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Evaluation of the Caveolin-1 expressions in uterine cervical carcinomas

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Summary

Aim: Caveolin-1 (Cav-1) is an important regulator of cellular processes and it involves several biological and metabolic functions, including cell growth, apoptosis, angiogenesis, and also carcinogenesis. This retrospective study was designed to evaluate the differences of tissue expressions of Cav-1 in a spectrum of cervical neoplasms. *Materials and Methods:* Tissue expression of Cav-1 was studied in a total of 107 formalin-fixed, paraffin-embedded uterine cervical tumors specimens and its association with different clinicopathologic parameters was evaluated. *Results:* In this series, there were 30 low- and 29 high-grade cervical intraepithelial neoplasms, 27 squamous cell carcinomas (SCCs), 15 adenosquamous carcinomas (ASCs), and six adenocarcinomas (ACs). Epithelial Cav-1 expression was determined in most SCCs, while it was negative in all ACs and most ASCs ($p < 0.001$). Statistically it was determined that the expression of stromal Cav-1 was significantly upregulated in invasive carcinomas when compared with non-invasive squamous cell carcinomas ($p < 0.001$), and this finding was inconsistent with literature. *Conclusions:* The present findings demonstrated a link between epithelial Cav-1 expression and the squamous differentiation of uterine neoplasms, as well as the relationship between invasion and stromal Cav-1 expression. Therefore it may be suggested that Cav-1 may play a role in the pathogenesis of cervical SCCs.

Key words: Uterine cervix; Caveolin-1 (Cav-1); Squamous cell carcinomas (SCC); Adenosquamous carcinoma; Adenocarcinoma.

Introduction

After the determination of etiological association between HPV and cervical cancer, cervical cancer screening methods and prophylactic HPV vaccine were developed [1]. Despite the preventing policies, cervical cancer is the fourth most common cancer in women worldwide. Squamous cell carcinoma (SCC) is the most common histologic subtype accounting for 70-80% of invasive carcinomas [2]. Adenosquamous carcinoma (ASC) and adenocarcinoma (AC) of the uterine cervix are relatively infrequent histological subtypes of cervical cancers [3-5]. ASCs are defined as tumors that contain an admixture of histologically malignant squamous and glandular cells, and associated with aggressive behavior and reduced survival rates [6]. For example ASCs metastasize to pelvic lymph nodes twice as frequently as SCCs or ACs [2-4]. Prevalence of ASCs has been reported between 3.6% and 25% of all cervical cancers [4]. Primary ACs are also rare tumors make up 5-15% of all uterine cervical carcinomas and its incidence is on the rise in the general population, particularly in young women [6, 7]. It was thought that squamous and glandular components are monoclonal in origin and arise from the pluripotential subcolumnar reserve cells of the endocervical mucous epithelium [6].

Caveolin-1 (Cav-1) is the major structural protein in caveolae which are small, flask-shape invaginations of the plasma membrane [8, 9]. Expression of Caveolin-2 (Cav-2) always mirrors that of Cav-1 [8]. Cav-1 and Cav-2 are widely co-expressed in fully differentiated mesenchymal and endothelial normal tissues, as well as in many solid tumors. It was reported that they are important regulators of cellular processes, such as signal transduction and endocytosis. In manner this they involve several biological and metabolic functions, including cell growth, apoptosis, and angiogenesis [8]. Previous studies revealed that levels of Cav-1 in epithelial cells of some carcinomas increase during tumor progression [9-13]. Conversely, Cav-1 expression in the peritumoral stromal cells can decline in advanced and metastatic cancer [13-15]. Similar results also were determined in sarcomas [8, 16].

The aim of the present study was to investigate the immunohistochemical expressions of Cav-1 in intraepithelial and invasive SCCs, ASCs, and ACs of uterine cervix. In addition, the authors aimed to conclude the relationship with these expressions and adenomatous differentiation of cervical cancers.

