WT1 in endometrial carcinoma

An Coosemans[1,2,3], Philippe Moerman[4], Godelieve Verbist[1], Wim Maes[2], Patrick Neven[1,3], Ignace Vergote[1,3], Stefaan W Van Gool[2,3], Frédéric Amant[1,3]

[1] Laboratory of Experimental Gynaecology, 6th floor (O&N 1), KatholiekeUniversiteit Leuven, Herestraat 49, 3000 Leuven, Belgium
[2] Laboratory of Experimental Immunology, 8th floor (O&N 1), KatholiekeUniversiteit Leuven, Herestraat 49, 3000 Leuven, Belgium
[3] Department of Child and Women, UZ Gasthuisberg, KatholiekeUniversiteit Leuven, Herestraat 49, 3000 Leuven, Belgium
[4] Department of Pathology, UZ Gasthuisberg, Katholieke Universiteit Leuven, Minderbroederstraat 12, 3000 Leuven, Belgium

Abstract

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

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 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 4.1**) (15–24). According to a metaanalysis 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 determineWT1 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.

Table 4.1. Literature review on WT1 expression in endometrial carcinoma

	Rackley 1995* (27)	**Goldstein 2002** (20)	**Zhang 2003** (24)	**Hashi 2003** (21)	**Shevchuk 2003** (23)	**Kiyokawa 2004** (22)	**Dupont 2004** (17)	**Acs 2004** (15)	**Egan 2004** (18)	**Al-Hussaini 2004** (16)	**Euscher 2005** (19)	**Nakatsuka 2006** (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
* only paper based on PCR data

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 benign tissue was available. There was also no material available for RT-PCR analysis. The characteristics of the samples are presented in **Tables 4.2** and **4.3**.

Immunohistochemistry (WT1)

WT1 staining was performed and interpreted semiquantitatively as described earlier (14). A scoring system, as published before [14], was based on the multiplication of percentage and intensity of positive cells, being negative (0–20), weak (21–80), moderately (81– 180) and strong (181–300). After examining each slide individually, the mean was calculated by adding the individual results of the 3 biopsies, proportional to their size.

RNA reverse transcriptase

RNA was isolated from snap frozen tissue using the RNeasy Mini Kit (Qiagen, Maryland, USA), according to the company's guidelines. The RNA was dissolved in RNase free water. Spectrophotometry (260 nm/280 nm) was used to determine the concentration. RNA was converted into cDNA as described earlier (14).

Real time polymerase chain reaction (RT-PCR)

RT-PCR was performed as described earlier (14). Primers and probe used were manufactured by Eurogentec (Seraing, Belgium): WT1-FW (CCA CAC AAC GCC CAT CCT) (FAM-dye), WT1-RV (TGA ATG CCT CTG AAG ACA CCG) (FAM-dye), WT1-TP (TGC GGA GCC CAA TAC AGA ATA CAC ACG). The cDNA plasmid standard, consisting of purified WT1 plasmid DNA, was used to quantify the target gene in the unknown samples. To analyse, WT1 value was divided by 18S
value. This was used to compare the different samples. To distinguish positive from negative samples the CT value of cultured human fibroblasts (purified from a nasal concha) was defined as negative, meaning the cutoff point in this study was considered as more than WT1/18S=1E-06.

Table 4.2.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	I	21	0	0
	II	3	9	3

EC : endometrial carcinoma; SEM: standard error of the mean

Table 4.3. Benign endometrial samples

Subtype		**Number**
Atrophic menopauzal endometrium without post-menopauzal bleedings		**7**
Atrophic menopauzal endometrium with post-menopauzal bleeding		**3**
Hormonally influenced endometrium		**13**
	Menopause with HRT	2
	Proliferative endometrium	10
	Secretory endometrium	1
Benign tumoral endometrium		**6**
	Simple hyperplasia	1
	Non atypic polyp	5
TOTAL		**29**

