Caveolin-1 Expression is Associated with Tumor Size and Therapy Response in Wilms Tumor

Wilms Tümöründe Kaveolin-1 Ekspresyonu Tümör Boyutu ve Tedaviye Yanıt ile İlişkilidir

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ABSTRACT Objective: Although importance of expression status of caveolin-1 protein was established in different cancers including the renal cell carcinoma, its significance has not been evaluated in Wilms tumor. The aim of this study was to determine the prognostic value of caveolin-1 in Wilms tumor. **Material and Methods:** Immunhistochemical caveolin-1 expression was studied in a total of 50 formalin-fixed, paraffin-embedded Wilms tumor specimens and its association with different clinicopathologic parameters was evaluated. **Results:** Caveolin-1 expression was positive in 24 tumors (48%), while was absent in 26 tumors (52%). Using Mann Whitney U Analysis, Caveolin-1 expression was found to be associated with the weight of the kidney (p=0.013). In addition; there was statistical significance with Caveolin-1 expression and therapy response (p=0.049). But there were no statistical relationships between with Caveolin-1 and some clinical prognosis factors such as stage (p=0.093) and survive (p=0.256). **Conclusion:** In the present study, it was shown that the presence of caveolin-1 expression in Wilms tumor associated with both tumor size and therapy response. These findings were thought that Caveolin-1 expression may play a role in Wilms tumor evolution.

Key Words: Wilms tumor; caveolin 1; nephroblastoma overexpressed protein

ÖZET Amaç: Kaveolin-1 protein ekspresyonu renal hücreli karsinom da dâhil birçok kanserde araştırılmış olsa da, Wilms tümöründeki önemi değerlendirilmemiştir. Bu çalışmanın amacı, Wilms tümöründe kaveolin-1'in tanısal değerini belirlemektir. **Gereç ve Yöntemler:** İmmünhistokimyasal kaveolin-1 ekspresyonu ve değişik klinikopatolojik parametrelerle ilişkisi, formalinle fikse parafinize 50 Wilms tümör örneğinde araştırıldı. **Bulgular:** Kaveolin-1 ekspresyonu 24 (%48) tümörde güçlü pozitif iken, 26 (%52) tümörde negatif bulundu. Mann-Whitney U analizinde kaveolin-1 ekspresyonunun böbrek ağırlığıyla ilişkili olduğu saptandı (p=0,013). Ek olarak kaveolin-1 ile tedavi yanıtı arasında istatistiksel olarak anlamlı ilişki vardı (p=0,049). Buna karşılık kaveolin-1 ekspresyonu ile evre (p=0,093) ve sağkalım (p=0,256) benzeri prognostik faktörler arasında ilişki saptanmadı. **Sonuç:** Bu çalışmada, Wilms tümöründe kaveolin-1 ekspresyonunun hem tümör boyutu hem de tedavi yanıtıyla ilişkili olduğu gösterilmiştir. Bu bulgular, kaveolin-1 varlığının Wilms tümörünün gelişiminde rolü olabileceğini düşündürmektedir.

Anahtar Kelimeler: Wilms tümörü; kaveolin 1; nefroblastom aşırı üretilen protein

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Caveolea, specialized micro domains, are flask-shape invaginations of the plasma membrane and have three different coat proteins, named as caveolin. Caveolin-1 (Cav-1) is widely co expressed in fully differentiated mesenchymal and endothelial normal tissues as well as in many solid tumors.[1] It was reported that they are important regulators of cellular processes such as signal transduction and endocytosis. By this way they in-

volve several biological and metabolic functions, including cell growth, apoptosis, and angiogenesis.[2] Previous studies revealed that levels of Cav-1 in epithelial cells of some carcinomas increase during tumor progression. Contrary, Cav-1 expression in the peritumoral stromal cells can decline in advanced and metastatic cancer as well as in sarcoma.[3,4]

Wilms tumor (WT) is the most common primary malignant renal tumor of childhood and has an incidence of 1 in 10 000.[5] At the current time, high cure rates can be achieved and multimodality treatment has resulted in a significant improvement in outcome.[5-7] Hitherto, many parameters were suggested as relevant markers for assessing the proliferative activity and tumor cell dynamics of the WT.[6-9] But the presence of Cav-1 expression in WT has not been investigated widely. The aim of this study was both to explore the importance of Cav-1 expression in Wilms tumor and also investigate the relationships between them, and some clinical prognostic factors such as tumor size, stage and histological features.