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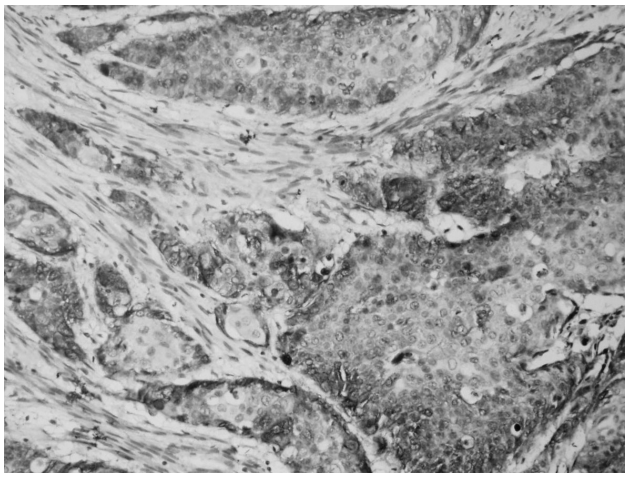

Figure 1. — High Cav-1 expression in a SCC sample (DAB ×200).

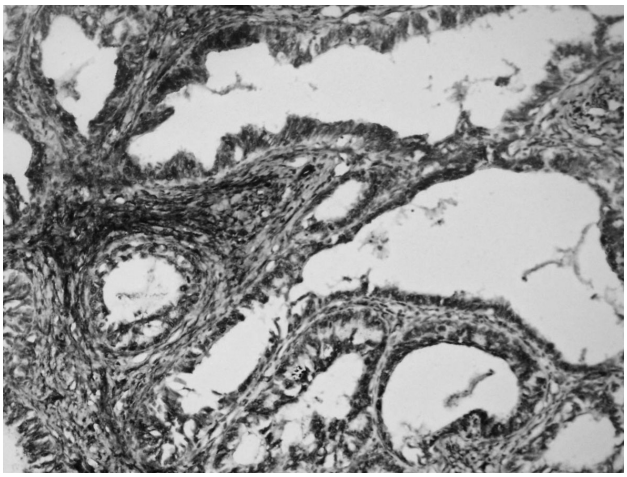

Figure 2. — Negative tumoral Cav-1 expression in an AC sample (DAB ×200).

Materials and Methods

One hundred and seven patients recently diagnosed with cervical intraepithelial neoplasms (CIN), invasive SCC, ASC, and AC at the Tepecik Training and Research Hospital were identified using pathology databases. The study was approved by the Local Ethic Committee of Hospital. For immunhistochemistry (IHC), 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. Serial five-µm sections were obtained and these slides were baked overnight at 60°C, 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 Cav-1 was 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 (tumoral). Spearman Correlation analysis, Mann Whitney U test, and Chi square test were performed for statistical analysis with SPSS 15.0. *P* values less than 0.05 were considered to be statistically significant.

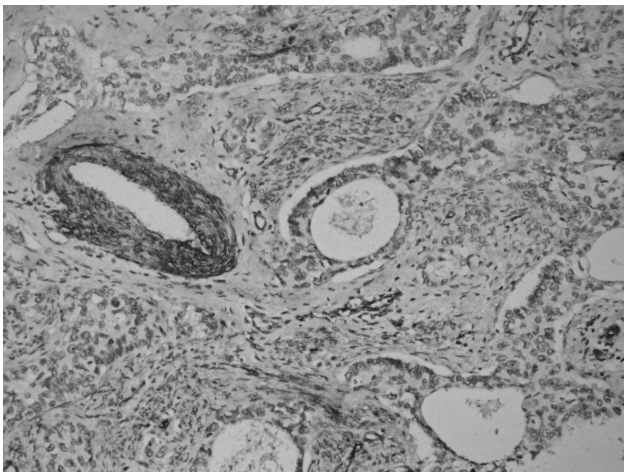

Figure 3. — A strong Cav-1 expression in a vessel wall next to the Cav-1 negative ASC sample can be onserved (DAB ×100).

Results

In this series, there were 30 (28%) low grade squamous intraepithelial lesions (L-SIL), 29 (27.1%) high grade squamous intraepithelial lesions (H-SIL), 27 (25.2%) SCCs, 15 (14%) ASCs, and six (5.6%) ACs. Mean age of all cases was 47.9 ± 12.8 (ranging from 20 to 80) years. The cases with invasive carcinomas (52.6 ± 13.2 years / 30 to 80) were older than cases with intraepithelial neoplasms (44.01 ± 11.1 years / 20 to 66 years). Contrarily, the mean age was similar in all tumor groups. For example, in women with SCC, the mean age was 52.8 ± 14.8, years, while it was 52.5 ± 11.1 years in women with AC and ASC.

