

Immunohistochemical Detection of Alpha Catenin in Wilms Tumor

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Introduction

Wilms tumor (nephroblastoma) is embryonic kidney tumor, which develops from metanephric blastem. It is one of the most frequent tumors among children which appears in 1 out of 10.000 children. Most cases of Wilms tumor are sporadic and unilateral, whereas in 1% of cases it is family tumor and in 7% of cases it is bilateral. There are several histological types of Wilms tumor. Classical (three-phase) type has three components: epithelial, blastemal and stromal, whereas two-phase and one-phase types are not common [1,2,3].

Adhesive molecules enable cell-cell and cell-matrix interaction. Cell-cell and cell-matrix interactions influence tissue architecture, changing cell proliferation, differentiation and apoptosis. Cadherins are the family of adhesive molecules which are involved Ca-dependent intercellular adhesion. On the long extracellular part of cadherin, there are places to which Ca is bound, and in cytoplasmic tail there are places to which catenins are bound. Catenins are cytoplasmic cadherin-binding proteins, and they mediate cadherin-cytoskeleton binding. However, it has been shown that cadherin-catenin complex binds actin components of cytoskeleton, and in that way it plays an important role in cell-cell adhesion. These molecules together with cadherin molecules are localized in zonula adherens of epithelial cells and they participate in the formation of intercellular connections. There 3 types of catenin and there are marked as α , β and γ catenin. Alpha-catenin serve to obtain the close contact between two cells, namely between cadherin and beta-catenin. A reduction of alpha-catenin expression in different types of malignant tumors is shown [4,5].

Materials and Methods

Tumour specimens used in this study were obtained from 28 patients undergoing surgery for Wilms' tumour (F:M ratio 36:20; age 7-132 months), 2 metastases from Wilms' tumour found in lungs and 5 normal kidney specimens. As a positive control we used normal lymph node tissue. According to SIOP classification from 2002 (6), 17 (60.7%) out of 28 cases was classified as Wilms tumour stage I, 4 (14.3%) as stage II, 4 (14.3%) as stage III and 2 (7.1%) as stage IV. One case (3.6%) with bilateral Wilms tumour was analysed (stage V) and it was found that the tumour in the left kidney was classified as stage I, while tumour in the right kidney was stage II. Two (7.1%) out of 28 analysed

cases were predominantly epithelial type, 11 (39.3%) blastemal, 6 (21.4%) stromal, and 4 (14.3%) were typical mixed type. Five cases were composed of anaplastic cells, 4 (14.3%) were diffusely anaplastic, and 1 (3.6%) contained focal anaplasia. In consideration with prognostic groups, 18 (64.3%) cases were classified as intermediate prognostic group, and 10 (35.7%) were in high risk group.

For immunohistochemistry, 5 μ m-thick sections were cut from three blocks per case and following the procedure incubated with the primary polyclonal antibody against Alpha-catenin (C-19, Santa Cruz Biotechnology, USA). A standard peroxidase-conjugated streptavidin-biotin labeling (DAKO LSAB+ kit) was used for visualization, with 3,3-diaminobenzidine as chromogen.

The results of immunohistochemical staining were scored by semiquantitative technique: negative staining -, positive staining involving 10% positive cells (focal expression) +, 10%-50% positive cells ++ (moderate expression), and more than 50% +++ (diffuse expression). Fisher's test, Mann-Whitney's and Student's T- test were used to do the statistic analysis, considering $P < 0.05$ as a significant finding. Cases with negative and focal expression were compared with those of moderate and diffuse expression.

Figure 1. Expression of alpha catenin in well differentiated cells of epithelial component (classical type of Wilms tumor)

(Figure not received.)

