ORIGINAL ARTICLE

Endometrial cancer – prospective potential to make diagnostic process more specific

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Abstract

OBJECTIVE: The incidence of carcinoma of endometrium in younger patients has increased tendency. Experimental data support that mutation of tumor suppressor gene TP53 plays an important role in endometrial carcinogenesis
MATERIAL AND METHODS: Exons 2–11 of the gene *TP53* into tissues of endometrial carcinoma and precancerous lesions were analyzed by DNA sequencing and restriction analysis.
RESULTS: A polymorphism CCC/CGC at codon 72 was identified in exon 4 of *TP53* gene of all precancerous lesions and carcinomas of endometrium.
CONCLUSION: Our results also suggest presence of endometrial glandular dysplasia or serous histological type of endometrial carcinoma into examinated samples. Given the course and prognosis of serous endometrial carcinoma, it is necessary and useful to identify this type of cancer in the mixed types of endometrial carcinomas. DNA analysis has potential to make diagnostic process more specific and affect to adjuvant therapy and survival of patients.

INTRODUCTION

The incidence of carcinoma of endometrium (CaE) in younger patients has increased tendency (Nakagawa-Okamura *et al.* 2002). A widely accepted dualistic model, which has been established on a morphological basis into two broad categories: Type I and Type II. The type I arise in pre- and post-menopausal women and has a strong etiological association with either endogenous or exogenous, unopposed oestrogen exposure. The type II arise in relatively older women and rather from resting or atrophic endometrium or often preceded by endometrial glandular dysplasia (EmGD). Generaly type II has worse prognosis (Doll *et al.* 2008).

MATERIAL AND METHODS

Endometrium tissues of women were analyzed using DNA sequencing and restriction analysis. Samples of CaE have had various histological diagnoses (Table 1).

DNA was isolated from 25 mg of endometrial tissue gathered in sterile tubes using isolation kits (*Qiagen, QIAamp DNA Mini Kit*). Examined segments of gene *TP53* were amplified by polymerase chain reaction. Amplification was performed in the thermal cycler (*Eppendorf Mastercycler*) with repetition of 30 cycles (Wasko *et al.* 2005). The conditions of PCR amplification were denaturation 96ºC, 2 min., denaturation 94 °C, 15 sec., annealing 20 sec., gradient 50–65 °C, extension 72 °C, 30 sec. and final extension 72 °C, 10 min. Samples were sequenced in the genetic analyzer with capillary electrophoresis (*ABI PRISM™ 310 Genetic Analyzer*). Exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 of the *TP53* gene were directly sequenced.

Restriction analysis of the tested samples was done with restriction endonuclease *BtgI* (*Biotech*). Reaction was conducted for 16 hours at 37 °C and inactivation of the enzyme ran for 20 min. at 80 °C.

RESULTS

A polymorphism (homozygous form in 14 cases, heterozygous in 10 cases) CCC/CGC at codon 72 was identified in exon 4 of gene *TP53* of all precancerous lesions and CaE. Any other genetic changes was not identified into gene *TP53* (exons 2–11). We validated the same representation of forms of polymorphism CCC/CGC by both methods.

Detected CCC/CGC polymorphism causes a substitution of amino acids in polypeptide chain of p53 protein; neutral Proline is replaced by basic Arginine. This might have possible affect to structure and function of the protein p53. We have started analysis how this frequent polymorphism correlates with clinical features and how DNA analysis would be useful in this way.

DISCUSSION

Based on several studies, a preoperative diagnosis of complex atypical hyperplasia is followed frequently by carcinoma after hysterectomy. The concomitant rate of endometrial carcinoma in patients with atypical endometrial hyperplasia varies from 15% to 52% (Trimble *et al.* 2006). Our samples of CaE can contain component of EmGD or serous CaE (ESC). Genetic changes of *TP53* are more frequent in these lesions and generally CaE has tendency to be mixed by histological types.

TP53 gene mutations occur frequently in EmGD, which provides a solid genetic evidence that EmGD is the precancer of ESC. Mutation of the *TP53* gene is probably one of the most important factors in initiating an endometrial serous carcinogenesis (Jia *et al.* 2008).

Endometrial glandular dysplasia, possibly included in our samples, is a strong candidate for a precursor lesion that is not malignant but harbors the potential to progress to serous intraepithelial carcinoma of endometrium (Fadare & Zheng 2008). This finding shows importance to identify this structure into mixed tumors. More specific diagnosis should leads to more specific therapy. Comparison of adjuvant therapy was made in Japan. Authors showed that chemotherapy group had a significantly higher progression free survival and overall survival rate than the pelvic radiotherapy group among patients in the high to intermediate risk group (Susumu *et al.* 2008).

Association between polymorphisms and colorectal cancer were already observed and these polymorphisms may be used as markers for diagnosis of this cancer (Wachsmannova-Matelova *et al.* 2009). Some other cancer tissues have well-recognized genetic markers and due to increasing incidence of CaE, there is possible expectation that genetic markers will be discover soon.

Tab. 1. Data of patients (EECa-Endometrioid carcinoma of endometrium, GlCH-Glandular cystic hyperplasia of endometrium, CoH-Complex hyperplasia of endometrium, SiH-Simple hyperplasia of endometrium).

No.	Age	Type	Invasion into myometrium
1	44	EECa	none
2	47	EECa	none
3	50	EECa	none
4	55	GlCH without atypia	none
5	57	EECa	minimum
6	58	EECa	minimum
7	59	EECa	minimum
8	60	EECa	minimum
9	60	SiH	none
10	63	CoH with atypia	none
11	64	EECa	minimum
12	65	EECa	minimum
13	66	EECa	minimum
14	67	EECa	minimum
15	70	EECa	high
16	70	EECa	minimum
17	74	EECa	minimum
18	75	EECa	minimum
19	77	EECa	minimum
20	77	EECa	none
21	77	EECa	minimum
22	82	EECa	minimum
23	83	EECa	minimum
24	88	EECa	minimum

Our DNA analysis would help to reach this goal. Resting endometrium (RE) compared with EmGD, serous EIC and ESC; RE had a significantly lower *TP53* mutation frequency. The concordance rate in neoplastic uteri between immunohistochemical and sequence-proven analysis was 85.2% which was significantly correlated (Jia *et al.* 2008).

CONCLUSION

Our results also suggest presence of EmGD or serous histological type of CaE into examinated samples. Given the course and prognosis of serous CaE, it is necessary and useful to identify this type of cancer in the mixed types of endometrial carcinomas. DNA analysis has potential to make diagnostic process more specific and affect to adjuvant therapy and survival of patients. Results of our started prospective database should support the potential use of DNA analysis in the differential diagnosis of carcinoma of endometrium.

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