HRT : hormonal replacement therapy

Results

Malignant tissue samples

Results are listed in **Table 4.4**. WT1 positivity was observed in the cytoplasm of endothelial cells lining intratumoral blood vessels and in the cytoplasm of endometrial tumor cells (**Figure. 4.1 A and B**). Comparing the three bioptic sites immunohistochemically, heterogeneity in WT1 positivity could be observed either in the tumors cells and/or the endothelial cells in 26 of the 36 tumor samples (72%). These samples clearly demonstrated that within one tumor WT1 positivity of endothelial cells and/ or tumor cells can range from negative to strongly positive. However, considering only tumor cells, only 25% (9/36) of samples showed this heterogenic pattern (sample 9, 13, 15, 24, 28, 30, 32, 33, 34).

Pooled data (mean results) revealed WT1 positivity in 7/36 samples (19%) in the tumor cells, while 22/35 samples (63%) were positive if only WT1 positivity in the blood vessels was taken into account. Finally, 24/32 samples (75%) showed WT1 positivity with RT-PCR. Comparing IHC with RT-PCR results only 44% (14/32) of samples were concordant for both results.

Adding together the mean of immunohistochemical results of tumor and endothelial cell positivity (of 3 bioptic sites), 72% of all tumors were WT1 positive (26/36). Of these, 39% were weakly positive and 33% were moderately positive.
Histological subtype analysis showed WT1 positivity in 78% and 71% of serous carcinoma and in 71% and 61% of endometrioid carcinoma for IHC and RT-PCR, respectively.

Benign tissue samples

The results are presented in **Table 4.5**. When normal endometrium was examined, again, the tissue cells were negative for WT1. However, when blood vessel positivity was analysed, a difference could be noticed. The blood vessels were 100% positive, if the endometrium was triggered (by hormones (spontaneous reproductive cycle or hormonal replacement therapy (HRT) during menopause) or by benign tumoral growth (atypic hyperplasia or benign, atypic polyps). If, on the other hand, the endometrium was atrophic blood vessel positivity remarkably reduced (**Figure 4.2 A** and **B**).
Since both in malignant and benign endometrial tissue samples the intensity of WT1 in the majority of endothelial cells was 2 (moderate) [normal endometrium 78% of all samples, endometrial carcinoma 81% of all samples], the only variable to compare benign and malignant is the percentage of positive blood vessels. These results are presented in **Table 4.6**. The percentage of positive cells is remarkably higher in triggered endometrium, benign tumoral endometrium and endometrial carcinoma type I.

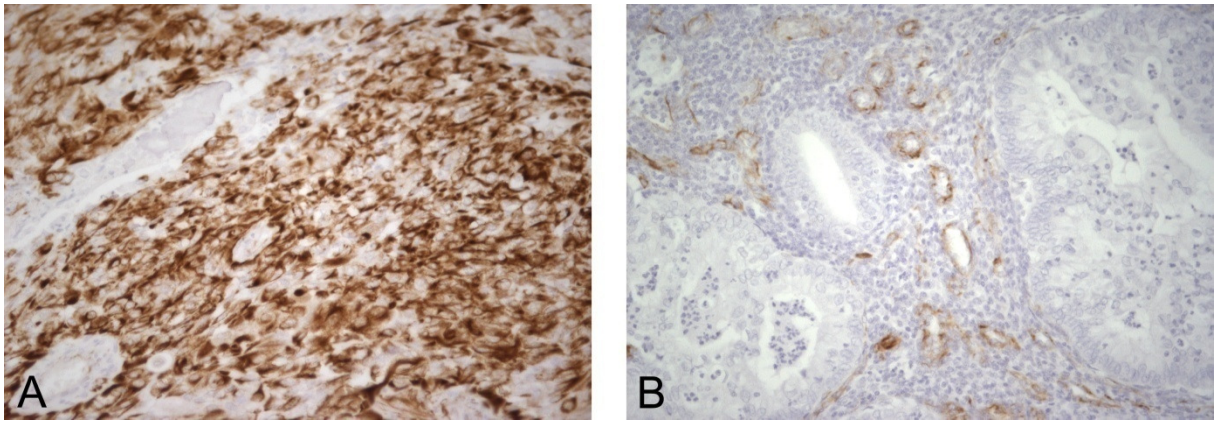

Figure4. 1. A. Cytoplasmic expression of WT1 in tumor cells of an endometrial carcinoma. B. Cytoplasmic expression of WT1 in endothelial cells of the same tumor.

