Original paper

Prognostic potential of PRMT5 and DSG2 proteins in pre-malignant cervical lesions

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Precancerous cervical lesions are metaplastic alterations of epithelial cells of the cervix, eventually developing into cervical cancer. Despite primary and secondary prevention, the burden of cervical cancer remains high globally. Protein arginine methyltransferases (PRMT) represent post-translational modifications that interact with multiple signalling pathways, playing a role in epithelial-mesenchymal transition. In complex with desmoglein-2 (DSG2), a cell adhesion protein, both participate in the progression of dysplastic changes with potential malignant development.

The presented study was performed on archival paraffin-embedded blocks from adult women. The studied samples were categorised into low-grade and high-grade intraepithelial lesions. Immunohistochemical analysis was used to observe subcellular localisation, immunoreaction intensity, and percentage of PRMT5- and DSG2-expressing cells, followed by statistical analysis.

Preliminary results identified statistically significant differences between the expression and subcellular localisation of proteins in question in low-grade and high-grade squamous intraepithelial lesions. The primary goal of the presented study is to perceive the involvement of PRMT5 and DSG2 in the initiation and progression of cervical lesions.

Our observations indicate the potential of the assessed proteins as prognostic markers. However, further studies of PRMT5 and DSG2 are required to provide greater insight into cervical carcinogenesis.

Key words: cervical lesions, PRMT5, DSG2, immunohistochemistry, prognosis.

Introduction

Cervical cancer is the fourth most common cancer among females worldwide, affecting women´s lives, especially in developing countries. In 2018 there were approximately 569,847 newly diagnosed cases of cervical cancer, but that number rose to 604,000 globally in 2020 [1, 2]. Among numerous risk factors, human papillomavirus (HPV) is highly associated with the initiation and progression of precancerous and cancerous cervical lesions. Persistent infection of high-risk subtypes of HPV (e.g. HPV 16, 18) is assigned a main but not exclusive cause of precancerous and cancerous cervical lesions [3, 4]. Arginine methylation, as a common post-translational modification, is one of the most prevalent epigenetic dysregulations playing a role in cancer promotion and progression. The importance of the occurrence of arginine modification has been compared to phosphorylation and ubiquitination. Protein arginine methyltransferases (PRMT) catalytic activity generates 3 classes: monomethylarginines, symmetric

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dimethylarginines, and asymmetric dimethylarginines. Protein arginine methyltransferases, the major regulators of arginine methylation, are categorised into 3 types depending on methylation arginine products: type I (PRMT1, 2, 3, 4, 6, and 8), type II (PRMT5 and PRMT9), and type III (PRMT7) [5].

Protein arginine methyltransferase 5 (PRMT5) belongs to the type II class. It consists of 2 domains, including the N-terminal domain responsible for its methyltransferase activity and the C-terminal catalytic domain. PRMT5 affects gene expression by modifying arginine residues on histone molecules. It catalyses symmetric dimethylation of histone H2A on Arg3 residue (H2AR3me2s), H3 on Arg2 and Arg8 residue (H3R2me2s, H3R8me2s), and H4 on Arg3 residue (H4R3me2s) in promoter regions of target genes, including tumour-suppressor p53 [6]. By regulation of many signalling pathways, PRMT5 is involved in cellular processes such as proliferation, apoptosis, cell cycle, DNA replication, and growth. Recent studies have demonstrated the upregulation of PRMT5 expression in cervical cancer in correlation with the poor prognosis of the disease. Its overexpression was observed in different types of cancers: B- and T-cell lymphoma, metastatic melanoma, neuroblastoma and glioblastoma, germ cell tumours, ovarian, nasopharyngeal, breast, colorectal, and gastric cancer [7, 8].