Results

Alpha-catenin was present on tumor cells in all examined cases of Wilms tumor. However, expression of these adhesive molecule was reduced in tumor cases examined, compared to the expression in normal kidney tissue. Expression of α -catenin was reduced in Wilms tumor compared to normal kidney tissue ($p < 0.05$). We detected α -catenin in 35% cases of Wilms tumor. Expression of alpha catenin was more frequent in stage III and IV than in stage I and II, showing no significant statistic correlation ($p > 0.05$). Intermediate risks group of Wilms tumour showed more frequent alpha catenin expression in comparison with high risks cases, showing no statistic significance ($p > 0.05$).

In blastemal component of Wilms tumor complete loss of α -catenin expression was observed. Epithelial and stromal

component showed reduced immunoreactivity of α -catenin. Expression of α -catenin was completely lost in poor differentiated cells of epithelial component, while well differentiated cell showed decreased α -catenin expression compared to normal kidney tissue ($p < 0.05$). 3 of 5 cases of Wilms tumor with regions of anaplasia (all 3 with diffuse anaplasia), showed total lost of α -catenin, while in another 2 cases (1 with focal and 1 with diffuse anaplasia) weak expression was detected.

Alpha catenin expression was detected in all histologic types of Wilms tumour but without statistic significance in intensity and distribution of expression ($p > 0.05$).

Discussion

Alpha- catenin expression is sensitive indicator of of altered E-cadherin dependent extracellular adhesion. In tumors with normal expression of E-cadherin, disturbance of cell adhesion may occur due to altered function of catenins.

We have observed that normal kidney tissue shows diffuse alpha catenin expression.

Until now, alpha-catenin expression was studied more in epithelial tumors [7,8] and less in mesenchymal tumors [9]. The alpha-catenin expression was reduced in all primary tumors as well as in their metastases [8]. We detected loss of alpha-catenin in 55% cases of Wilms tumour which confirms that alpha-catenin acts as sensitive indicator of altered cell adhesion in Wilms tumor too.

In blastemal component of Wilms tumor complete lost of α -catenin expression was observed. Epithelial and stromal component showed reduced immunoreactivity of α -catenin. Expression of α -catenin was completely lost in poor differentiated cells of epithelial component, while well differentiated cell showed decreased α -catenin expression compared to normal kidney tissue ($p < 0.05$). This level of alpha-catenin in epithelial component of Wilms tumor was expected, considering that the epithelial component is the best differentiated, so connection between the tumor cells are the most coherent.

Decreasing, and loss of alpha -catenin expression was not correlated with tumor stage nor with prognostic group. All histologic types, examined in this study, showed significant decrease, and loss of alpha-catenin expression, but there was no correlation between this loss and histologic type of tumor.

In all examined cases of Wilms tumour metastases we have detected absence or focal alpha catenin expression that corresponded to alpha catenin expression in primary tumour. Knowing this, we assume that increased alpha catenin expression in Wilms tumour could be a predictive parameter for its metastatic ability (the stronger expression, the higher risk of metastases).

Anaplastic Wilms' tumors are commonly believed to be rare forms of progression, driven by p53 mutations, of the more common classical Wilms' tumor or nephroblastoma. Contrary to classical Wilms' tumors, anaplastic tumors traditionally tend to metastasize, to be drug-resistant and to have

a poor prognosis [10]. 3 of 5 cases of Wilms tumor with regions of anaplasia (all 3 with diffuse anaplasia), showed total lost of α -catenin, while in another 2 cases (1 with focal and 1 with diffuse anaplasia) weak expression was detected. From four cases of diffuse anaplasia two cases were classified as stage I, and two of diffuse anaplasia as stage III. As anaplastic Wilms tumour more often tends to metastasize than classic Wilms tumour, our lack of alpha catenin expression may be associated with tendency of tumour to metastasize. This observation has to be tested on the greater number of cases.

Conclusion

There is alpha-catenin expression in Wilms tumor and it exists in epithelial and stromal components. Intensity of expression and distribution of alpha-catenin are reduced in tumor tissue compared to normal kidney tissue. Our results suggest that the α -catenin expression is reduced in Wilms tumor (especially in poor differentiated cells) and that implies reduced cell adhesion, which contributes enhanced tumor cell migration and proliferation.

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