Higher expression rate of epithelial (tumoral) Cav-1 (Figure 1) was determined in squamous neoplasms (n=62, 72.1%). Contrarily, negative expressions were determined in all ACs (Figure 2) and in the most (n=12/ 80%) ASCs (Figure 3). In summary, the authors determined if the tumor gains adenomatous components, it loses tumoral Cav-1 expression (Figure 4). They also determined that the expression of stromal Cav-1 was significantly upregulated in invasive carcinomas (n=29, 60.5%) when compared with non-invasive SCCs (n=13, 22.1%). With Chi square test (Figure 5), the expression of stromal Cav-1 was significantly associated with invasion ($p < 0.001$). Interestingly, this finding was inconsistent with the literature.

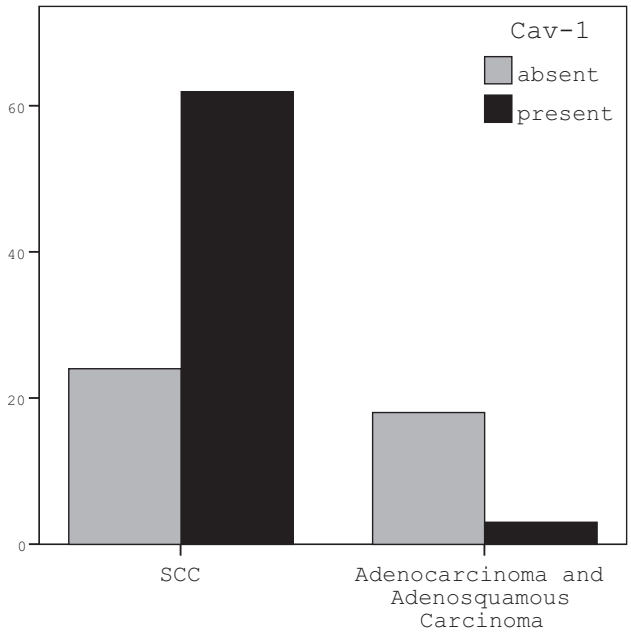

Figure 4. — Tumoral Cav-1 expression was determined in most SCCs, while it was negative in all ACs and most ASCs ($p < 0.001$).

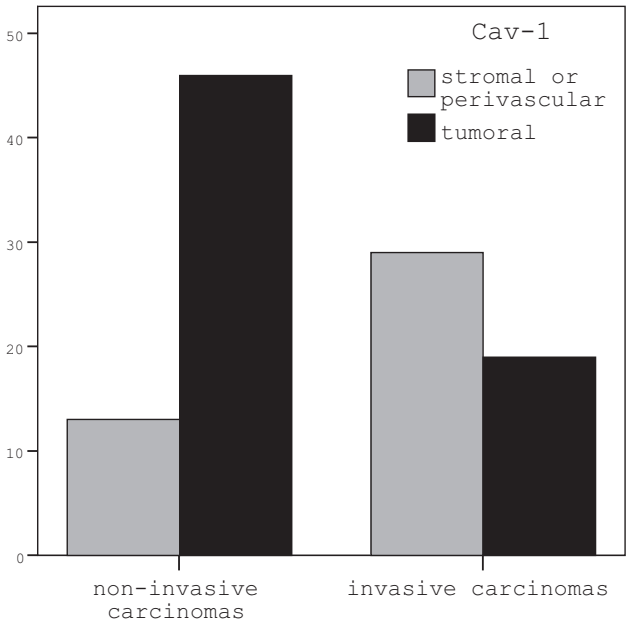

Figure 5. — Tumoral Cav-1 expression was determined in most non-invasive carcinomas, while stromal or perivascular Cav-1 expressions were determined in most invasive tumors ($p < 0.001$).