Desmosomal cadherins are transmembrane proteins divided into groups of desmogleins (DSG1-DSG4) and desmocollins (DSC1-3). Desmoglein-2 (DSG2) with its adhesive and mechanical functions is responsible for the formation of desmosomes, maintaining cell-cell adhesion in the basal cells of stratified epithelial tissues, playing an essential role in many different cancers [9]. Contrary to normal tissues, the function of DSG2 was monitored in primary prostate cancer, colon cancer, and skin squamous cell carcinoma. Recent studies assign DSG2 expression in cervical neoplastic cells to its cooperation with signalling pathways influencing the dissemination of cancer cells, activation of multiple cellular growth, and survival [10].

Based on these findings, PRMT5 and DSG2 have emerged as potential biomarkers in several types of cancers, including cervical cancer. The presented study evaluates immunohistochemical (IHC) expression of PRMT5 and DSG2 in cervical low-grade (LSIL) and high-grade (HSIL) squamous intraepithelial lesions (SIL), and their correlation with other clinical-morphological parameters.

Material and methods

The present study was performed on 150 tissue samples obtained from the archives of the Pathology Department of Medicyt JSCo in Bratislava. The age of the patients ranges 20–70 years, with the predominance of women aged up to 50 years (137/150; 91%). Squamous intraepithelial lesions were assessed according to the Bethesda system and divided into LSIL and HSIL categories. Out of 150 FFPE samples, 129 FFPE were stained by anti-PRMT5 antibody (LSIL-49, HSIL-80) and 120 of these samples by anti-DSG2 antibody (LSIL-47, HSIL-73). For IHC analysis, formalin-fixed paraffin-embedded (FFPE) blocks were sectioned into 3-μm-thick sections and put onto FLEX slides. After deparaffinisation with xylene and rehydration in graded alcohol, antigens were retrieved (Target Retrieval Solution Low pH, Dako). Endogenous peroxidase activity was blocked by a 3% hydrogen peroxide solution for 10 minutes. The manual IHC staining was performed according to the manufacturer´s protocol, using a monoclonal anti-PRMT5 antibody (Abcam, EPR5772, 1 : 100 dilution) and monoclonal anti-desmoglein-2/DSG2 antibody (Abcam, EPR6768, 1 : 250 dilution). To visualise primary antibodies, the Envision TM FLEX/HRP system polymer technique (Dako) with peroxidase chromogen DAB (3,3-diaminobenzidine) was used. The sections were counterstained with Mayer´s Haematoxylin (Dako). Cervical disease-free tissues were used as positive controls, and the primary antibody was replaced with TRIS-buffered saline for negative controls. Inclusion criteria were based on the appropriate neoplastic lesion size to eligibly answer the research question. The small lesion size disqualified the sample from being included in the study group. To visualise and compare the expression pattern of the proteins in question with disease progression, the control samples of squamocellular cervical carcinoma were added, but they were not included in the statistical analysis (Fig. 1). Digital microphotographs were taken at a magnification of 400´ with a camera microscope (Axiocam 208 colour) in AxioScope.A1 microscope (ZEISS). QuickPhotoMicro Version 3.2 software (Promicra, Prague, Czech Republic) was used for the digital quantitative evaluation of PRMT5 and DSG2 expression. Analysed regions of interest showing the highest protein positivity were identified by 2 independent observers (BK, VM). The protein expression was described as a percentage of positively stained epithelial cells. The percentage of PRMT5-positive cells was expressed as follows: (1 = 1–25%, 2 = 25–50%, 3 = 50–75% and 4 = 75–100%) [6], and the percentage of DSG2-positive cells was expressed as follows: (1 = 1–10%, 2 = 11–50%, 3 = 51–70%, and 4 = 71–100%) [38]. Semiquantitatively, each tissue sample was assigned a score according to the immunoreaction intensity of PRMT5 and DSG2 (0 – no staining, * – weak, ** – moderate, and *** – strong). The values of $p16^{INK4a}$ and Ki-67 expression in cervical lesions were obtained from the bioptic pathological reports, following the routine diagnostic process.

