA, catalog No. M0879; dilution at ×150; Dako-Cytomation, Kyoto, Japan), anti-wild and mutant p53 (catalog No. NCL-p53-CM5p; dilution at ×200; NovoCastra Laboratories Ltd, Newcastle, UK) or p21 (catalog No. sc6246; dilution at ×100; Santa Cruz Biotechnology, Inc, California, USA). Before the incubation, the sections were heated with citrate buffer pH 7.0 using a microwave (antibodies against ERα, PCNA, p53 or p21 for 20, 2, 20 or 20 min, respectively). After the incubation with these antibodies during overnight at 4°C, the sections were processed using peroxidase labeled dextran polymers (Histofine, Nichirei, Tokyo, Japan) and were visualized with 3,3'-diaminobenzidine tetra hydrochloride (Wako Pure Chemical Industries, Osaka, Japan). Counterstaining was with hematoxylin. The uterus at proestrus in the normal cycling rat was used as a positive control for the ERα, PCNA and p21 antibodies. Repeated positive reactions in the nuclei of different poorly-differentiated endometrial adenocarcinoma cells were judged as positive. Sections incubated without these antibodies were used as the negative control.

Image-analysis of expression profiles

To compare ERα, PCNA and p53 immunohistochemical expression profiles in the endometrium of normal uteri in aged female rats with those of atypical hyperplasia and adenocarcinomas, the positive areas for these antibodies in total nuclear areas in the epithelium and lesions were calculated using an image analyzing system (IPAP, Sumika Technos, Osaka, Japan). After confirmation of the morphological features of each lesion by HE staining, the positive nuclear areas for these antibodies and total nuclear areas in

Table 1. Numbers of Uterine Samples Examined in the Present Study

Samples obtained	Number of samples
Normal areas	
Normal uteri showing no cyclicity (at 12 to 15 months of age)	50
Proliferative lesions	
Atypical hyperplasias (slight to severe, at 12 to 15 months of age) 27, 26 and 24 samples for slight, moderate and severe, respectively)	67
Adenocarcinomas (at 12 to 15 months of age, 28 well-differentiated and 3 moderately-differentiated)	31
Adenocarcinomas (poorly differentiated, at 12 to 15 months of age)	8

4 specimens of normal uteri and each lesion were counted, and the percentages of positive areas for the antibodies were calculated. The typical changes in the normal uteri and each lesion were determined using an automated cellular image analysis scanning system (SL-50, Applied imaging, Santa Clara, CA, USA). In addition, immunohistochemical staining with p21 antibody was performed if tumor cells were stained positive for p53.

Confirmation of hormone independence of poorly-differentiated endometrial adenocarcinomas by tumor graft implantation

Mature male and female Crj:Donryu rats demonstrating normal cycling were purchased from Charles River Laboratories Japan (Kanagawa, Japan) and maintained in an air-conditioned animal room. Commercial pellet diet (CRF-1, Oriental Yeast, Kanagawa, Japan) and drinking water were available *ad libitum* throughout the experiment. Half of the male and female rats (6 to 9 animals per sex) were gonadectomized under deep anesthesia. Two or 3 weeks after the gonadectomy, small grafts (3 mm cubes) from one poorly differentiated endometrial adenocarcinoma were implanted into the back subcutis of normal (gonad-intact) and gonadectomized animals. Two carcinomas identified as immunohistochemistry as ERα negative were selected from 4 samples. Growth of the grafts was checked daily by palpation and measured once a week up to 6 weeks after the implantation. Animal care and use followed the guide of the Animal Committee of Sasaki Institute.

Statistical analysis

Means and standard deviations (SD) of the individual values of positive nuclear areas and total nuclear areas in the normal uteri and each lesion were calculated. Growth of the grafts in the intact females was compared with those in other groups. In multiple comparisons, continuous data were analyzed with Barlett's test. When variances were homogenous, Dunnett's multiple comparison test was performed. The Steel's multiple comparison test was employed, when variances were not homogenous. The level of significance was set as $P<5\%$ and 1%.