MATERIAL AND METHODS

WT resection specimens of 50 cases diagnosed and treated in Dr.Behçet Uz Children's Training and Research Hospital between 1999 and 2013 were included in this study. The study was approved by the Local Ethics Committee. The staging system developed by the National Wilms Tumor Study Group (NWTS) was used to describe the extent of spread of these tumors.

For immunhistochemistry (IHC) studies, hematoxylin and eosin (HE) staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. IHC was performed by the streptavidine biotin peroxidase method (Invitrogen, Camarillo, 85-9043). Serial 5-µm sections were obtained and these slides were baked over-night at 60^0C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat- induced epitope retrieval in the microwave (in 10 mM/L citrate buffer, pH 6.0, for 20 minutes, followed by cooling at room temperature for 20 minutes) and blocked for endogenous peroxidase and biotin. An affinity purified monoclonal mouse antibody against Caveolin 1 (Novus Biologicals, Littleton, NB100-615) and used at a dilution of 1: 200. The evaluation was blinded to any of the clinical features and staining patterns were classified as stromal, perivascular or epithelial (Figure 1). Mann Whitney U test, Chi square test and Kaplan Meier survival analyses were performed for statistical analysis with SPSS 15.0. Descriptive analyses were performed and the mean values with standard deviations were presented. P values less than 0.05 was considered to be statistically significant.

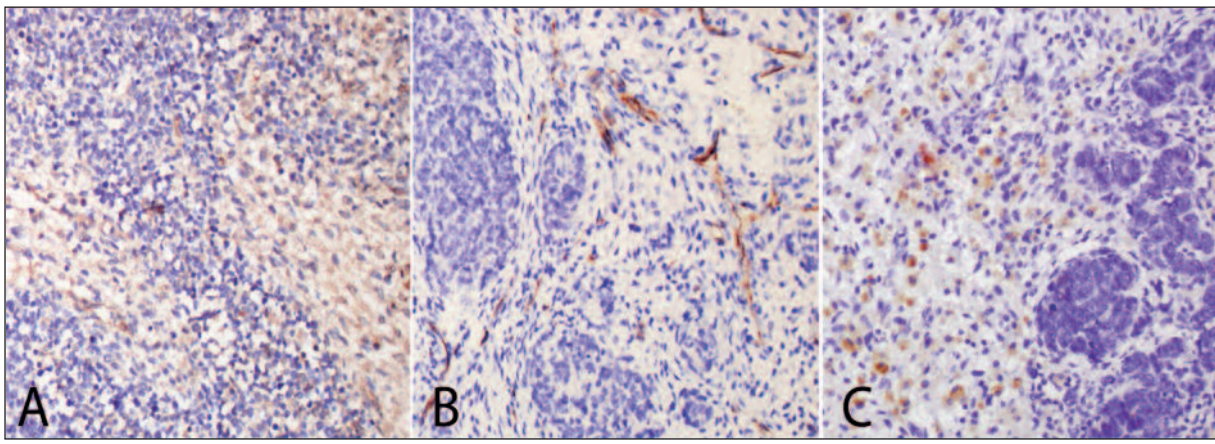

FIGURE 1: A) Cav-1 expression in mesenchymal and blastemal components of Wilms tumor (WT), **B)** Perivascular Cav-1 expression in WT, **C)** Cav-1 expression in mesenchymal component of WT (DAB, x200).