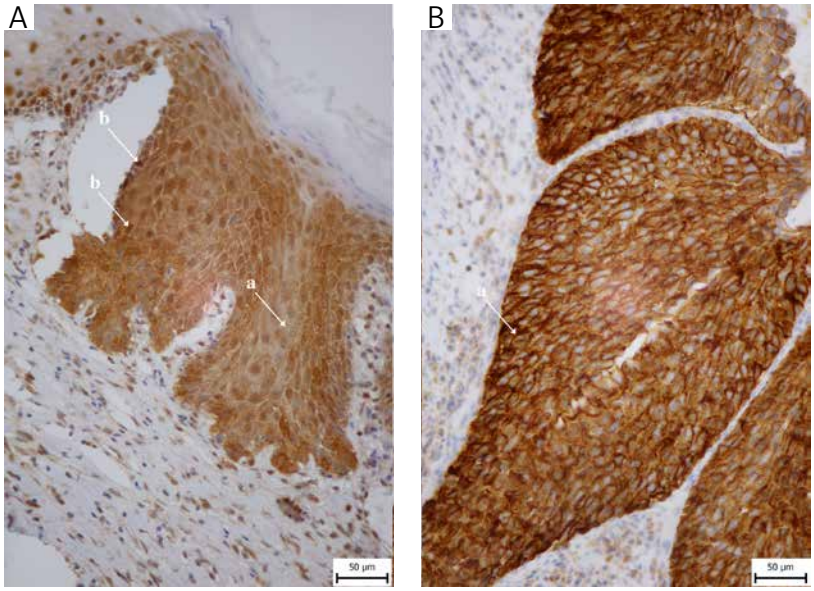

Fig. 1. Immunohistochemical expression of PRMT5 in squamocellular cervical carcinoma (SCCa) (A) has a similar pattern as high-grade squamous intraepithelial lesions with apparent translocation of nuclear PRMT5 positivity (B) into the invasive front of the lesion and moderate cytoplasmic expression in the central region (A). A) DSG2-positive cells with prevailing strong combined immunoreaction. B) DSG2-positive cells were detected throughout the thickness of the cervical SCCa

Table I. χ^2 test analysis of PRMT5

Parameters	Negative	Weak	Moderate	Strong
Cytoplasmic immunoreaction intensity				
LSIL, $n = 49$ (%)		14 (28.57)	**32 (65.31)**	3 (6.12)
HSIL, $n = 80$ (%)		**57 (71.25)**	18 (22.50)	5 (6.25)
$p < 0.001$, $\chi^2 = 24.42$				
Nuclear immunoreaction intensity				
LSIL, $n = 49$ (%)	4 (8.16)	**38 (77.55)**	7 (14.29)	0 (0)
HSIL, $n = 80$ (%)	0 (0)	9 (11.25)	**42 (52.50)**	29 (36.25)
$p < 0.001$; $\chi^2 = 72.64$				

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions

Statistical analysis

Statistical analysis was performed using JASP 0.15 software. Spearman´s correlation coefficient and the χ^2 test were used for statistical analysis. A p-value less than 0.05 was set as a statistical criterion for a significant result.

Results

During the IHC analysis, the combined cytoplasmic and nuclear (CN) subcellular localisation of PRMT5 was detected. PRMT5 immunoreaction intensities were evaluated separately for C and N positivity due to their different expression.

Statistically significant differences were confirmed by the χ^2 test in the immunoreaction intensity of PRMT5 cytoplasmic expression between LSIL and HSIL groups ($p < 0.001$). Cytoplasmic expression of PRMT5 showed a decreasing intensity with disease progression. In the LSIL group ($n = 49$), moderate intensity of PRMT5 cytoplasmic expression prevailed ($n = 32$; 65.31%) compared to the HSIL group ($n = 80$) with weak cytoplasmic positivity predominance ($n = 57$; 71.25%) (Table I, Fig. 2).