Results

Histopathologic changes in atypical hyperplasias and adenocarcinomas dependent were similar among the samples obtained, respectveily. Well-differentiated adenocarcinomas were characterized by the tumor cells arranged glandular or back-to-back pattern and cytoplasmic vacuolation was partly detected in some cases. In poorly differentiated ones, severe cellular atypia and structure destruction with cirrhosis including dissemination into the abdominal cavity or metastasis to the lungs were frequently observed.

Typical immunohistochemical profiles for ERα, PCNA and p53 in uterine proliferative lesions assessed using an automated cellular image analyzing system are shown in Fig. 1, and calculated percentage values for positive cells are shown in Fig. 2. ERα expression was constantly observed in most of the uterine epithelium in aged rats. In the aged uteri, severe fibrosis or cystic hyperplasia was common, so the glandular epithelium was often difficult to detect. ERα positive tumor cells were distributed in the atypical hyperplasias and well- and moderately-differentiated adenocarcinomas. Their intensities were similar to that in the aged uterus (Fig. 2). In clear contrast, no binding was detected in any of the poorly differentiated adenocarcinomas examined.

The numbers of PCNA-positive cells were elevated in uterine proliferative lesions, compared with the normal epithelia, and there was a tendency for an increase in the degree of atypical hyperplasia, and significantly increase in the adenocarcinomas with advancing malignancy (Fig. 1 and Fig. 2). There was no expression of p53 in normal epithelia and well- or moderately-differentiated adenocarcinomas of either the early and advanced types. On the other hand, strong antibody binding was evident in poorly differentiated adenocarcinomas, especially in cells with marked cellular atypia in invading areas, with or without abundant fibrous stroma, although p21 expression was not detectable in any tumor cells from poorly differentiated adenocarcinomas that were positive for p53.

Growth curves for implanted tumors are shown in Fig. 3. The tumor nodules grafted into the back skin showed rapid growth in all animals of both sexes, becoming detectable as palpable nodules after 2 weeks. The growth curves did not differ between males and females up to 6 weeks after the implantation and were not influenced by gonadectomy. In addition, metastasis to the lung and/or lymph nodes was observed in any groups with similar incidences (over 80%) at the termination.

The most common aging-associated changes in the endometrium were stromal fibrosis, cystic hyperplasia of the luminal epithelium, squamous metaplasia, atrophic cuboidal epithelium and inflammation. The ovaries of aged rats in the present study showed atrophic changes with lack of corpus lutea, cystic or atretic follicles and increased interstitial glands, which are typical of the observations for aged ovaries in this strain. A number of age-related nonneoplastic or neoplastic lesions were observed in other organs, but no clear relationship with the expression profiles of ERα, PNCA or p53 could be established.

Discussion

E2 plays a crucial role in proliferating activity in the uteri via ER in mammals[28–31]. In our previous studies, chronic exposure to estrogenic compounds or long-term elevation of the serum E2/progesterone ratio has enhanced the development of uterine neoplastic lesions in rats, whereas the lesions were not inducible in ovariectomized rats[7–9,32].

The present results of consistent ERα expression in the majority of the normal endometrial epithelium in aged uteri, uterine atypical hyperplasias and well- to moderately differentiated adenocarcinomas suggest that uterine proliferating lesions in rats were estrogen dependent event mediated by

