Summary. Uterine serous carcinoma (USC) is closely associated with advanced age in patients. The p53 signature (p53S) is considered the earliest indication for the presence of carcinogenesis of USC. Based on our previous studies, the presence of p53Ss have almost always been found in elderly women and are suspected of being responsible for the imbalance between the proliferation and apoptosis of endometrial epithelial cells with advanced age. We have summarized the current state of knowledge regarding the association between age and cancer and propose an age-related type of endometrial cancer instead of Type II estrogen-independent endometrial cancer.

Key words: Uterus, Serous carcinoma, Aging, p53, Apoptosis

Introduction

Aging is one of the most important risk factors for the development of neoplasia. Its status as a risk factor has been partially attributed to the cumulative exposure to carcinogens over time and the multiple hits required for the onset of neoplasia (Vogelstein et al., 2013). A patient’s age indicates the time interval during which driver genes are usually mutated (Vogelstein et al., 2013). The number of somatic mutations in tumors of self-renewing tissues is positively correlated with the age of patients at the diagnosis (Tomasetti et al., 2013).

Uterine serous carcinoma (USC) is closely associated with an advanced age of patients, with a mean age in the late 60s, and often arising against a background of inactive or atrophic endometrium (Longacre and Well, 2014). These findings suggest that USCs have a higher number of somatic mutations than endometrioid carcinomas of the endometrium that occur in relatively young women. However, integrated genomic and proteomic analyses of endometrial carcinoma showed that USC had extensive somatic copy number alterations (SCNAS), a low mutation rate, few DNA methylation changes, low estrogen/progesterone receptor levels, and frequent TP53 mutations (Cancer Genome Atlas Research Network et al., 2013). The authors suggested the ultramutated phenotype and the hypermutated type of endometrial cancers to be due to mutations in the exonuclease domain of POLE and deficiency of mismatch repair, respectively. A high degree of SCNAS was correlated with an unfavorable prognosis in endometrial cancer patients, while tumors with a high mutation rate were correlated with favorable prognosis in endometrial cancer patients. Another group reported that 9% of USCs have a very high number of somatic mutations, with many somatic mutations in mismatch repair and POLE genes in particular. These tumors had no identified SCNAS, despite the remarkable somatic mutation burden (Zhao et al., 2013). SCNAS play critical roles in activating oncogenes and inactivating tumor suppressors (Zack et al., 2013). The SCNA prevalence was found to be strongly positively associated with age by applying SNP microarray data from healthy individuals (Vattathil and Scheet, 2016).
The analysis of SCNA patterns in cancers showed that SCNAs were associated with TP53 mutations according to The Cancer Genome Atlas Pan-cancer data (Zack et al., 2013).

In this review, I have tried to adapt the concept of age-related cancer for ordinary Type II endometrial cancer based on the findings from recent studies on the association between age and cancer.

Materials and methods

The contents of three previous reports, including 82 postmenopausal endometria (Koi et al., 2015), 133 endometrial polyps (EMPs) (Sho et al., 2016), and 225 hysterectomy specimens of endometrial cancer (Nguyen et al., 2015), were reviewed. The postmenopausal women with a prolapsed uterus received a transdermal treatment consisting of 17β-estradiol (E2) 0.72 mg (3 cases) and E2 0.62 mg plus norethindrone acetate (P) 2.70 mg (12 cases) as a matrix patch for the treatment of severe vaginitis before surgery for 1 to 2 months. These 15 hysterectomy specimens have also been included in this review. The details regarding the methods were described in the previous studies (Koi et al., 2015; Nguyen et al., 2015; Sho et al., 2016). For the immunohistochemical analyses, 4-μm-thick sections were cut from formalin-fixed paraffin-embedded tissue blocks, deparaffinized in xylene, and rehydrated through sequential washes of alcohol and distilled water. Ki-67 and p53 were detected using the ready-to-use monoclonal antibodies against Ki-67 and p53 (clones MIB-1 and DO-7, respectively; DAKO, Kyoto, Japan). Estrogen receptor (ER)-alpha and progesterone receptor (PR) A were detected using monoclonal antibodies (clone 6F11, diluted 1:50, and clone 16, diluted 1:50, respectively; Novocastra, Fukuoka, Japan). The slides were heated in an autoclave at 120°C for 5 min in 0.01 M citrate buffer (pH=6.0) before immunostaining. The slides were incubated with these antibodies for 2 hours at room temperature. Antibody binding was visualized using the EnVision+ Dual link system and dianinobenzidine as a chromogen (Dako Cytomation, Kyoto, Japan). 3′-endlabeling of apoptotic cell DNA was performed using an Apop Tag in situ apoptosis detection kit (Millipore Corporation, Tokyo, Japan) according to the instructions of the manufacturer. The slides were counterstained with methyl green or hematoxylin and mounted. The interpretation of the immunohistochemical preparations and presence of apoptotic cells was assisted with the WinROOF image processing software program (Mitani Corp., Tokyo, Japan) (Koi et al., 2015). This review was approved by the Review Board of the University Hospital of Occupational and Environmental Health on Ethical Issues.