The χ^2 test also confirmed statistically significant differences in the immunoreaction intensity of PRMT5 nuclear expression between LSIL and HSIL groups ($p < 0.001$). PRMT5 nuclear expression showed an increasing intensity of immunoreaction with disease progression. In the LSIL group ($n = 49$), the weak intensity of PRMT5 nuclear expression prevailed ($n = 38$; 77.55%) compared to the HSIL group ($n = 80$), with moderate nuclear positivity dominance ($n = 42$; 52.50%). In the LSIL group, negative nuclear expression of PRMT5 protein was noted in 4/49 cases (8.16%), in contrast to HSIL, where not

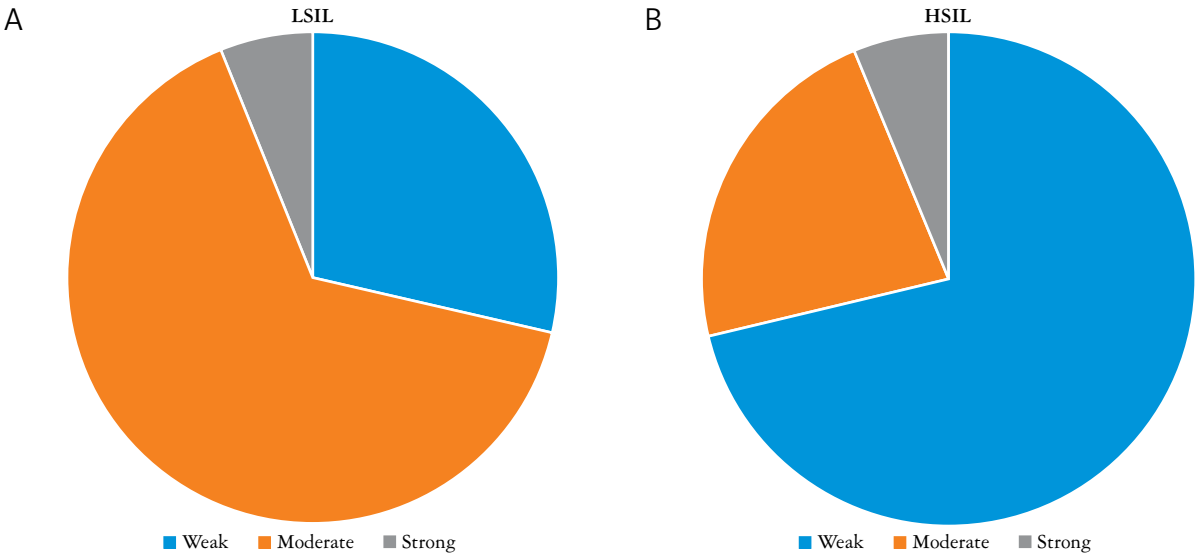

Fig. 2. Intensity of PRMT5 cytoplasmic immunoreaction in low-grade squamous intraepithelial lesions (A) and highgrade squamous intraepithelial lesions (B) group. A) Dominant moderate intensity. B) Dominant weak intensity

