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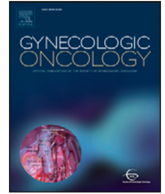

Immunohistochemical and genetic profiles of endometrioid endometrial carcinoma arising from atrophic endometrium

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HIGHLIGHTS

- Endometrium next to endometrioid endometrial carcinomas are mostly hyperplastic, but sometimes atrophic, which predicts a worse prognosis.
- The immunohistochemical and genetic profiles of endometrioid carcinomas next to hyperplastic and atrophic endometrium was assessed and compared.
- Carcinomas next to atrophic endometrium were associated with fewer *KRAS* mutations and loss of E-cadherin.

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ABSTRACT

Objective. Endometrial carcinomas are divided into type I endometrioid endometrial carcinomas (EECs), thought to arise from hyperplastic endometrium, and type II nonendometrioid endometrial carcinomas, thought to arise from atrophic endometrium. However, a minority (20%) of EECs have atrophic background endometrium, which was shown to be a marker of a worse prognosis. This study compares the immunohistochemical and genetic profiles of this possible third type to that of the known two types.

Methods. 43 patients with grade 1 EEC and hyperplastic background endometrium (type I), 43 patients with grade 1 EEC and atrophic background endometrium (type III) and 21 patients with serous carcinoma (type II) were included (n = 107). Tissue microarrays of tumor samples were immunohistochemically stained for PTEN, L1CAM, ER, PR, p53, MLH1, PMS2, β-catenin, E-cadherin and MIB1. The *BRAF*, *KRAS*, and *PIK3CA* genes were analyzed for mutations.

Results. A significantly higher expression of ER and PR, and a lower expression of L1CAM, p53 and MLH1 were found in type I and III compared to type II carcinomas. Expression of E-cadherin was significantly reduced in type III compared to type I carcinomas. Mutation analysis showed significantly less mutations of *KRAS* in type III compared to type I and II carcinomas ($p < 0.01$).

Conclusion. There appear to be slight immunohistochemical and genetic differences between EECs with hyperplastic and atrophic background endometrium. Carcinogenesis of EEC in atrophic endometrium seems to be characterized by loss of E-cadherin and a lack of *KRAS* mutations. As expected, endometrioid and serous carcinomas were immunohistochemically different.

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Introduction

Cancer of the uterine corpus is the most common gynecologic malignancy among women in the developed world. In 2012, it affected 47,130 women and caused the death of 8010 women in the US [1].

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It is generally accepted that endometrial carcinomas (EC) can be divided into two subtypes [2]. Type I endometrial carcinoma is the most common subtype. It affects women at a median age of 60 years and has a good prognosis. These tumors are usually related to unopposed estrogen stimulation and show endometrioid histology, arising from hyperplastic endometrium. In contrast, the less common type II carcinomas affect older women and have a poor prognosis. These tumors are not related to unopposed estrogen stimulation and are characterized by clear cell or serous histology, arising from atrophic endometrium [3–5]. Distinct carcinogenic pathways have been described in each subtype. Type I carcinomas are characterized by microsatellite instability and alterations of the *PTEN*, *KRAS*, *PIK3CA* and *CTNNB1* genes, whereas type II carcinomas are often aneuploid and show over expression of p53 and Her2/neu [6–9].

However, some tumors do not fit within this dualistic model. In a recent study we reviewed slides from 527 patients with grade 1 endometrioid endometrial carcinomas and found that 17% of these carcinomas had atrophic background endometrium [10]. Furthermore, the presence of atrophic background endometrium adjacent to EEC was associated with several predictors of poor survival, and an independent predictor of reduced progression free survival in endometrioid endometrial carcinomas. Moreover, recent studies looking at the molecular basis of endometrial carcinomas also show that this dualistic model is too simplistic and propose to categorize them based on their molecular profile [11–13]. These studies show that within the two groups as defined by the dualistic model, it is able to distinguish certain groups solely based on their molecular pattern. It might be possible that endometrioid endometrial carcinomas with atrophic background endometrium have a different molecular and therefore immunohistochemical pattern when compared to endometrioid endometrial carcinomas with hyperplastic background endometrium.

The aim of the present study was to analyze the hypothesis that endometrioid endometrial carcinomas with a background of atrophic endometrium arise through different carcinogenic pathways than type I and II endometrial carcinomas. Therefore, the expression of several immunohistochemical markers and the presence of distinct genetic mutations in endometrioid endometrial carcinoma with a background of atrophic endometrium was compared to those of type I and II carcinomas.