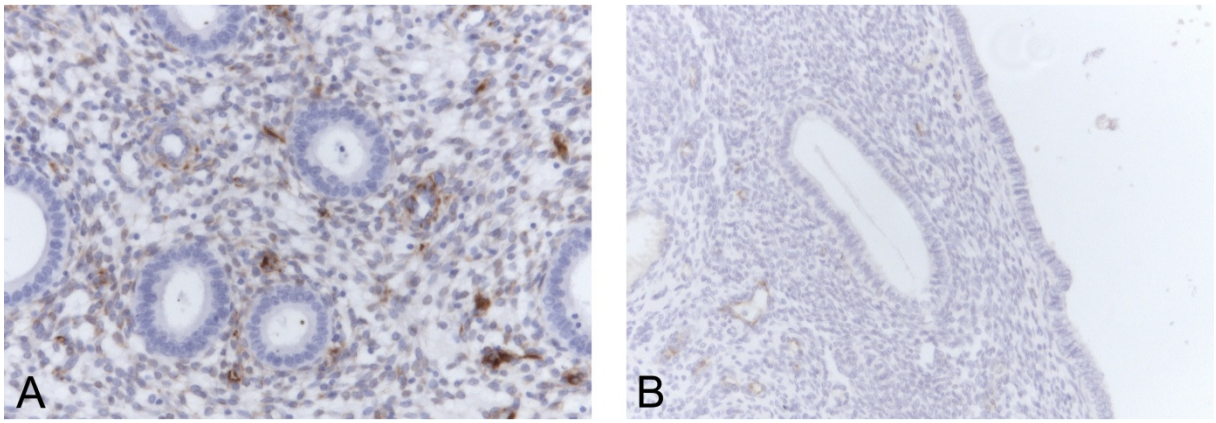

Figure 4.2. A. Presence of WT1 expression in endothelial cells of proliferative endometrium. B. Absence of WT1 expression in endothelial cells of atrophic endometrium.

Table 4.4.WT1 expression in endometrial carcinoma, determined by immunohistochemistry and real-time polymerase chaine reaction (RT-PCR).

Sub-type	Stage/grade	Sample	Immunohistochemistry									RT-PCR
			Tumor cells				Blood vessels				TOTAL	
			B1	B2	B3	mean	B1	B2	B3	mean	Positive samples	
ENDOMETRIOID	IB/M	1	-	-	-	-	++	++	+	++	Pos	0,00001
	IB/W	2	-	-	-	-	+	++	++	++	Pos	0,0001
	IA/W	3	-	-	-	-	+	+	+++	++	Pos	0,0002
	IB/W-M	4	-	-	-	-	-	-	-	-	Neg	0,00002
	IC/W	5	-	-	-	-	-	+	+++	++	Pos	0,000002
	IC/W	6	-	-	-	-	+	+	++	+	Pos	-
	IB/P	7	-	-	-	-	-	-	-	-	Neg	0,000002
	IB/P	8	-	-	-	-	-	++	-	-	Neg	-
	IB/M	9	+	-	++	+	-	+	-	-	Pos	0,000002
	IB/M	10	-	-	-	-	-	+	+	+	Pos	0,000003
	IB/P	11	-	-	-	-	+++	++	+	++	Pos	0,00001
	IC/W	12	-	-	-	-	+	-	+	+	Pos	-
	IB/M	13	+	+	-	-		+	-	-	Neg	0,000006
	IC/M	14	-	-	-	-	-	-	-	-	Neg	-
	IIB/W	15	+	+	-	+	++	+++	++	++	Pos	-
	IIB/W	16	-	-	-	-	-	+	-	-	Neg	0,000003
	II/W	17	-	-	-	-	+	+	+	+	Pos	0,000002
	IIA/M	18	++	++		++	+	-		-	Pos	-
	IIIA/P	19	-	-	-	-	-	-	-	-	Neg	0,000003
	IIIC/W	20	-	-	-	-	+	-	++	+	Pos	
	IIIB/P	21	-	-	-	-	+	+	++	+	Pos	-
	IIIB/W	22	-	-	-	-	++	+++	++	++	Pos	0,00001
	IVB/P	23	++	++	++	++					Pos	-
	IVB/P	24	++	-	++	++	-	+		-	Pos	-