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions

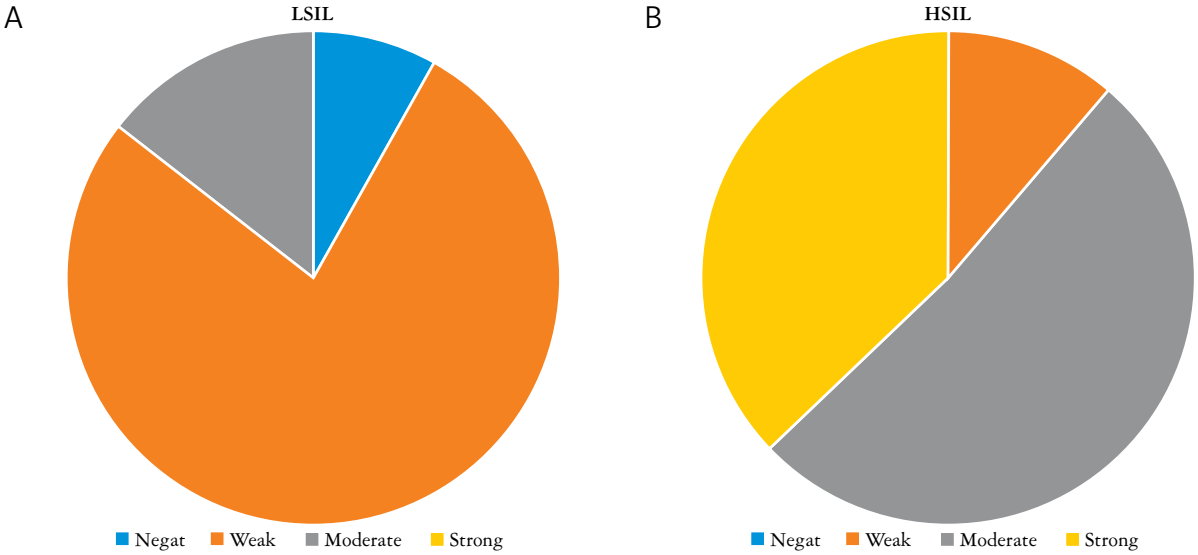

Fig. 3. Intensity of PRMT5 nuclear immunoreaction in low-grade squamous intraepithelial lesions (A) and high-grade squamous intraepithelial lesions (B) group. A) Dominant weak intensity. B) Dominant moderate intensity

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions

a single case showed negative nuclear expression, i.e. 0/80 (0%). Strong nuclear expression in LSIL was negative 0/49 (0%), compared to the HSIL group, where up to 29/80 (36.25%) cases showed strong PRMT5 protein expression (Table I, Fig. 3). The difference between the percentage of PRMT5-positive cells in the LSIL and HSIL groups was not statistically significant ($p > 0.001$).

Spearman´s correlation analysis confirmed a statistically significant negative correlation between the intensity of PRMT5 nuclear immunoreaction and the intensity of PRMT5 cytoplasmic immunoreaction in the HSIL group ($R = –0.34$; $p < 0.05$). In the LSIL group, a weak negative correlation was also confirmed between assessed parameters. However, the results were considered statistically non-significant.

Correlation analysis of both studied groups (LSIL + HSIL) confirmed a significant negative correlation between the intensity of PRMT5 CN immunoreaction ($R = 0.46$; $p < 0.001$); a significant positive correlation between the percentage of PRMT5 positive cells and intensity of PRMT5 nuclear immunoreaction ($R = 0.18$; $p < 0.001$); a significant positive correlation between $p16^{Ink4a}$ expression and the intensity of PRMT5 nuclear immunoreaction ($R = 0.45$; $p < 0.001$), and a significant negative correlation between $p16^{Ink4a}$ expression and the intensity of PRMT5 cytoplasmic immunoreaction ($R = –0.25$; $p < 0.001$). The expected positive correlation was proven between

Table II. Spearman´s correlation analysis of PRMT5 and DSG2 with other parameters between low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions groups

Parameters	LSIL			HSIL		
	N	*R*	*P*	*N*	*R*	*P*
PRMT5						
Cytoplasmic/nuclear immunoreaction intensity	49	0.03	> 0.05	80	–0.34	< 0.05
DSG2						
Percentage of positive cells/immunoreaction intensity	45	–0.08	> 0.05	73	0.33	< 0.05

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions, n – number of samples, r – Spearman´s correlation coefficient