Materials and methods

Patients

For this study, patients with endometrial carcinoma from two cohorts, who were at least treated with a hysterectomy and bilateral salpingo-oophorectomy and who did not have a personal history of malignancy, were evaluated for inclusion. The first cohort is comprised of patients treated for grade 1 endometrioid endometrial carcinoma at the Radboud University Medical Center (Radboudumc) and the Canisius-Wilhelmina Hospital in Nijmegen, The Netherlands, between January 1999 and December 2009, and at the Mayo Clinic in Rochester, Minnesota, USA, between January 2002 and December 2008 [10]. The second cohort is comprised of patients with uterine serous carcinoma treated at the Radboud University Medical Center and the Canisius-Wilhelmina Hospital, Nijmegen between January 1999 and December 2009 [14,15].

Slides of the primary carcinoma and background endometrium from the cohort of patients with grade 1 endometrioid endometrial carcinoma were reviewed with special attention to the nature of the background endometrium by experienced pathologists (JB, SB, DV) who were unaware of the original pathology results and clinical outcome. In the case of doubt or discrepancy with the original pathology report a second review was performed by another pathologist and consensus was reached. Background endometrium was categorized as simple hyperplasia, simple atypical hyperplasia, complex hyperplasia, complex atypical hyperplasia, disordered proliferative, atrophic, and normal proliferative as previously described [5,10,16]. Some cases had to be excluded because the tumor covered the entire cavity of the uterus and there was no background endometrium to be evaluated.

All patients with grade I endometrioid endometrial carcinoma and a background of pure (100%) atrophic endometrium (abbreviated to type III) as well as a similar amount of patients with grade I endometrioid endometrial carcinoma and a background of hyperplastic endometrium (type I) were included. Subsequently, all patients from the uterine serous carcinoma (type II) cohort of whom uterine tissue could be retrieved from the archive were included. This cohort consisted of carcinomas with both pure and mixed serous histology. It has been previously described that only about half of the serous carcinomas have pure serous histology [17].

Tissue microarray and immunohistochemistry

Tissue microarrays were created from the primary carcinoma [18]. Two representative areas of the carcinoma were selected on hematoxylin and eosin-stained slides. For the type II cases, areas with pure serous histology were selected. Two cylinders with a diameter of 2 mm were punched out of every donor block from the selected areas, and mounted into a recipient paraffin block using the Tissue-Tek Quick-Ray (Sakura Finetek, Torrance, CA, U.S.) manual tissue microarrayer.

The tissue microarrays were cut in 4 μm slides and immunohistochemically stained. Several markers were selected to be stained, based on the difference in their expression in type I and type II endometrial carcinoma [6–9,19,20]. An overview of the antibodies and dilutions used as well as the area of the cell which was evaluated when scoring is shown in Table 1.

Immunohistochemical analysis of PTEN, L1CAM, ER, PR, p53, MLH1, PMS2, β-catenin, E-cadherin and MIB1 expression was performed according to the local protocols. These markers were chosen because previous literature has shown that their expression is different in type I and II EC [8,9,20]. In short, formalin fixed paraffin sections were stained with the primary antibody following EDTA antigen retrieval, blocking of endogenous background with Peroxidase Blocking Reagent and protein blocking using horse serum. Subsequently, a secondary antibody was added and visualization was performed with Vectastain and 3,3′-Diaminobenzidine (Zymed Lab. California, USA) as a substrate. Staining was enhanced in $CuSO_4$ and slides were counterstained with Mayer's hematoxylin. Finally, slides were dehydrated and mounted.

Tumor samples were given a score ranging from 0–9 by two independent evaluators (YG, AT) which was the product of the percentage of cells stained (0% = 0; 1–10% = 1; 11–50% = 2; 51–100% = 3) and intensity of staining (none = 0; weak = 1; moderate = 2; strong = 3) [21]. The evaluators were unaware whether the tissue cylinders were from type I, type II or type III carcinomas. Samples with too little tissue to assess or samples not containing any malignant tissue were not included in the calculations. In the case of a large discrepancy between the scores of the two evaluators (i.e., a difference in percentage or intensity score >2 or disagreement on the presence of malignant tissue) a third independent reviewer (JB), who was unaware of the score given by the first evaluators, scored the sample as well.

The final score per case (range 0–9) was calculated by adding all scores given to the two tissue samples and dividing them by the number of scores in the sum (which varied depending on the presence of tumor tissue in the sample and the need for a third review). The final semi-quantitative score was used for analysis of PTEN, ER and PR according to the literature as well as for β-catenin and E-cadherin, because there is no consensus as to which scoring system should be used [22,23]. L1CAM was considered positive when there was staining seen in at least 10% of the malignant cells [24]. Cases with a final score of four and up concerning p53 were considered to be positive while those with a score below four were negative [25]. MLH1 and PMS2 were considered lost when there was no scoring at all, according to the

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