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Wilms' tumor gene 1 (WT1) in endometrial carcinoma

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Abstract

Objective. Wilms' tumor gene (WT1), located on chromosome 11, encodes a transcription factor that contributes to the carcinogenesis of uterine sarcomas. To expand the knowledge on the biological role of WT1 in other uterine cancers, we focused on its detection in endometrial carcinoma.

Methods. In total, 36 paraffin-embedded tumors were available for WT1 immunohistochemical (IHC) analysis including endometrial endometrioid carcinoma ($n=24$), serous carcinoma ($n=9$) and clear cell carcinoma ($n=3$). Three slides from different sites of the tumor were analysed. Of these tumors, 32 snap frozen tissue samples were available for RT-PCR (endometrioid carcinoma (23), serous carcinoma (7) and clear cell carcinoma (2)). To compare, WT1 expression was also evaluated by IHC in benign endometrium (12) and benign endometrial polyps (5).

Results. WT1 positivity was noticed in tumor cells and endothelial cells, lining the intratumoral blood vessels. Overall, 72% (26/36) of tumors stained positive for WT1. RT-PCR results showed WT1 positivity in 75% (24/32) of samples. Comparing the staining patterns of the 3 different bioptic sites, tumor heterogeneity was demonstrated in the majority (72%) of samples.

Conclusion. Although WT1 is expressed in a majority of endometrial carcinomas, a heterogeneous staining pattern is observed. This information is important for WT1-directed immunotherapy.

Keywords: Wilms' tumor gene 1 (WT1); Endometrial cancer; Uterus; Tumor

Introduction

Wilms' tumor gene 1 (WT1) is located on chromosome 11p13. It has many molecular functions [1], which are partially explained by different splicing of WT1 RNA, resulting in 36 protein isoforms. WT1 is thought to have a role in the regulation of transcription, RNA metabolism (possibly splicing) and in translation. It has a central role in embryonic development [2–5] while its overexpression in several malignancies suggests a role in tumorigenesis [6].

Uterine cancers encompass epithelial (carcinomas) and mesenchymal (sarcomas) entities. The former is the most common pelvic tumor in women. Although the overall prognosis is fair, systemic relapse is invariably fatal and better treatment modalities are needed [7]. Active immunotherapy can induce a tumor-specific immune response against one or several tumor associated antigens (TAA). Theoretically, this allows a systemic immune surveillance against tumor recurrence and even spreading tumor cells. One of the relevant TAA that might be considered for uterine cancer immunotherapy is WT1. Preliminary data from pilot clinical studies for other types of malignancy, expressing WT1, showed encouraging results by immunotherapeutic targeting of WT1 [8–13].

Our group previously investigated the biological role of WT1 in uterine sarcomas [14]. In a large sample study, we were able to demonstrate that WT1 was overexpressed in all

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Table 1
Literature review on WT1 expression in endometrial carcinoma

	Rackley et al.[a] [27]	Goldstein and Uzieblo [20]	Zhang et al. [24]	Hashi et al. [21]	Shevchuk et al. [23]	Kiyokawa et al. [22]	Dupont et al. [17]	Acs et al. [15]	Egan et al. [18]	Al Hussaini et al. [16]	Euscher et al. [19]	Nakatsuka et al. [6]	Total
Serous EC	–	0/18 with M+	9/13	0/5	2/6	2/12	3/9	10/16	2/31	5/25	1/9	2/3	36/147 (24%)
Endometrioid EC	–	–	0/10	–	0/32	–	20/99	0/35	0/39	0/7	–	13/16	33/238 (14%)
Mixed EC	–	–	–	–	0/6	–	1/5	–	–	–	–	–	1/11 (9%)
Clear cell EC	–	–	–	–	0/2	–	2/4	0/18	–	–	–	–	2/24 (8%)
Mucinous EC	–	–	–	–	0/3	–	–	–	–	–	–	–	0/3 (0%)
EC, not otherwise specified	8/14	–	–	–	–	–	–	–	–	–	–	–	–
Total	8/14 (57%)	0/18 (0%)	9/23 (39%)	0/5 (0%)	2/49 (4%)	2/12 (17%)	26/117 (22%)	10/69 (14%)	2/70 (3%)	5/32 (16%)	1/9 (11%)	15/19 (79%)	–

EC: endometrial carcinoma.
References are given in brackets.
M+ = metastasis.
[a] Only paper based on PCR data.

subtypes of uterine sarcomas. With immunohistochemistry (IHC), 76% of all leiomyosarcomata were positive (44% of carcinosarcomas, 47% of endometrial stromal sarcomas and 57% of undifferentiated sarcomas). In contrast, with RT-PCR all-but-one tested samples were WT1 positive and with Western blotting all samples showed positivity.

In endometrial carcinoma, WT1 staining using IHC ranges from 0% up to 79% (Table 1) [15–24]. According to a meta-analysis in 2005 by Heatley [25], including 7 of the 10 immunohistochemical studies, the detection rate of WT1 in endometrial carcinoma was 29.1% (20.5–39.4%). In 2004, Goldstein [26] concluded that the differences in methodology prohibited a good comparison between the studies. So far, only 1 study was conducted using PCR to determine WT1 expression at RNA level [27]. In total, 6/14 samples (subtype not specified) were WT1 positive.

The objective of the current retrospective study was to clarify the expression of WT1 in uterine carcinomas. The analysis was based on parallel protein (IHC) and RNA (RT-PCR) detection. Different sites from the tumor were used to adequately assess the protein.

Materials and methods

Patient samples

After approval of the local ethical committee, 36 patients with endometrial carcinoma were identified. Of all, a snap frozen biopsy from a hysterectomy specimen was collected from the central tissue bank. From the laboratory of pathology 3 additional, different biopsies from the resection specimen were collected for immunohistochemical analysis. Due to technical problems, 4 snap frozen samples had to be omitted.

Expression in malignant endometrial carcinoma was compared to WT1 expression in benign endometrium (12 samples) and benign endometrial polyps (5 samples). Of those, only 1 slide could be evaluated by IHC, since no more material of

Table 2
Most relevant patient characteristics

		Endometrioid EC	Serous EC	Clear cell EC
Number of patients	36	24	9	3
Mean age (years)±SEM		65±11.6	65±9.4	65±5.5
Surgical stage	I	14	5	1
	II	4	–	–
	III	4	2	2
	IV	2	3	–
Type [7]	I	21	0	0
	II	3	9	3

EC: endometrial carcinoma.

Table 3
Benign endometrial samples

Subtype		Number
Atrophic endometrium without post-menopausal bleeding		7
Hormonally influenced endometrium	Menopause with HRT	2
	Proliferative endometrium	11
	Secretory endometrium	1
Benign tumoral endometrium	Simple hyperplasia	1
	Non atypic polyp	5
Total		27

HRT: hormonal replacement therapy.