	IB	25	-	-	-	-	++	++	+	++	Pos	0,000003
	IB	26	-	-	-	-	-	-	-	-	Neg	0,000002
S	IB	27	-	-	-	-	+	-	-	+	Pos	-
E	IC	28		-	+	+		+	++	++	Pos	0,000009
R	IIIC	29		-	-	-		-	-	-	Neg	0,000003
O	IIIC	30	-	+	-	-	-	+	-	+	Pos	0,000002
U	IIIC	31	-	-	-	-	+	+	-	+	Pos	
S	IVB	32	-	-	+	-	-	+	+	+	Pos	
	IV	33	-	-	+	-	+	++	+	+	Pos	-
C	IB	34	+	+	-	+	+	++	+	+	Pos	-
C	IIIA	35			-	-			-	-	Neg	
C	IIIC	36	-	-	-	-	+	+	+	+	Pos	0,000001
SUMMARY of POSITIVE RESULTS		**36**				**19%**				**63%**	**72%**	**75%**

CCC : clear cell carcinoma
B1-3 : biopsy 1-3
W/M/P : well/moderately/poor differentiated
- : negative; + : weak positivity; ++ : moderate positivity; +++ : strong positivity
Pos : positive sample; Neg = negative sample (taken together tumor cell and endothelial cell positivity)
Due to technical problems, results were not always available (open bars)

Table 4.5. WT1 expression in benign endometrial tissue, determined by immunohistochemistry

	Subtype	Sample	Immunohistochemistry		
			Endometrial cells*	Blood vessels*	TOTAL
UNTRIGGERED	**ATROPHIC WITHOUT BLEEDING**	1	-	+	Pos
		2	-	+	Pos
		3	-	+	Pos
		4	-	-	Neg
		5	-	-	Neg
		6	-	-	Neg
		7	-	++	Pos
	SUMMARY of POSITIVE SAMPLES	**7**	**0%**	**57%**	**57%**
TRIGGERED	**HORMONALLY INFLUENCED ENDOMETRIUM**	8	-	++	Pos
		9	-	+	Pos
		10	-	++	Pos
		11	-	++	Pos
		12	-	++	Pos
		13	-	+++	Pos
		14	-	+	Pos
		15	-	++	Pos
		16	-	++	Pos
		17	-	+	Pos
		18	-	+	Pos
		19	-	++	Pos
		20	-	++	Pos
		21	-	+	Pos
	BENIGN TUMORAL ENDOMETRIUM	22	-	++	Pos
		23	-	+	Pos
		24	-	+	Pos
		25	-	++	Pos
		26	-	++	Pos
		27	-	+++	Pos
	SUMMARY of POSITIVE SAMPLES	**20**	**0%**	**100%**	**100%**

* one biopsy only

- : negative; + : weak positivity; ++ : moderate positivity; +++ : strong positivity

Pos : positive sample; Neg = negative sample (taken together tumor cell and endothelial cell positivity)

Discussion

In this study we are able to show that WT1 is expressed in endometrial carcinoma and that the WT1 expression pattern is heterogeneous. Therefore, determination of its expression based on IHC result of one sample can give an incorrect result (false positive or negative). In addition, our results clearly show that not only tumoral positivity, but also theWT1 positivity of blood vessels has to be taken into account when IHC is performed to determine WT1 expression. Finally, we confirm the results of Nakatsuka et al. (6) and also determined the subcellular localisation of WT1 in the cytoplasm of endometrial carcinoma cells.