RESULTS

Surgery, chemotherapy and radiotherapy were the treatment modalities which were applied alone or in combination to the total of 50 of patients according to their individual features. The patients with unilateral tumor underwent surgical resection directly without neo-adjuvant treatment. No children had associated specific syndromes or congenital major anomalies except for duplex ureter in one case. Twenty-three (46%) of the cases were male while 27 (54%) were female. The mean age was found to be 3.26±standard deviation (SD) 2 years (ranging from 5 months to 8 years). The tumor was right-sided in 25 (50%) cases, left-sided in 19 (38%) cases and 6 (12%) cases had bilateral tumors (stage V). The average tumor size was 9.16±2.9 cm in diameter and the average weight of kidney was 478±312 g.

Thirteen (26%) cases were stage I (Cav-1 positivity rate= 23.1%, n=3), 18 (36%) cases were stage II (Cav-1 positivity rate= 55.6%, n=10), 7 (14%) cases were stage III (Cav-1 positivity rate= 42.9%, n=3), 6 (12%) cases were stage IV (Cav-1 positivity rate= 83.3%, n=5). Thirty-nine cases (78%) were alive (Cav-1 positivity rate= 43.6%, n=17), while 11 cases (22%) were deceased (Cav-1 positivity rate=63.6%, n=7). Mean overall survival time was 71.3± standard error (SE) 5.2 (2-136) months.

The frequency of Cav-1 expression varied between different components in the same tumor and the highest expression rate was shown in the stromal component. The Cav-1 expressions were determined in only mesenchymal component of most tumors. In addition, there was also positive perivascular Cav-1 staining in the peritumoral stroma in some cases (Figure 1).

We used the NWTS protocol with surgery approach first, but the bilateral tumors, pre-operative chemotherapy was added and the combination of drugs were changed. In addition, the unfavorable histology was required the radiation therapy, even in some localized diseases. Therefore we classified as favorable or unfavorable histology of all tumors. Thirty-nine (78%) cases had triphasic tumors, while 11 (22%) of them had biphasic and the blastemal component was predominant. These latter 11 cases were evaluated as showing unfavorable histology. The Cav-1 positivity rate was 43.6% (n=17) in tumors with favorable histology, while it was 63.6% (n=7) in tumors with unfavorable histology. But there was no relationship between histology and Cav-1 expression (p=0.270). In the whole series, 11 patients died, 3 of these died because of bilateral tumor, and 4 from conditions apparently unrelated to WT such as pneumonia, sepsis, hepatic insufficiency and veno-occlusive disease.

The mean weight of kidneys with Cav-1 positive tumors was 546,46±337.9 g, was 403,8± 269.5 g in Cav-1 negative tumors. Similarly in Cav-1 positive groups, therapy response rate was 83.3%, while it was 96.1% in others. Both the kidney weight (p=0.013) and the therapy response (p=0.049) were found to be associated with the Cav-1 expression by the Mann Whitney U and Chi-square analyses. Contrary there were no relationship between the Cav-1 expression and the survival (Log Rank, p=0.339) by Kaplan Meier Survival analysis (Figure 2). The Chi square test also revealed that the Cav-1 expression didn't affect the survival rate (p=0.256). The overall survival was 50.83±35.1 months in patients with Cav-1 positive tumors while it was 66.85±37 months in Cav-1 negative tumors. In addition; percent of the patients with

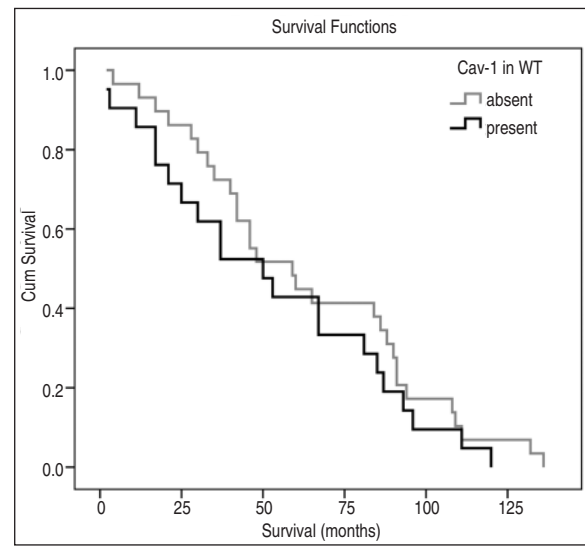

FIGURE 2: There were no statistical significant association with tumoral Cav-1 expression and survival (Log Rank, P= 0.339).

early-stage was 69.2% in Cav-1 negative tumors, while it was 54.2% in Cav-1 positive tumors. But the Chi square test revealed that there was no statistical significance between the Cav-1 expression and the stage (p=0.093).