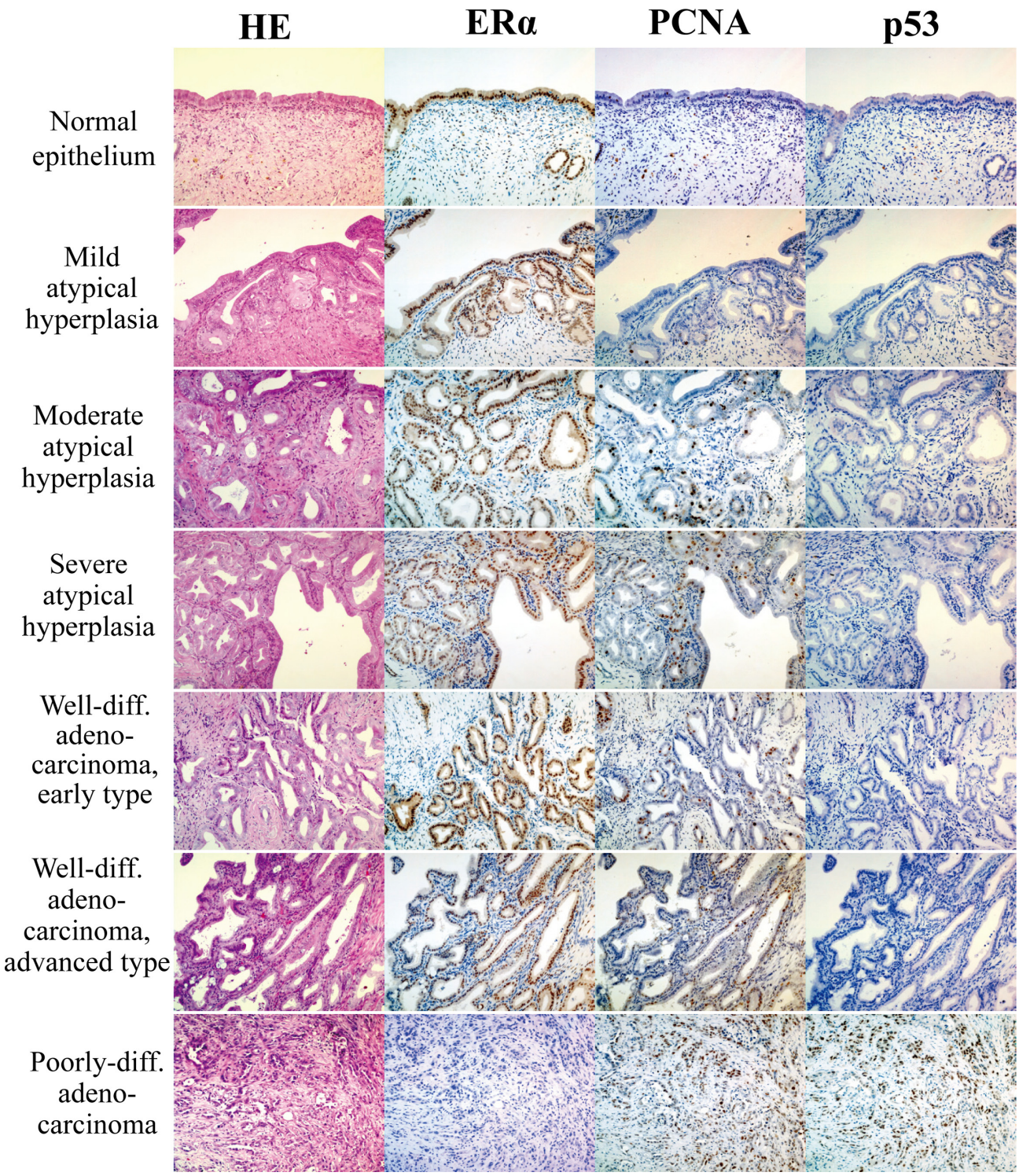

Fig. 1. Immunohistochemical expression profiles of ERα, PCNA and p53 and HE staining in representative areas of normal uteri (at 12 to 15 months of age) and various neoplastic lesions in the uteri. Well-diff., well-differentiated adenocarcinoma; Poorly-diff., poorly-differentiated adenocarcinomas.

ERα. The high yield of endometrial adenocarcinomas in the Donryu rats is considered to be linked to continuous stimulation of E2, and our results added that ERα-expressing cells might be necessary for the initiation and promotion steps of uterine adenocarcinoma development. The loss of ERα in poorly differentiated adenocarcinomas supported by estrogen-independent growth of the implanted tumors might indicate that the expression of ERα is not necessary for the progression step of uterine cancer in rats. It has already been established for human endometrial adenocarcinomas that hormone therapy has no effect on advanced malignancies[33, 34]. The involvement of another estrogen re-