Results with comments

Proliferation and apoptosis in the endometrium

Endometrium and endometrial polyps during the menstrual cycle

The immunohistochemical data of the benign endometrium using antibodies of Ki-67 and p53 and the apoptotic kit are shown in Table 1. In our previous study of endometrial glandular cells with regular cyclic menstruation, the labeling index (LI) for Ki-67 in the proliferative phase was significantly higher than that of Ki-67 in the secretory phase. The apoptotic activity in endometrial glandular cells increased during the late secretory phase with a low Ki-67 index (Udo et al., 2004). Among premenopausal EMPs, the LI for Ki-67 was more frequently detected during the proliferative phase than during the secretory phase and was not correlated with the expression of Ki-67 (Sho et al., 2016). Maia et al. (2004) said that it was impossible to discern whether the increased expression of p53 in endometrial polyps during the proliferative phase resulted from a direct effect of estrogen on gene transcription or as the consequence of the accumulation

Table 1. Immunohistochemical data of the benign endometrium.

<table>
<thead>
<tr>
<th></th>
<th>Number of cases</th>
<th>ki-67 median (range)</th>
<th>p53 median (range)</th>
<th>apoptosis median (range)</th>
<th>Number of p53S(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postmenopausal endometrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-user</td>
<td>72</td>
<td>6% (0-47)</td>
<td>2% (0-37)</td>
<td>1% (0-12)</td>
<td>8 (11.1)</td>
</tr>
<tr>
<td>E2 user</td>
<td>8</td>
<td>16% (5-49)</td>
<td>0% (0-8)</td>
<td>5% (0-9)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>E2+P user</td>
<td>17</td>
<td>3% (0-82)</td>
<td>0% (0-28)</td>
<td>0% (0-4)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Posmenopausal EMP</td>
<td>71</td>
<td>15% (0-76)</td>
<td>3% (0-38)</td>
<td>1% (1-8)</td>
<td>9 (12.6)</td>
</tr>
<tr>
<td>Premenopausal EMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferative phase</td>
<td>41</td>
<td>48% (14-81)</td>
<td>7% (0-32)</td>
<td>2% (0-8)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Secretory phase</td>
<td>21</td>
<td>0% (0-15)</td>
<td>0% (0-8)</td>
<td>2% (0-8)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

p53S: p53 signature, E2: 17β-estradiol, P: norethindrone acetate, EMP: endometrial polyp. aP<0.005 (E2 users versus non-users), bP=0.02 (E2 users versus non-users), cP<0.001 (postmenopausal EMP versus non-users).
of DNA errors caused by the high rates of cell division, which in turn indirectly increased the intracellular p53 in order to correct them or to induce apoptosis.

Endometrium and endometrial polyps in postmenopausal women

The LI for Ki-67 was significantly positively correlated with the apoptotic index in postmenopausal women not using hormones (Koi et al., 2015). Furthermore, the LI for p53 was significantly correlated with the LI for Ki-67 but not with the apoptotic index (Koi et al., 2015). Postmenopausal endometria of E2 users showed significantly higher LIs for Ki-67 and apoptotic index than those of non-users (P<0.005 and P=0.02, respectively, Fig. 2), while the expression of p53 did not differ significantly between postmenopausal endometria with and without E2 use (P=0.605). The postmenopausal endometria of E2 + P users showed no significant difference in the LIs for Ki-67 and p53 or in the apoptotic index from non-hormone users (P=0.431, 0.095 and 0.634, respectively). Among postmenopausal EMPs, a univariate analysis showed that the LI for Ki-67 was inversely correlated with p53 and significantly correlated with apoptosis (Sho et al., 2016). Furthermore, a multivariate analysis showed that the significance of the correlation between the LI of Ki-67 and apoptosis was preserved, whereas that of the inverse correlation between the LI for Ki-67 and p53 disappeared. Postmenopausal EMPs also showed a significantly higher LI for Ki-67 than did postmenopausal endometria (P<0.001). A positive correlation between the LIs of p53 and Ki-67 was found in postmenopausal endometria (Koi et al., 2015) but not in the postmenopausal EMPs (Sho et al., 2016).

