EXPRESSION PROFILE OF TIGHT JUNCTION PROTEINS IN PREMALIGNANT CERVICAL LRESIONS AND CERVICAL CANCER

Ph.D. Thesis Synopsis

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Budapest
2009

1. INTRODUCTION

Cervical cancer is the 2nd most common cancer in women worldwide, the 3rd in developed and the 1st in developing countries. The number of new cases diagnosed in Hungary is about 1500 yearly, unfortunately, 500 lives are lost each year. Cervical cancer screening significantly reduced the incidence of cervical cancer, recognizing the premalignant lesions, as cervical intraepithelial neoplasia (CIN), or the term used in cytology as squamous intraepithelial lesion (SIL) and in situ carcinoma (CIS). The role of human papilloma virus (HPV) as etiological agent of cervical cancer helped to understand the pathogenesis. The significance of this discovery was recognized by donating the 2008 Nobel Prize to professor Harald zur Hausen.

For the organization of epithelial tissues, the interaction of epithelial cells with each other and the extracellular matrix (ECM) components through specialized adhesive junctions is highly important and critical. Although several events contribute to cancer development, progression and metastasis, it is accepted that the loss of cell-to-cell adhesion is one of the important mechanisms in carcinogenesis.

The intercellular junctions consist of zonula occludens or tight junctions (TJs), adherens junctions, desmosomes and gap junctions. TJs are band-like circumferential structures at the most apical part of the cells and consist of integral membrane (transmembrane) and cytoplasmic proteins. TJ interacts with actin cytoskeleton through an intracellular domain and acts as selective barrier controlling paracellular diffusion and plays a role in maintaining cellular polarity and participates in signal transduction. Cells and tissues are characterized by individual claudin patterns; the composition and levels of expression changes during differentiation and carcinogenesis.

The first TJ protein, occludin was revealed in 1993 by S.Tsukita and coworkers, followed by the discovery of the claudin family in 1998. By now, 24 members of the claudin family have been isolated in humans. Claudins are integral membrane proteins with four transmembrane regions, two extracellular loops and N- and C-terminal cytoplasmic domains.

Alterations in the expression of individual claudins have been detected in several cancers and shown to be related with progression and invasion.

2. AIM OF THE STUDY

The above mentioned data proved that the expression of TJ proteins changes during carcinogenesis. The TJ undergoes rapid and dynamic molecular remodeling in response to extracellular stimuli.

The cervical suqamous epithelium represents a highly polarized „resistant" epithelium in which cell adhesion, the normal function of TJs are highly important. During carcinogenesis, however, interruption of normal cell-to-cell contact occurs with dissociation of premalignant and malignant cells.

Based on these data, the following **hypothesis** has been established:

The alterations in the composition of TJ proteins occur in the early stages of cervical carcinogenesis. The expression of the backbones of TJs, the claudins, can be followed through the multistep process of carcinogenesis from premalignant lesions to invasive cancer. It is assumed that well characterized claudin patterns can be detected in different stages of cancer development, which are highly characteristic to the stages.

To prove the hypothesis the following **questions** have been asked:

2.1. How characteristic is the claudin pattern in normal cervical squamous epithelia? What types of claudins characterize the normal epithelium?

2.2. Are there any changes in claudin pattern in the premalignant CIN lesions?

2.3. What are the most characteristic changes in claudin expression comparing the different types in early neoplasia and invasive cancer?

2.4. Are there any differences in claudin pattern and level of expression of claudins in CIN I/II, CINIII/CIS lesions and invasive cancer?

2.5. Are there any changes in occludin expression during cervical carcinogenesis? Is it parallel with the changes detected in claudin expression?

2.6. Is there any correlation between the HPV receptor heparan sulphate proteoglycan, syndecan-1 and the expression of claudins and occludin?

2.7. Could the alterations in the expression of TJ proteins, the characteristic claudin pattern be used as diagnostic marker in the differential diagnosnosis of premalignant cervical lesion?