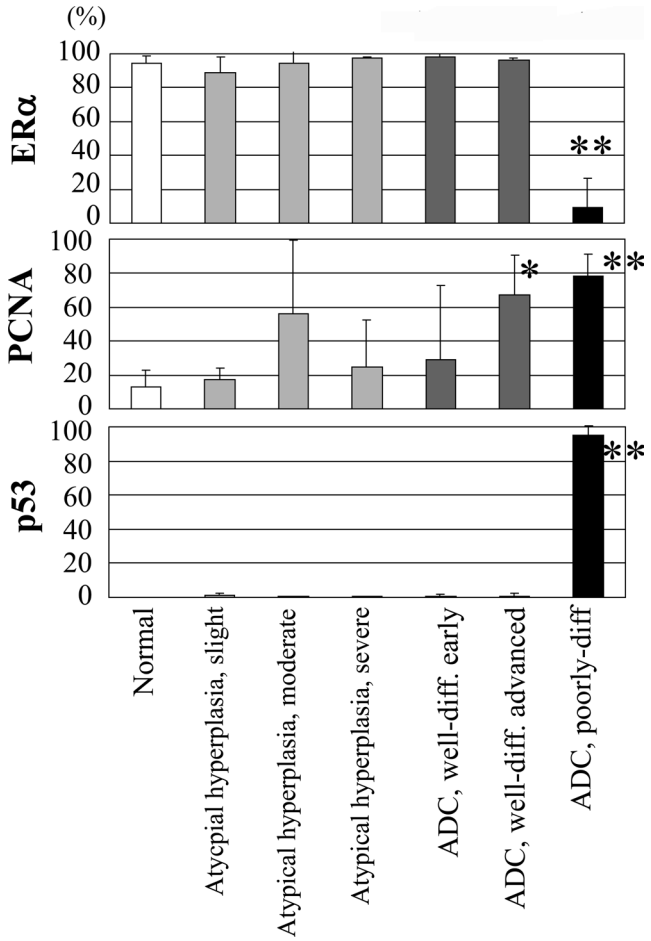

Fig. 2. Percentages of immunohistochemically positive cells for ERα, PCNA and p53 in representative areas of normal uteri (at 15 months of age) and various neoplastic lesions in the uteri. *P<0.05 and **P<0.01 indicate significant differences in the expression of endometrial epithelium (normal) and uterine proliferative lesions compared with that in normal aged rats at 5% and 1%, respectively. The column and bar are represent the mean and SD, respectively.

ceptor form, ERβ, to uterine cancer development in rodents remains to be established, but recent investigations have pointed to a relationship between ERα and β expression in human endometrial adenocarcinomas[35,36].

In the present study, the PCNA labeling index was increased in advanced proliferating lesions, correlating with cellular atypia and/or tumor invasion. Only the poorly-differentiated tumors were positive for p53 accumulation, and their negative reactions for p21 suggested that the cells positive for p53 were mutated. In endometrial adenocarcinomas in women, mutation in the p53 tumor suppressor gene and accumulation of p53 protein are detected in approximately 90% of serous adenocarcinomas[11,12]. In endometrioid adenocarcinomas, p53 expression is not common, but the p53 accumulation is considered to indicate progression to uterine carcinoma or large high-grade tumors[11,19]. It was recently reported that p53 is not related to ERα expression status in women[37], but any links to the question in rodents remain to be clarified.

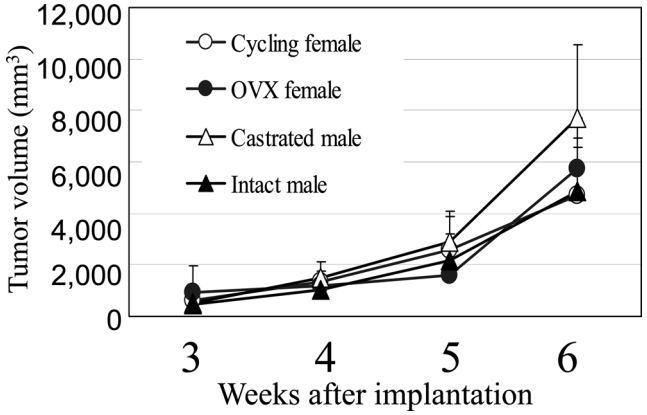

Fig. 3. Growth curves of tumor grafts after implantation. OVX, ovariectomy.