Discussion

It was previously documented that the most important role of the Cav-1 protein is a regulator of signal transduction and cytoskeletal dynamics in several neoplasms. This role is very controversial [17, 18]. In some cell types, Cav-1 interacts with multiple members of the EGF-R/RAS/ERK and PI3/AKT pathways to modify signaling activity [18-21]. Many studies showed that Cav-1 facilitate both ERK and AKT signaling in cancer cells from kidney, colon, prostate, epidermis, muscle, brain, and is associated with cellular proliferation, angiogenic processes, acceleration of cell invasion, and multi-drug resistance [19-23]. Most authors suggested that Cav-1 positive tumor cells served as tumor promoters by these signaling pathways [12-20]. In the present study, the authors determined higher tumoral expression of Cav-1 protein in most of the SCCs. Therefore they speculated a relationship between the Cav-1 and expression and the carcinogenesis of SCCs.

It is now clear that the microenvironment of tumors plays a crucial role in the initiation and progression of malignancies. Because cancer promotes increased microvessel density, recruits reactive stromal fibroblasts, and different inflammatory cells, and releases peptide-signaling molecules and proteases [21-27]. The exact mechanisms of this role are still largely unknown. However, a major role seems to be played by the transforming growth factor β (TGF-β) which is involved in modulation of cell growth and tumorigenesis [19-23]. In a recent study, Atala *et al.* [19] evaluated the relationship between cancer cells and the tumor microenvironment. Their results indicated that Cav-1 downregulated in the stroma, which coincides with increased expression of the some reactive marker, induces a number of gene alterations, including upregulation of TGF-β1 and downregulation of some genes related with a pro-angiogenesis effect. They also showed that Cav-1 silencing stimulates proliferation and provokes oncogenic cell signaling in prostate stromal cells, coincident with increased levels of intracellular cholesterol and activation of steroidogenic enzymes. These stromal alterations result in increased tumor cell migration [19]. In many studies, reduced levels of Cav-1 in the extratumoral stroma have been reported [23]. Similarly it was shown by functional studies that downregulation of stromal Cav-1 expression is likely to alter stromal influences on tumor epithelium, tumor angiogenesis, cholesterol, and androgen metabolism. Most authors suggested that decreasing stromal Cav-1 expression has promoting effects on tumorigenesis [15, 16, 19, 22, 23]. However, in this study, the authors' observation was not similar with theirs. They determined that the expression of stromal Cav-1 was significantly upregulated in invasive carcinomas when compared with in non-invasive SCCs.

The use of the tissue expression of Cav-1 as markers of cancer progression is controversial. Definition of expression status in the peritumoral stromal cells has been accepted as a better parameter. In most English literature, stromal Cav-1 appears to be downregulated and the decreasing expression seems to play a negative role in cancer transformation. Many oncogenes such as SRC, RAS, BCR-

ABL, transcriptionally downregulate Cav-1 expression [8, 13, 19, 23]. Recent studies have focused their attention on Cav-1 expression in the peritumoral stromal cells rather than Cav-1 expression in the tumor cells. However findings are also contradictory. For example, Goetz *et al.* [22] suggest that there may be an important role for stromal Cav-1 in promoting tumor progression and metastasis. Similarly the present authors demonstrated that stromal expression of Cav-1 increased in uterine cervical carcinomas with invasive behavior, while in 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 [8, 13, 19-28]. In the current study, the authors only evaluated the histopathological parameters of cases. Therefore they could not arrive at a conclusion regarding the association between Cav-1 expression and the prognosis of cervical cancers.

In this study, the authors evaluated the expression of Cav-1 in the uterine cervical tumors and determined that the stromal and perivascular expression of Cav-1 is almost always present in invasive tumors. Although epithelial Cav-1 expression has been extensively studied in several carcinomas, there is no data on the expression and significance of Cav-1 in uterine cervical tumors [29]. Inconsistent with the most published findings, the present authors observed in this study that expression Cav-1 in the peritumoral stroma increased according to the tumor invasiveness. In addition, Cav-1 is overexpressed in tumor cells of SCCs. In conclusion, the results of the present study demonstrate that altered expression of Cav-1 protein in the stroma of uterine cervix carcinomas may be a component tumor aggressiveness. Similarly tumoral Cav-1 expression may play a role in carcinogenesis of SCCs. Therefore it may be suggested that Cav-1 may act as a marker in uterine cervical tumors, especially for differential diagnosis of the invasive and non-invasive squamous cell neoplasms; these findings, however, require further investigation with larger series.

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