p53 signature in endometrium

In a recently proposed model of the carcinogenesis of USC, the lesion was suggested to arise predominantly in the resting endometrium, manifesting first as p53 signature (p53S) and then subsequently evolving to endometrial glandular dysplasia (EmGD), followed by serous endometrial intraepithelial carcinoma (SEIC), and finally presenting as fully developed USC (Zheng et al., 2011). The p53S was found in the background endometrium of endometrial carcinomas in 20 (10.5%) postmenopausal patients (mean age 63.0 years) except for 2 premenopausal patients with Lynch syndrome in our previous study (Nguyen et al., 2015). The p53S was more frequently detected in non-endometrioid tumors than in endometrioid tumors. EIC was found to be strongly associated with non-endometrioid tumors with the p53S (Fig. 3). The mean age of women with p53S was 68.7 years under a benign condition of the endometrium. Among endometrial samples, including 97 postmenopausal endometria and 71 postmenopausal EMPs, p53S was associated with high proliferative

Fig. 1. The endometrial polyp in a 36-year-old woman during the proliferative phase (A). The endometrial glands showed a high proliferative activity for Ki-67 (B) and moderate expression of p53 (C). The endometrial polyp in a 32-year-old woman during the secretory phase (D). The endometrial glands showed no expression of Ki67 (E) or p53 (F). These samples were collected via the hysteroscopy.
Aging and uterine serous carcinoma

Fig. 2. The postmenopausal endometrium in a 79-year-old woman using estrogen (A). The endometrial glands showed high expression of Ki-67 (B) and several apoptotic bodies (arrow), (C) and weak positivity for p53 (D) and high positivity for estrogen receptor-alpha (E) and progesterone receptor (F).

Fig. 3. An endometrial polyp with uterine serous carcinoma (USC) in a 72-year-old woman (A). The arrow indicates B. The p53 signature (p53S) and serous endometrial intraepithelial carcinoma (SEIC) associated with USC (B). The p53S showed low expression of Ki-67 (C), and high expressions of p53 (D) and estrogen receptor-alpha (E). SEIC with high expressions of Ki-67 (C) and p53 (D), and a weak expression of estrogen receptor-alpha (E).
activity of the surrounding postmenopausal endometrial gland (P<0.001) but was not associated with the LI of p53 or the apoptotic index (Table 2). The p53S itself showed a relatively low LI for Ki-67 and a low apoptotic index compared with the surrounding endometrial glands (Fig. 4). The p53S is responsible for the imbalance between the proliferation and apoptosis of endometrial epithelial cells with advanced age.

**Tamoxifen and endometrium**

Tamoxifen (TAM) is a member of a class of agents known as selective estrogen receptor modulators (SERMs) and is now widely used for the treatment and prevention of breast cancer. However, TAM use has been associated with a variety of gynecologic problems (ACOG committee opinion, 2006). EMPs and diffuse cystic change are often found in TAM users. The endometrial carcinomas of TAM users are more often limited to a polyp than those of non-users (Hoogendoorn et al., 2008), suggesting that the polyp-carcinoma sequence plays an important role in the development of endometrial cancer among postmenopausal breast cancer patients treated with TAM. However, no marked difference was observed in the incidence of the p53S between postmenopausal EMPs of TAM users and non-users (Sho et al., 2016).