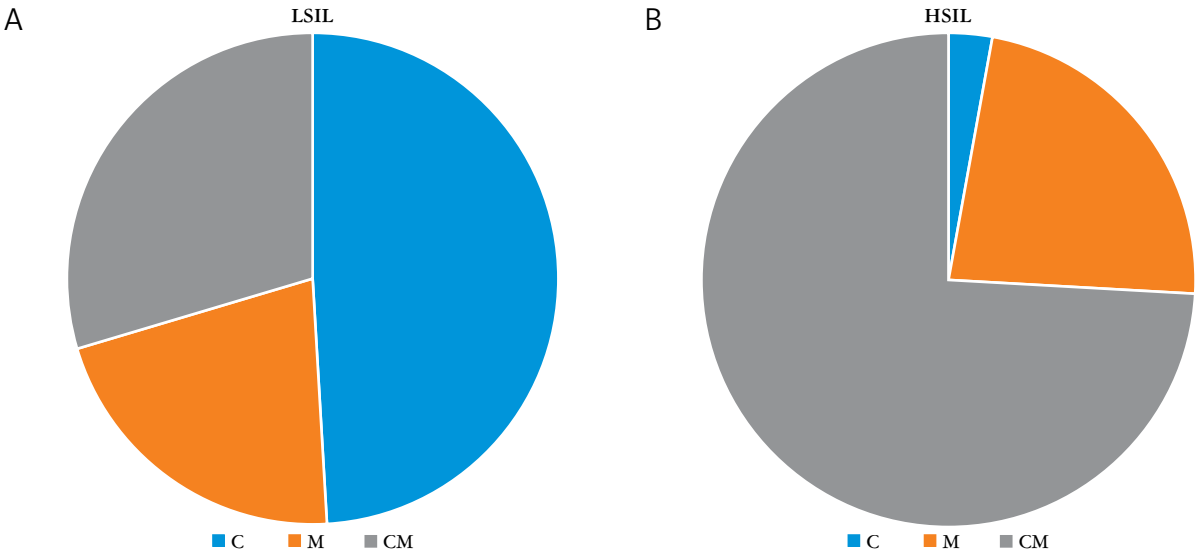

Fig. 4. Subcellular localisation of DSG2 in low-grade squamous intraepithelial lesions (A) and high-grade squamous intraepithelial lesions (B) group. A) Dominant cytoplasmic. B) Dominant combined

C – cytoplasmic, H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions, M – membrane, CM – combined

Table III. χ2 test analysis of DSG2

Subcellular localization	C	M	CM
LSIL, n = 47 (%)	**23 (48.94)**	10 (21.27)	14 (29.79)
HSIL, n = 73 (%)	2 (2.74)	17 (23.29)	**54 (73.97)**
$p < 0.001$; $\chi^2 = 39.19$			
Immunoreaction intensity	**Weak**	**Moderate**	**Strong**
LSIL, n = 47 (%)	15 (31.91)	**25 (53.19)**	7 (14.90)
HSIL, n = 73 (%)	5 (6.85)	27 (36.99)	**41 (56.16)**
$p < 0.001$; $\chi^2 = 24.69$			

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions.

the Ki-67 expression and the intensity of PRMT5 nuclear immunoreaction ($R = 0.56$; $p < 0.001$) (Table II).

Using IHC analysis, cytoplasmic, membrane, and combined subcellular localisation of DSG2 were assessed (Fig. 4). According to the χ^2 test, differences in the DSG2 subcellular localisation between the LSIL and HSIL groups were statistically significant ($p < 0.001$). In the LSIL group, the highest percentage was observed in DSG2 cytoplasmic expression (48.94%) with a total of 23/47 samples. In the HSIL group, the highest combined DSG2 expression was observed in the 54/73 (73.97%) samples (Table III, Fig. 4).

Regarding DSG2 detection, the largest number of samples in the LSIL group showed moderate immunoreaction intensity (25/47; 53.19%), whereas the strong intensity of immunoreaction was noted in samples within the HSIL group (41/73; 56.16%) (Table III, Fig. 5).