Current literature on the role of WT1 in endometrial cancer biology is inconsistent and flawn by different methodologies. We investigated the presence of WT1 in four different sites from each tumor (3 times IHC and once RT-PCR). Based on 36 immunohistochemical samples we encountered WT1 positivity in 72% of endometrial carcinoma samples. As we know today, WT1, expressed in tumor cells, is an attractive target for immunotherapy (8–13). However, the same might be true for WT1 expressed in endothelial cells, making endometrial carcinoma a tumor that can be targeted with immunotherapy.

Our current data show a higher WT1 positivity when compared to previously published studies (15–24). Focusing on the methodology and staining patterns, we encountered three potential causes for this difference.

Firstly, our results are based on the interpretation of the immunohistochemical analysis of 3 different bioptic sites. Goldstein already shortly suggested the possible heterogeneity of the tumor (26), however, this was never clearly demonstrated in previous studies. In this study, we are able to prove that the tumor is heterogeneous for WT1 expression. The previous data on WT1 positivity were based on a single tumor site assessment and thus sensitive for variability. Consequently, we suggest to analyse at least 3 different tumor sites on WT1 positivity before a firm conclusion on WT1 presence can be drawn.

The second reason for the discrepancy between the results of previous studies and this study is the subcellular localisation of WT1. In this study, only cytoplasmic sublocalisation was noticed whereas in all previous immunohistochemical studies, except for the most recent study of Nakatsuka et al. in 2006 (6), only nuclear staining was considered as WT1 positivity. Goldstein and Ubliezo (20) published the first immunohistochemical study on uterine tumors and WT1. These authors also stated that WT1 nuclear, based on previously published immunohistochemical studies in lung, pleura, peritoneum and ovarian carcinoma and mesothelioma, in which all WT1 was localised in the nucleus. However, the study of Nakatsuka et al. clearly showed that WT1 is mainly localised in the cytoplasm. Our group was also able to demonstrate WT1 cytoplasmic localisation in uterine sarcoma (14). It is known that the function of WT1 is threefold (1) and that the protein shuttles between the nucleus and the cytoplasm (28). Since the protein can shuttle, one can find WT1 in the nucleus and in the cytoplasm. Hence in concordance with Nakatsuka et al., we also suggest to include cytoplasmic positivity into the global assessment of WT1 expression.

Thirdly, endothelial WT1 positivity was included. In the past, WT1 positivity in endothelial cells was considered to be a universal phenomenon and it was used as an internal positive

control during staining (20). However, we noticed (**Table 4.4**) that not all blood vessels were WT1 positive, proving that this is not universal. The biological role of WT1 positive endothelial cells remains enigmatic but the increased WT1 RNA expression suggests a biological role. Since blood supply is essential for tumor growth, WT1 blood vessel positivity is important when immunotherapy is aimed for. Our way of sampling the 4 different biopsies (see materials and method) can explain the finding why results of tumor IHC, blood vessels IHC and RT-PCR differ (19%, 63% and 75% respectively). RT-PCR is the result of one biopsy taken at random. By definition, this should reflect the mean result of 3 biopsies (IHC). No matter how heterogeneous the tumor is, if only tumor cell positivity is taken into account, the discrepancy is too large (19% vs 75%). This suggests that WT1 positivity of endothelial cells is a substantial, RNA-based positivity (63% vs 75%) and thus essential to take into account when WT1 positivity of an endometrial carcinoma is determined. Recent work of the group of Wagner (29) supports this. They demonstrated by immunohistochemistry that WT1 is expressed in intratumoral endothelial cells in different tumor types (their study did not include endometrial carcinoma) and this can be linked to regulation of endothelial cell proliferation and migration.