3. MATERIAL AND METHODS

3.1. Patients and tissue samples

Tissue samples were obtained from patients of the 2nd Department of Obstetrics and Gynecology and from the 2nd Department of Pathology of the Semmelweis University, with the permission of the Regional Ethical Committee (#172/2003). The portio tissues were removed for diagnostic or curative purposes. The tissue blocks were fixed in 10% buffered formalin for 24 hrs and were then embedded in paraffin. Diagnosis was based on hematoxylin and eosin (HE) stained slides. A total of 205 samples (biopsy and surgical specimens of lesions of the uterine cervix) consisting of normal squamous epithelia (n=20), cervical intraepithelial neoplasia (CIN) grade I (n=10), II (n=17) and III (n=10), carcinomas in situ (CIS) (n=15) and T1 and T2 invasive squamous carcinomas (n=33) were analysed.

3.2. Histology and immunohistochemistry

The slides were treated in a target retrieval solution in a microwave oven followed by incubation with the primary antibodies (Zymed, San Francisco, CA, USA) at a 1:80, 1:100 dilution for 1 hr, at room temperature. For visualization, a standard avidin-biotin-peroxidase technique (ABC system, Dako, Glostrup, Denmark) was used with diaminobenzidine as chromogen. For each reaction, a negative control with the omission of the primary antibody or replacing it with a non-immune sera was included. Colon carcinoma and endometrial tissue previously shown to express different claudins and occludins served as positive control. The distribution, intensity, and subcellular location (membranous, apical, cytoplasmic, nuclear, etc) of the staining were all recorded.

A semiquantitative evaluation was used for the claudin immunohistochemical reaction, analysing 10 randomly selected areas per slide using a x20 objective and counting 100 cells in each field. Scoring was as follows: 5=80%-100%, 4= 60%-80%, 3= 40%-60%, 2=20%-40%, 1= 5%-20%, and 0= <5% of the cells showed positive reactions.

3.3. Statistical analysis

The Mann-Whitney *U* test was used to compare immunohistological expression of individual claudins in different groups. Two values were considered significantly different at $p < 0.05$. Correlation coefficients were calculated using Spearman's rank method.

4. RESULTS

4.1. Expression of claudins in normal cervical squamous epithelia

The expression and distribution of the individual claudins were characteristic. Cells of the parabasal and intermedier layers stained strongly for claudin-1, -4 and -7, giving a membranous linear reaction. Claudin-2 showed strong positivity.

Certain TJ molecules showed different expression in various layers of the epithelium: claudin-1, -4 and -7 in the suprabasal and intermediate layer, claudin-2 and occludin in the basal cell layer. With the exception of claudin-2 which showed granular reaction, all other studied molecules exhibited membranous pattern by immunohistochemical method. Claudin-3 was not present in the epithelium. Distribution of claudin showed no essential difference in the studied samples.

4.2. Expression of claudins in cervical premalignant alterations (CIN) and in situ carcinomas (CIS)

In the normal epithelia, a change in the pattern of TJ proteins was observable during the course of cervical carcinogenesis. Strikingly increased expression of claudin-1 was detected in the CIN lesions. The honeycomb reaction pattern was similar to that seen in normal epithelia, although much stronger. Expression of claudin-1 was significantly greater in CINI/II (mean score 4.00+/-) and CIN III/CIS (mean 4.88 +/- 0.33) than in the normal epithelia (mean 2.05 +/-; $p < 0.0001$). Claudin-2 was extended further toward the upper layers in CIN lesions than in normal epithelia. Claudin-3 was not detected. Claudin-4 was expressed either weakly or not at all in the basal layer, showing gradual intensity toward the surface. The localization, distribution and pattern of claudin-7 expression were very similar to those observed for claudin-1. Statistically, all CIN lesions

ahowed a significant increase in claudin expression compared with normal cervical tissues.

4.3. Expression of claudins in invasive cervical squamous carcinomas

The expression of claudins except for claudin-4 was significantly greater in the 33 invasive cancer samples than in the normal epithelia ($p<0.0001$). A decrease of claudin expression in the invasive carcinomas was observed, however, compared with CIN I/II and CIN III/CIS lesions, respectively. The most significant decreases comparing the CIN III/CIS lesions and invasive carcinomas were detected for claudin-1 ($p<0.0001$), followed by claudin-2 ($p=0.0006$). The changes were not significant for claudin-4 and -7, however, decreasing tendency was noted as well.