Gonadotropin-releasing hormone analogues (Gn-RHAs) act on the hypothalamic-pituitary axis and reduce estradiol to postmenopausal levels. Our previous study showed increased endometrial thickness (<0.5 cm or more using transvaginal sonography) in over half of TAM-treated postmenopausal breast cancer patients but rarely in premenopausal women with temporary amenorrhea induced by Gn-RHa plus TAM adjuvant therapy (Fig. 5) (Hachisuga et al., 2005). Gn-RHa plus

<table>
<thead>
<tr>
<th>Variable</th>
<th>p53 signature</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>presence</td>
<td>absence</td>
</tr>
<tr>
<td>Number of cases</td>
<td>20</td>
<td>148</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>60-69</td>
<td>9</td>
<td>72</td>
</tr>
<tr>
<td>70-79</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>80-89</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Ki-67 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>16 (4-62)</td>
<td>8 (0-72)</td>
</tr>
<tr>
<td>p53 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>3 (0-18)</td>
<td>1 (0-52)</td>
</tr>
<tr>
<td>Apoptosis (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>median (range)</td>
<td>1 (0-8)</td>
<td>1 (0-12)</td>
</tr>
</tbody>
</table>

*cases including 72 postmenopausal women, 25 postmenopausal women with hormone use and 71 postmenopausal women with endometrial polyp

Fig. 4. The p53S (arrow) in the endometrium of a 67-year-old woman (A). The p53S with low expression of Ki-67 (B) and no apoptotic body (C), and positivity for p53 (D), estrogen receptor-alpha (E) and progesterone receptor (F).
TAM adjuvant therapy induced the downregulation of the estradiol level, followed by a significant reduction in the endometrial thickness (Yang et al., 2013). Most studies have found that the relative risk of developing endometrial cancer for TAM users is two or three times higher than that of an age-matched population (ACOG committee opinion, 2006). This increased risk was limited in postmenopausal women taking TAM. The median age at the diagnosis of TAM-related endometrial cancer was 69 years in the pooled results from 3 countries (Jones et al., 2012). An older age at the endometrial cancer diagnosis was associated with a greater risk of dying from endometrial cancer. Recent large case studies have shown a high incidence of non-endometrioid histological subtypes with a poorer prognosis among long-term TAM users (Hoogendoorn et al., 2008; Jones et al., 2012). The effects of TAM on the endometrium are closely related to the age of patients.

Age and endometrial carcinomas

Activation of p53 can induce several responses in cells, including cell-cycle inhibition, apoptosis, genetic stability and inhibition of blood-vessel formation (Vogelstein et al., 2000). Several experimental studies using breast cancer cells have reported that estrogen receptor (ER)-alpha binds to p53 and represses its transcriptional function, resulting in the inhibition of p53-mediated cell cycle arrest and apoptosis (Shirley et al., 2009; Konduri et al., 2010). E2 enhanced ER-alpha binding to p53 and inhibited p21 transcription. ER-alpha-positive breast cancer patients with tumors expressing wild-type p53 respond better to TAM therapy (Konduri et al., 2010). The inhibition of p53-mediated transcription by ER-alpha binding to p53 may or may not explain the overexpression of p53 in endometrium during the proliferative phase.

Feng et al. (2007) reported that the efficiency of the p53 pathway declines with age as a function of the life span of the organism. The enhanced fixation of mutations in older individuals is suggested to be associated with the declining fidelity of p53-mediated apoptosis or senescence in response to stress. DeGregori (2013) said that the age-dependent accumulation of mutations plays a relatively minor role in the increased incidence of cancer with age. Instead, other aging-associated changes, such as alterations in tissues that influence selection for oncogenic events, largely underlie the aging-associated cancer. The author suggests that the p53S is responsible for the imbalance between the proliferation and apoptosis of endometrial epithelial cells with advanced age and the p53S is thought to be partly associated with precancerous lesions of USCs.

Therefore, the concept of age-related endometrial cancers is proposed instead of Type II (estrogen-independent) endometrial cancers, based on decline in the p53 functions with age associated with the subsequent increased number of SCNA (Fig. 6). One epidemiological study showed that Type I and II endometrial cancers share many common etiologic factors. The etiology of type II tumors is not completely estrogen-independent, as previously believed (Setiawan et al., 2013).