To find an explanation for the WT1 positivity in intratumoral endothelial cells, a comparison between normal endometrium, benign tumors (endometrial polyps) and endometrial carcinoma was made. Since 'normal' endometrium is subject to a hormonal cycle during the reproductive age and can become stimulated again in the (post)menopause due to hormonal replacement therapy or bleeding, 'normal' endometrium does not exist. Unfortunately, we were not able to analyse this 'normal' tissue the same manner as the endometrial carcinoma tissue since this was a retrospective trial and only one biopsy was available for IHC and none for RT-PCR. We are aware that this is a shortcoming. A prospective study to the expression of WT1 in benign endometrium should therefore be performed.

Comparing (**Tables 4.4 and 4.5**) the endometrial cells in these benign tissues, they only become positive if the endometrium becomes a malignant tumor. The intra-endometrial endothelial cells have the lowest positivity in atrophic endometrium (57%). This increases in endometrial carcinoma (63%) and triggered benign endometrium (100%). Since the intensity of staining is the same in the groups, the variability lies in the percentages of positive cells, as demonstrated in **Table 4.6**. From this table, one could suggest that the underlying cause for the rise in WT1 positive endothelial cells is an excess in estrogens.

Table 4.6 : Percentage of WT1 positive endothelial cells in the different groups of benign endometrium.

	% of positive endothelial cells
Atrophic endometrium	16,4
Type II endometrial carcinoma	20,8
Type I endometrial carcinoma	35
Hormonally stimulated endometrium	52
Benign tumoral endometrium	53

To our belief, testing for WT1 expression is important, because WT1 is an attractive TAA to target in an immunotherapeutic strategy. WT1 peptide based vaccination therapy was used in several animal and human phase I/II trails with proven clinical response in lung and breast cancer, glioblastoma and renal cell carcinoma (8–13). Since the immunotherapeutic technique is more or less similar in the different protocols, WT1 immunotherapy might be applicable on endometrial carcinomata as well, nevertheless the tumor heterogeneity (as it was proven for a different tumor antigen by Hunder et al. (30)).

Here, we show that uterine carcinomata are heterogeneous for WT1 expression in tumor cells and intratumoral endothelial cells and we suggest to determine WT1 on at least 3 different bioptic sites from the same tumor in order to conclude on WT1 positivity.