Endometrial adenocarcinomas in humans have been classified into two broad categories: type I estrogen-dependent adenocarcinoma with an endometriod morphology and type II non-estrogen-dependent adenocarcinoma with a serous, papillary or clear cell morphology[11,12,19–21]. The present study and our previous studies described in the introduction indicate that the endometrial adenocarcinomas in the Donryu rat strain have many similarities to type I, endometrioid adenocarcinoma in women. Samuelson *et al.* reported that spontaneous endometrial adenocarcinomas in BDII rats are also similar to type I tumors in women[38]. On the other hand, a possibility might exist that the poorly differentiated types in the present study might be biologically different from well-differentiated adenocarcinomas, because of the similarity of the p53 profile in the poorly-differentiated adenocarcinomas in rats to that of type II carcinomas in women. Whereas type I cancer develops in menopausal women, type II is mainly detected in elderly women[11]. Further investigation might be required to clarify the suggestion above; however, the samples of adenocarcinomas in the present study were obtained from rats at similar ages supporting the idea that poorly-differentiated adenocarcinoma represent the late stage of well- or moderate-differentiated adenocarcinomas.

In conclusion, the present study provided evidence of the involvement of consistent ERα expression in the initiation and promotion steps of uterine carcinogenesis in rats. The loss of expression was linked to malignant progression and hormone independence. PCNA is related to tumor development and the expression of p53 might be a late event leading to malignancy. The data point to a number of similarities with endometrioid adenocarcinomas, the major type of corpus uterine cancer in women.

