WT1 positivity in endothelial cells surrounding epithelial uterine tumors.

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Wilms' tumor gene 1 (WT1) is located on chromosome 11p13. It is overexpressed in several tumoral cells, suggesting a role in tumorigenesis(1). This overexpression and its high immunogenicity have led to the conclusion that WT1 is an attractive target for immunotherapy. Tumoral blood vessels (endothelial cells) have been considered to be inherently WT1 positive(2). As immunotherapeutic strategies attacking WT1 positive endothelial cells might offer an attractive approach, we intended to explore this further.

Paraffin embedded tissue samples of 89 patients with different primary tumors were collected from our central tissue bank after approval of the local ethical committee (**Table 4.7**). Additionally, 7 human myocardial infarction samples (2 acute, 5 old) were analysed. From 10 samples of endometrial carcinoma, 4 serial slides per tumor were made. Each slide was stained with 1 antibody: CD31 (endothelial cell marker) or podoplanin (marker of endothelial cells of lymphatic vessels) or Ki67 (proliferation marker) or WT1, according to the standard techniques. All other samples were only stained for WT1. Immunohistochemical stainings were interpreted by 3 independent observers.

All endothelial cells were positive for CD31, but endothelial cells positive for podoplanin were negative for WT1 and the other way around (**Figure 4.3**). This indicated that WT1 positive vessels thus could be identified as blood vessels. Ki67 stainings were negative.

An overview of the WT1 staining results in different tumoral samples is presented in **Table 4.8**. In malignant tissue, intratumoral blood vessels were WT1 positive in 86% of samples. Surprisingly, detailed analysis of the distribution pattern of the WT1 positive blood vessels in the tumor-surrounding tissue revealed that the vessels were also positive for WT1 in the close periphery of the tumor and became negative with increasing distance from the tumor (**Figure 4.4**). This 'gradient'-phenomenon was observed in 67% of all epithelial malignant tumors. It was absent in almost all (93%) uterine sarcomata. Benign tumors (polyps and

myomata) behaved similarly as their malignant counterparts. A comparison of endothelial cell staining in normal tissue surrounding normal versus tumoral tissue of colon and uterus in the same specimen clearly showed that the amount of WT1 positive blood vessels was much less in tissue surrounding normal colonic mucosa and endometrium compared to the tissue surrounding colorectal and endometrial carcinoma, respectively. Staining of both acute and old human myocardial infarction specimens showed WT1 positive blood vessels in the periphery of the lesion in all-but-one of the samples.

Table 4.7. Sample overview

Tumor	Subtype	Number per subtype	Total number
EPITHELIAL TUMORS			
Malignant			
Endometrial carcinoma	Endometrioid	21	34
	Serous	10	
	Clear Cell	3	
Cervical carcinoma	Spinocellular	4	6
	Adenocarcinoma	2	
Vulvar carcinoma	Spinocellular	8	8
Colorectal carcinoma	Adenocarcinoma	8	8
Breast carcinoma	Ductal carcinoma	4	8
	Lobular carcinoma	2	
	Basal-like carcinoma	2	
Benign			
Endometrial polyp	Non-atypic	5	5
MESENCHYMAL TUMORS			
Malignant			
Uterine sarcoma	Leiomyosarcoma	6	15
	Carcinosarcoma	5	
	Endometrial stromal sarcoma	2	
	Undifferentiated sarcoma	2	
Benign			
Uterine myoma		10	10

Figure 4.3. Serial staining of endothelial cells with CD31 (A), podoplanin (B) and WT1 (C). Podoplanin and WT1 show a complementary staining pattern.

Table 4.8. WT1 positivity of endothelial cells lining blood vessels. Results are shown as n (%)

Tumor	samples with tumoral WT1 positive blood vessels	samples with WT1 positive blood vessels in the periphery	samples with the presence of a gradient in the periphery
EPITHELIAL TUMORS			
<i>Malignant</i>			
Endometrial carcinoma*	88 (29/33)	97 (33/34)	88 (30/34)
Cervical carcinoma*	100 (6/6)	83 (5/6)	67 (4/6)
Vulvar carcinoma*	62 (5/8)	50 (4/8)	33 (2/6)
Colorectal carcinoma*	100 (8/8)	100 (8/8)	86 (6/7)
Breast carcinoma*	72 (6/8)	0 (0/8)	0 (0/8)
<i>Benign</i>			
Endometrial polyp	10 (5/5)	100 (5/5)	100 (5/5)
MESENCHYMAL TUMORS			
<i>Malignant</i>			
Uterine sarcoma*°	-	53 (8/15)	7 (1/15)
<i>Benign</i>			
Uterine myoma	90 (9/10)	70 (7/10)	10 (1/10)

* mean percentage of all subtypes together.

° We are aware that in the group of the sarcomata, carcinosarcomata have a different origin (13). However, for this study, they are considered as one group.

Graybar : not to be interpreted due to extreme WT1 positivity of the tumor

Figure 4.4. Endometrial carcinoma with surrounding normal myometrium, displaying the 'gradient'-phenomenon. Endothelial cells of blood vessels are positive close to the tumor and become negative towards the serosal site.

A mechanism controlling WT1 expression has to be supposed. It might be that these blood vessels are neo-angiogenic. Wagner and colleagues (3) showed a rise of new blood vessels, WT1+/VEGF+, in the periphery of myocardial infarction zone in rats. However, we could not confirm that the blood vessels were proliferating, since Ki67 was negative. Another hypothesis might be that WT1 expression is switched-on in pre-existing blood vessels, derived from WT1 expressing endothelial progenitor cells(4). This action mechanism was already proven by Wagner et al in tubular cells of kidneys of hypoxic rats (5). These differentiated cells retained the capacity to express WT1 in adult organs. Hypoxia could be the driving factor. From literature we know that the most prominent increase in vascular density is situated in the normal tissue surrounding the tumor (6). Consequently, this area does not suffer from hypoxia. However, it is very likely we are looking to a 'compensated' hypoxia and thus hypoxic signals from intratumorally could still be the driving factor, which is confirmed by the observation of the same phenomenon in the periphery of a myocardial infarction in humans.

Although we are aware of the limitations of this observational study, the results demonstrate that WT1 expression in endothelial cells is not an inherent/universal phenomenon as reported before(2). Moreover, a gradient can be noted in the majority of epithelial tumors. This might have important further implications since WT1 has become an attractive target for immunotherapy.

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