References

1. Hohenstein P, Hastie ND. The many facets of the Wilms' tumorgene, WT1. Hum Mol Genet 2006;15:R196-R201.
2. Herzer U, Crocoll A, Barton D, et al. The Wilmstumor suppressor gene wt1 is required for development of the spleen. Curr Biol 1999;9:837-40.
3. Kreidberg JA, Sariola H, Loring JM, et al. WT-1 is required for early kidney development. Cell 1993;74:679-91.
4. Moore AW, Schedl A, McInnes L, et al. YAC transgenic analysis reveals Wilms' tumor 1 gene activity in the proliferating coelomic epithelium, developing diaphragm and limb. Mech Dev 1998;79:169-84.
5. Moore AW, McInnes L, Kreidberg J, et al. YAC complementation shows a requirement for Wt1 in the development of epicardium, adrenal gland and throughout nephrogenesis. Development 1999;126:1845-57.
6. Nakatsuka S, Oji Y, Horiuchi T, et al. Immunohistochemical detection of WT1 protein in a variety of cancer cells. Mod Pathol 2006;19:804-14.
7. Amant F, Moerman P, Neven P, et al. Endometrial cancer. Lancet 2005;366:491-505.
8. Iiyama T, Udaka K, Takeda S, et al. WT1 (Wilms' tumor 1) peptide immunotherapy for renal cell carcinoma.Microbiol Immunol 2007;51:519-30.
9. Mailander V, Scheibenbogen C, Thiel E, et al. Complete remission in a patient with recurrent acute myeloid leukemia induced by vaccination with WT1 peptide in the absence of hematological or renal toxicity. Leukemia 2004;18:165-6.
10. Oka Y, Tsuboi A, Taguchi T, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. ProcNatlAcadSci U.S.A 2004;101:13885-90.
11. Oka Y, Tsuboi A, Elisseeva OA, et al. WT1 peptide cancer vaccine for patients with hematopoietic malignancies and solid cancers. ScientificWorldJournal 2007;7:649-65.
12. Van Driessche A, Gao L, Stauss HJ, et al. Antigen-specific cellular immunotherapy of leukemia. Leukemia 2005;19:1863-71.
13. Izumoto S, Tsuboi A, Oka Y, et al. Phase II clinical trial of Wilms tumor 1 peptide vaccination for patients with recurrent glioblastomamultiforme. J Neurosurg 2008;108:963-971.
14. Coosemans A, Nik SA, Caluwaerts S, et al. Upregulation of Wilms' tumor gene 1 (WT1) in uterine sarcomas. Eur J Cancer 2007;43:1630-7.
15. Acs G, Pasha T, Zhang PJ. WT1 is differentially expressed in serous, endometrioid, clear cell, and mucinous carcinomas of the peritoneum, fallopian tube, ovary, and endometrium. Int J GynecolPathol 2004;23:110-8.
16. Al Hussaini M, Stockman A, Foster H, et al. WT-1 assists in distinguishing ovarian from uterine serous carcinoma and in distinguishing between serous and endometrioid ovarian carcinoma. Histopathology 2004;44:109-15.
17. Dupont J, Wang X, Marshall DS, et al. WilmsTumor Gene (WT1) and p53 expression in endometrial carcinomas: a study of 130 cases using a tissue microarray. Gynecol Oncol 2004;94:449-55.
18. Egan JA, Ionescu MC, Eapen E, et al. Differential expression of WT1 and p53 in serous and endometrioid carcinomas of the endometrium.Int J GynecolPathol 2004;23:119-22.
19. Euscher ED, Malpica A, Deavers MT, et al. Differential expression of WT-1 in serous carcinomas in the peritoneum with or without associated serous carcinoma in endometrial polyps. Am J SurgPathol 2005;29:1074-8.
20. Goldstein NS, Uzieblo A. WT1 immunoreactivity in uterine papillary serous carcinomas is different from ovarian serous carcinomas. Am J ClinPathol 2002;117:541-5.
21. Hashi A, Yuminamochi T, Murata S, et al. Wilmstumor gene immunoreactivity in primary serous carcinomas of the fallopian tube, ovary, endometrium, and peritoneum. Int J GynecolPathol 2003;22:374-7.
22. Kiyokawa T, Kanetsuna Y, Harada T, et al. WT-1 Expression in Ovarian Epithelial Tumors and Endometrial Serous Carcinoma. Mod Pathol16(A837). 2003.
Ref Type: Abstract
23. Shevchuk MM, Lallas TA, DaSilva M. Endometrial Carcinoma : Do Oncogene Studies Separate Types I and II. Mod Pathol16(A216). 2003.
Ref Type: Abstract

24. Zhang Q, Wright T, Alexis D, et al. Differential Expression of WT1 in Uterine Serous and Endometrioid Carcinoma. Mod Pathol16(A988). 2003.
Ref Type: Abstract
25. Heatley MK. WT-1 in ovarian and endometrioid serous carcinoma: a meta-analysis. Histopathology 2005;46:468.
26. Goldstein NS. WT-staining in endometrial serous carcinomas.Int J GynecolPathol 2004;23:405-7.
27. Rackley R, Casey G, Zhao XL, et al. Analysis of the Wilmstumor suppressor gene WT1 in endometrial carcinoma. Genes Chromosomes Cancer 1995;14:313-5.
28. Niksic M, Slight J, Sanford JR, et al. The Wilms' tumor protein (WT1) shuttles between nucleus and cytoplasm and is present in functional polysomes. Hum Mol Genet 2004;13:463-71.
29. Wagner N, Michiels JF, Schedl A, et al. The Wilms' tumor suppressor WT1 is involved in endothelial cell proliferation and migration : expression in tumor vessels *in vivo*. Oncogene2008;27:3662-3672.