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References

1. Pintér A, Torok G, Borzsonyi M, Surjan A, Csik M, Kelecsenyi Z, and Kocsis Z. Lon-term carcinogenicity bioassay of the herbicide atrazine in F344 rats. Neoplasma. **37**: 533–544. 1990. [Medline]
2. Irwin RD, Haseman JK, and Eutis SL. 1,2,3-Trichloropropane: a multiple carcinogen in rats and mice. Fundam Appl Toxicol. **25**: 241–252. 1995. [Medline] [CrossRef]
3. Picut CA, Aoyama H, Holder JW, Gold LS, Maronpot RR, and Dixon D. Boromoethane, chloroethane and ethylene oxide induced uterine neoplasms in B6C3F1 mice from 2-year NTP inhalation bioassays: pathology and incidence data revised. Exp Toxicol Pathol. **55**: 1–9. 2003. [Medline] [CrossRef]
4. Nagaoka T, Takeuchi M, Onodera H, Matsushima Y, Ando-Lu J, and Maekawa A. A sequential observation of spontaneous endometrial adenocarcinoma development in Donryu rats. Toxicol Pathol. **22**: 261–269. 1994. [Medline] [CrossRef]
5. Niwa K, Murase T, Mirishita S, Tanaka T, Mori H, and Tamaya T. Enhancing effect of estrogens on endometrial carcinogenesis initiated by N-methyl-N-nitrosourea in ICR mice. Jpn J Cancer Res. **84**: 951–955. 1993. [Medline] [CrossRef]
6. Niwa K, Tanaka T, Yokoyama Y, Mori H, and Tamaya T. Rapid induction of endometrial carcinoma in ICR mice treated with N-methyl-N-nitrosourea and 17B-estradiol. Jpn J Cancer Res. **82**: 1391–1396. 1991. [Medline] [CrossRef]
7. Nagaoka T, Takeuchi M, Onodera H, Mitsumori K, Lu J, and Maekawa A. Experimental induction of uterine adenocarcinoma in rats by estrogen and N-methyl-N-nitrosourea. In vivo. **7**: 525–530. 1993. [Medline]
8. Maekawa A, Takahashi M, Ando J, and Yoshida M. Uterine carcinogenesis by chemicals/hormones in rodents. J Toxicol Pathol. **12**: 1–11. 1999. [CrossRef]
9. Katsuda S, Yoshida M, Kuroda H, Ando J, Takahashi M, Kurokawa Y, Watanabe G, Taya K, and Maekawa A. Uterine adenocarcinoma in N-ethyl-N'-nitro-N-nitrosoguanidine-treated rats with high-dose exposure to p-terty-octylphenol during adulthood. Jpn J Cancer Res. **93**: 117–124. 2002. [Medline] [CrossRef]
10. Maekawa A, Yoshida M, Katsuda S-I, and Imai K. Toxicologic/carcinogenic effects of endocrine disrupting chemicals on the female genital organs of rodents. J Toxicol Pathol. **17**: 69–83. 2004. [CrossRef]
11. Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. Mod Pathol. **13**: 295–308. 2000. [Medline] [CrossRef]
12. Albertini AF, Devouassoux-Shesheboran M, and Genesite C. Pathology of endometrioid carcinoma. Bull Cancer. **99**: 7–12. 2012. [Medline]
13. Maekawa A, Onodera H, Tanigawa H, Furuta K, Kanno J, Matsuoka C, Ogiu T, and Hahashi Y. Spontaneous neoplastic and non-neoplastic lesions in aging Donryu rats. Jpn J Cancer Res. **77**: 882–890. 1986. [Medline]
14. Nagaoka T, Onodera H, Matsushima Y, Todate A, Shibutani M, Ogasawara H, and Meakawa A. Spontaneous uterine adenocarcinomas in aged rats and their relation to endocrine imbalance. J Cancer Res Clin Oncol. **116**: 623–628. 1990. [Medline] [CrossRef]
15. Ando-Lu J, Takahashi M, Imai S, Ishihara R, Kitamura T, Iijima T, Takano S, Nishiyama K, Suzuki K, and Maekawa A. High-yield induction of endometrial adenocarcinomas in Donryu rats by a single intra-uterine administration of N-ethyl-N'-nitro-N-nitrosoguanidine. Jpn J Cancer Res. **85**: 789–793. 1994. [Medline] [CrossRef]
16. Nishiyama K, Ando-Lu J, Nishimura S, Takahashi M, Yoshida M, Sasahara K, Miyajima K, and Maekawa A. Initiating and promoting effects of concurrent oral administration of ethylenethiourea and sodium nitrite on uterine endometrial adenocarcinoma development in Donryu rats. In vivo. **12**: 363–368. 1998. [Medline]
17. Yoshida M, Kudoh K, Katsuda S, Takahashi M, Ando J, and Maekawa A. Inhibitory effects of uterine endometrial carcinogenesis in Donryu rats by tamoxifen. Cancer Lett. **134**: 43–51. 1998. [Medline] [CrossRef]
18. Katsuda S, Yoshida M, Saarinen N, Smeds A, Nakae D, Santti R, and Maekawa A. Chemopreventive effects of hydorxymatairesinol on uterine carcinogenesis in Donryu rats. Exp Bio Med. **229**: 417–424. 2004.
19. Abal M, Planaguma J, Gill-Moreno A, Monge M, Ganzales M, Baro T, Garcia A, Castellvi J, Cajal SR, Xercavinzs J, Alameda F, and Reventos J. Molecular pathology of endometrial carcinoma: transcriptional signature in endometrioid tumors. Histol Histopathol. **21**: 197–204. 2006. [Medline]
20. Cerezo L, Cárdenes H, and Michael H. Molecular alterations in the pathogenesis of endometrial adenocarcinoma. Therapeutic implications. Clin Transl Oncol. **8**: 231–241. 2006. [Medline] [CrossRef]
21. Doll A, Abai M, Rigau M, Monge M, Gonzales M, Demajo S, and Colás E. Llauradò M, Alazzouzi H, lanagumá J, Lohmann MA, Garcia J, Roman CJ, Gil-Moreno A, Xercavins J, Alameda F, and Reventòs J. Novel molecular profiles of endometrial cancer-new light through old windows. J Steroid Mol Biol. **108**: 221–229. 2008. [CrossRef]
22. Halperin R, Zehavi S, Habler L, Hades E, Bukovsky I, and Schneider D. Comparative immunohistochemical study of endometrioid and serous papillary carcinoma of endometrium. Eur J Gynaecol Oncol. **22**: 122–126. 2001. [Medline]
23. Tashiro H, Isacson C, Levine R, Kurman RJ, Cho KR, and Kedrick L. p53 Gene mutations are common in uterine serous carcinoma and occur early in their pathogenesis. Am J Pathol. **150**: 177–185. 1997. [Medline]
24. Demopoulos RI, Mesia AF, Mittal K, and Vamvakas E. Immunohistochemical comparison of uterine papillary serous and papillary endometrioid carcinoma: clues to pathogenesis. Int J Gynecol Pathol. **18**: 233–237. 1999. [Medline] [CrossRef]
25. Sherman ME, Bur ME, and Kurman RJ. p53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorigenesis. Hum Pathol. **26**: 1268–1274. 1995. [Medline] [CrossRef]
26. Tanoguchi K, Yaegashi N, Jiko K, Maekawa A, Sato S, and Yajima A. K-ras point mutations in spontaneously occurring endometrial adenocarcinomas in the Donryu rat. Tohoku J Exp Med. **189**: 87–93. 1999. [Medline] [CrossRef]