DISCUSSION

Kidney development is a complex process regulated by transcription factors, proto-oncogenes, and several growth factors which act as signaling molecules and their receptors. WT can be considered as a failure of this transition.[10] A number of genes involved in nephrogenesis as well as in Wilms tumorigenesis.[7,9,10] WT1 gene is the most famous gene involved in WT tumorigenesis. Recently Vicent et al. suppose that WT1 inactivation was predictor of survival in lung cancer patients exhibiting evidence of oncogenic K-RAS activation.[11] Interestingly, Cav-1 interacts with multiple members of the EGFR/RAS/ERK and PI3/AKT pathways to modify signaling activity.[11,12] These findings reveal an unexpected role for WT1 as a key regulator of the genetic network of oncogenic K-RAS and provide important insight into the mechanisms that regulate proliferation in response to oncogenic signals.[11-14] In the present study, molecular genetics analyses were not performed and this is a limitation of the study. Therefore we don't know the WT1 gene status of the patients.

Cav-1 protein has been documented in different neoplasm with a controversial role in cell proliferation, tumor development and progression. This role is both complex and multifaceted. Previous studies showed that Cav1 facilitate both ERK and AKT signaling in cancer cells from kidney, ovary, colon, prostate, epidermis, muscle, brain and is associated with promoting cell invasion, proliferation, angiogenesis and multi-drug resistance.[12-20] Most authors suggested that Cav-1 positive tumor cells served as tumor promoters by these signaling pathways.[3,12-19] In the present study, we determined Cav-1 expression nearly in the half of tumors. But we couldn't determine any statistical significant relationship between the presence of Cav-1 expression in the tumor cells and the tumor behaviors.

In the most English literature, stromal Cav-1 appears to be downregulated and the decreasing expression seems to play a negative role in cancer transformation. The exact mechanisms of this role are still largely unknown. Many oncogenes such as SRC, RAS, BCR-ABL, transcriptionally down-regulate Cav-1 expression.[1,2] Definition of expression status in the peritumoral stromal cells has been accepted as a better parameter. Recent studies have also focused their attention on Cav-1 expression in the peritumoral stromal cells rather than Cav-1 expression in the tumor cells.[12-17] For example Goetz et al. suggest that there may be an important role for stromal Cav-1 in promoting tumor progression and metastasis.[20] But in the most other studies, loss of stromal Cav-1 expression in association with the high tumoral Cav-1 expression, has been reported to be closely related with poor outcome in different malignancies.[1,3,12-14,16,18,21] Similarly we demonstrated that the Cav-1 expression in WT associate with histological grade and stage. However, in survival analysis, Cav-1 expression was not an independent prognostic factor for patient outcome.

The three histological components of WT have different proliferation potentials and different responses to therapy. Hitherto, many studies have revealed these differences. In most reports, the lowest proliferation index was determined in the stromal component and this component generally survived after chemotherapy.[7,9,10] In the present study, we determined Cav-1 expressions confined the mesenchymal component of WTs with or without perivascular expression in the most cases. The importance of Cav-1 expression is novel and potentially relevant in WT progression, since WT1 has been identified as a suppressor of K-RAS oncogene.[12] Our results have two important implications. Firstly, the relationship between the Cav-1 expression in tumors and tumor size suggests that Cav-1 may play an important role in WT evolution. Secondly, our findings support the value of Cav-1 expression as an important predictive factor for therapy response of WT. However, further research is required to define how Cav-1 expression status can be used as a clinical advantage in WT.

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