The endometrium undergoes cyclical changes in proliferation, differentiation and apoptosis in response to...
the rise and fall of ovarian estrogen and progesterone levels. The loss of oocytes, which occurs with age, is responsible for menopause (Pelosi et al., 2015). The lifetime risk of cancers of many different types is suggested to be strongly correlated with the total number of divisions of the normal self-renewing cells maintaining that tissue’s homeostasis (Tomasetti and Vogelstein, 2015). Most cancer risk is due to random mutations arising during DNA replication in normal, noncancerous stem cells (Martincorena and Cambell, 2015). The division activity of normal endometrial self-renewing cells is dramatically changed from active to stationary due to menopause, and the phenotypes of endometrial cancers reflect these age-dependent factors. POLE is a catalytic subunit of DNA polymerase epsilon involved in nuclear DNA replication and repair. Tumors with mutations in the exonuclease domain of POLE were classified into the ultramutated group of the endometrial carcinoma and showed an endometrioid histologic type with a favorable prognosis among endometrial cancer patients (Cancer Genome Atlas Research Network et al., 2013). The POLE subtype may or may not be associated with a high division activity of normal endometrial self-renewing cells in the menstrual cycle, in contrast to USC, which is associated with a declining p53 function during aging.

USC and high-grade ovarian serous carcinoma

High-grade serous ovarian carcinoma was characterized by TP53 mutations in almost all tumors (96%). The inactivation (germline or somatic mutation or promoter methylation) of BRCA1/2 was found in nearly half of high-grade serous ovarian carcinomas (Cancer Genome Atlas Research Network et al., 2013). Based on the evaluation of risk-reducing salpingooophorectomy specimens in women with BRCA mutations, the concept of a high-grade pelvic serous carcinogenic sequence was proposed, in which the spectrum of fallopian tube (FT) epithelial transformation ranges from normal epithelium through p53 S and serous tubal intraepithelial carcinoma (STIC), ultimately presenting as invasive carcinoma (Crum et al., 2012). STICs show high rates of cellular proliferation, p53 mutations, significant cytologic atypia, and a secretory phenotype and are commonly located in the tubal fimbria, frequently presenting in association with p53Ss (Lee et al., 2007). In nonprophylactic settings, STIC has been observed in the FT in up to 60% of women with high-grade serous carcinoma (Crum et al., 2012), but in only 0.8% of non-neoplastic cases in the FT (Seidman et al., 2016). These data suggest that the TP53 mutation closely associated with the BRCA1/2 mutation in high-grade pelvic serous carcinogenic sequences. The p53Ss of the FT also showed a high rate of p53 mutations (Lee et al., 2007). However, p53S was reported to be equally prevalent in the FTs of BRCA mutation carriers and controls, with no particular association with age (Mehra et al., 2011).

The most common somatic mutations in USC were TP53, PIK3CA, FBXW7 and PPP2R1A (Cancer Genome Atlas Research Network et al., 2013). Although the association between germline BRCA1/2 and the development of USC is still controversial, genomic changes in BRCA1/2 were rare in USC (Cancer Genome Atlas Research Network et al., 2013). Two of four p53Ss
in the benign endometrial polyps showed TP53 mutations according to a DNA sequence analysis (Sho et al., 2016). Sixteen (42%) of the 38 samples of p53Ss showed at least 1 TP53 mutation from 8 uteri with either USC or SEIC (Zhang et al., 2009). The decline in the p53 functions with age, along with the accumulation of genomic alterations, including TP53 mutations, may be one reason why USCs occur in only a small portion of older individuals. Although the rate of point mutations in tumors is similar to that of normal cells, the rate of chromosomal changes in cancer is elevated. Cancer cells can survive such chromosome aberrations more easily than normal cells because they contain mutations that incapacitate genes like TP53 (Vogelstein et al., 2013). Systematic sequencing studies of normal tissues have also provided clues regarding the evolution from normal tissues to cancers. Half or more of the somatic mutations in certain tumors of self-renewing tissues are estimated to occur before the onset of neoplasia (Tomasetti et al., 2013). Martincorena and Campbell (2015) said that the study of established cancers has provided many clues about the temporal evolution of cancers, but many gaps in our understanding remain. The age-related decline in the functions for maintaining homeostasis of human cells may be a clue to resolving the gap between genomic features of established cancers and normal tissues.

Conclusions

We herein propose an age-related type of endometrial cancer, instead of Type II estrogen-independent endometrial cancer, based on the current state of knowledge regarding the association between age and cancer. High-throughput DNA sequencing has enabled the systematic sequencing of more than 10,000 cancer exomes and 2,500 whole cancer genomes (Martincorena and Campbell, 2015), however, we should focus more on the age-related decline in the functions for maintaining homeostasis in normal human cells in order to understand the process by which normal cells transform to invasive carcinomas.

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