4.4. Expression of occludin in CIN/CIS lesions

Intensive occludin reaction was localized in the basal cell layers in the normal cervical epithelia. The intensity of the reaction decreased in CIN/CIS lesions, however, more cells were reacting positively. The reaction in CIN III/CIS lesions were focal.

4.5. Expression of syndecan-1 in CIN/CIS lesions

The immunohistochemical reaction for syndecan-1 was intensive in the parabasal and intermedier layers of the normal cervical epithelia. The intensity of the reaction decreased toward CIN/CIS lesions, however, it was more extended.

5. NEW FINDINGS AND CONCLUSIONS

5.1. The TJ proteins, claudin and occludin patterns of the normal human cervical epithelia are characteristic and stable.

The claudins and occludin were detected for the first time in the normal cervical squamous epithelia. It was proved that the individual claudins are expressed differently in the different layers of the cervical epithelia. The typical claudin pattern characterized well the normal epithelium.

5.2. The claudin pattern changes in cervical premalignant lesions (CIN I-III) and in CIS. A significant increase in claudin expression was detected in the premalignant lesions and in CIS as compared with the normal epithelia. Our results proved for the first time that the increase in claudin expression occurs in the early premalignant lesions.

5.3. The claudin expression significantly decreases in invasive carcinomas compared with CIN/CIS lesions. The most significant increase was detected in the expression of claudin-1. The level of claudin expression, however, was still significantly higher in cancer than in the normal cervical epithelia.

5.4. Occludin reacted in the basal cell layer of normal epithelia, showing decreased intensity in the CIN lesions. The number cells showing weaker positivity was, however, also observable focally in the upper epithelial layers during the neoplastic progression.

The expression of occludin in normal epithelia corresponded with claudin-2, with both proteins detected in the basal layers. Increased number of positive cells was seen in CIN regarding both TJ proteins, however, contrary to claudin-2, the intensity of occludin staining was found significantly decreased in CIN and was of focal nature in CIS.

5.5. Syndecan-1 showed intense membranous staining reaction in the parabasal and intermediate layers of the normal cervical epithelia. The intensity did not change in the CIN I-II lesions and was found expanded to the upper layers of the epithelia, whereas becoming decreased and focal in CIS.

The syndecan-1 reaction showed similar localization to claudins-1, -4, and -7 in the normal epithelia. Extensive reaction, i.e. the number of cells showing positivity was also observable, similar to the above claudins.

5.6. To summarize the results, we can assume that our studies proved for the very first time that the expression of all studied claudins present in normal cervical epithelia, occludin and syndecan-1 increased significantly in CIN/CIS lesions

compared with the normal epithelia. The expression of all claudins in the invasive cancers decreased as compared with the CIN/CIS lesions, however, it was still higher than in the normal epithelia.

FINAL CONCLUSIONS, SIGNIFICANCE OF THE STUDIES

Our study proved for the very first time that the expression of several TJ proteins significantly altered during cervical carcinogenesis. Increased expression of claudin expression can be noted in the premalignant cervical lesions. These data suggest that the modification of TJ-associated proteins does not necessary mean the loss of individual types of proteins, but an increase in the expression also occurs, at least transitionally. Loss of differentiation and cohesion and increased invasiveness might be associated with increased expression of certain TJ components. Claudin overexpression seems to be an early alteration in cervical epithelium.

Because of the strong expression of the epithelial cell adhesion molecules in CIN lesions, it has been suggested for use as an early marker of cervical premalignant stages. The altered expressions of claudins displayed in our study seem to be closely associated with premalignant lesions and carcinomas of the uterine cervix and are most likely related to progression and invasion. Because of the significantly increased expression of claudin-1 in abnormal epithelia, this transmembrane adhesion molecule may serve as a good marker for the detection of premalignant lesions or early invasive cancers of the cervical squamous epithelia.

5. LIST OF PUBLICATIONS

Total Impact Factor (IF): 14.847

Publications related to the thesis:
(IF: 6.004)

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