27. Pecorelli S, Benedet JL, Creasman WT, and Shepherd JH. FIGO staging of gynecologic cancer. Int J Gynaecol Obstet. **64**: 5–10. 1999. [Medline] [CrossRef]
28. Ando-Lu J, Sasahara K, Nishiyama K, Takano S, Takahashi M, Yoshida M, and Maekawa A. Stain-differences in proliferative activity of uterine endometrial cells in Donryu and Fischer 344 rats. Exp Toxicol Pathol. **50**: 185–190. 1998. [Medline] [CrossRef]
29. Katsuda SI, Yoshida M, Watanabe T, Kuroda H, Ando-Lu J, Takahashi M, Hayashi H, and Maekawa A. Estrogen receptor mRNA in uteri of normal estrous cycling and ovariectomized rats by in situ hybridization. Proc Soc Exp Biol Med. **221**: 207–214. 1999. [Medline] [CrossRef]
30. Yoshida M, Katsuda S, Ando J, Kuroda H, Takahashi M, and Maekawa A. Subcutaenous treatment of p-tert-octylphenol exerts estrogenic activity on the female reproductive tract in normal cycling rats of two different strains. Toxicol Lett. **116**: 89–101. 2000. [Medline] [CrossRef]
31. Cooke PS, Buchanan DL, Young P, Setiawan T, Brody J, Korach KS, and Taylor J. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. Proc Natl Acad USA. **94**: 6535–6540. 1997. [CrossRef]
32. Yoshida M, Katashima S, Ando J, Tanaka T, Uematsu F, Nakae D, and Maekawa A. Dietary indole-3-carbinol promotes endometrial adenocarcinoma development in rats initiated with N-ethyl-N'-nitro-N-nistrosoguanidine, with induction of cytochrome P450s in the liver and consequent modulation of estrogen metabolism. Carcinogenesis. **25**: 2257–2264. 2004. [Medline] [CrossRef]
33. Pertschuk LP, Masood S, Simone J, Feldman JG, Fruchter RG, Axiotis CA, and Greene GL. Estrogen receptor immunohistochemistry in endometrial carcinoma: a prognostic marker for survival. Gynecol Oncol. **63**: 28–33. 1996. [Medline] [CrossRef]
34. Susumu N, Aoki D, Suzuki N, and Nozawa S. Hormonal therapy for endometrial adenocarcinoma. Gan To Kagaku Ryoho. **28**: 934–945 (Abstract in English) 2001. [Medline]
35. Utsunomiya H, Suzuki T, Harada N, Ito K, Matsuzaki S, Konno R, Sato S, Yajima A, and Sasano H. Analysis of estrogen receptor α and β in endometrial carcinomas: correlation with ERβ and clinicopathologic findings in 45 cases. Int J Gynecol Pathol. **19**: 335–341. 2000. [Medline] [CrossRef]
36. Takama F, Kanuma T, Wang D, Kagami I, and Mizunuma H. Oestrogen receptor β expression and depth of myometrial invasion in human endometrial cancer. Br J Cancer. **84**: 545–549. 2001. [Medline] [CrossRef]
37. Maeda K, Tsuda H, Hashiguchi Y, Yamamoto K, Inoue T, Ishiko O, and Ogita S. Relationship between p53 pathway and estrogen receptor status in endometrial-type endometrial cancer. Human Pathol. **33**: 386–391. 2002. [CrossRef]
38. Samuelson E, Hedberg C, Nilsson S, and Behboudi A. Molecular classification of spontaneous endometrial adenocarcinomas in BDII rats. Endocr Relat Cancer. **16**: 99–111. 2009. [Medline] [CrossRef]