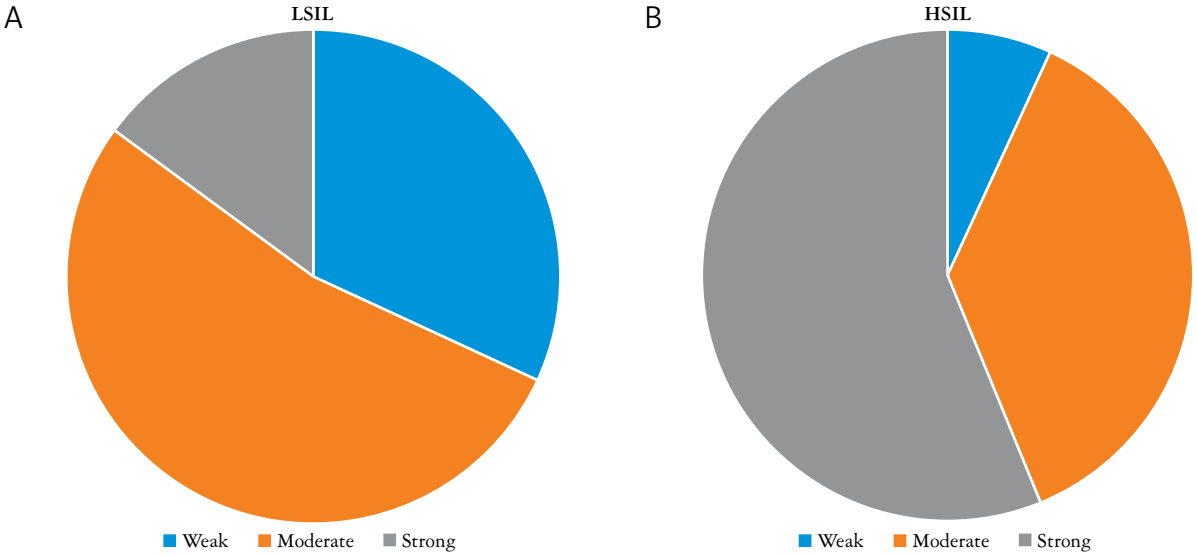

Fig. 5. Intensity of DSG2 immunoreaction in low-grade squamous intraepithelial lesions (A) and high-grade squamous intraepithelial lesions (B) group. A) Dominant moderate intensity. B) Dominant strong intensity

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions

Spearman´s correlation analysis confirmed a statistically significant positive correlation between the percentage of DSG2 positive epithelial cells and the intensity of DSG2 immunoreaction in the HSIL group ($R = 0.33$; $p < 0.05$), in comparison to the LSIL group (Table II).

Evaluating both studied groups as one set of samples (LSIL + HSIL), the correlation analysis confirmed a significant positive correlation between the percentage of DSG2-positive cells and the intensity of DSG2 immunoreaction ($R = 0.25$; $p < 0.05$); a significant negative correlation between the age of the patients and the percentage of DSG2-positive epithelial cells ($R = –0.23$; $p < 0.05$); a significant positive correlation between $p16^{Ink4a}$ expression and DSG2 immunoreaction intensity ($R = 0.37$; $p < 0.001$); a significant positive correlation between the expression of Ki-67 and the intensity of DSG2 immunoreaction ($R = 0.53$; $p < 0.001$), and between the expression of Ki-67 and the percentage of DSG2 positive cells ($R = 0.28$; $p < 0.05$) (Table IV).

Correlation analysis confirmed a statistically significant positive correlation between DSG2 and PRMT5 expression in both studied groups. More specifically, a significant positive correlation was seen between the percentage of DSG2-positive cells and PRMT5-positive cells in HSIL (Table V).

Discussion

PRMT5, an oncoprotein involved in many signalling pathways, promotes tumourigenesis by regulating cancer cell proliferation, survival, and migration in numerous human cancers. It participates in the methylation of histone and non-histone molecules and can affect the epithelial-mesenchymal transition (EMT) related to tumour progression. Moreover, PRMT5 plays a crucial role in the immune system by maintaining the tumour microenvironment (TME). It suppresses the presence of chemokines and interferons in TME and decreases the cancer cell recognition by regulatory T-cells (Tregs), compromising the tumour infiltration and escaping immune surveillance. Methyltransferase activity of PRMT5 probably influences a large variety of inflammatory diseases [11–16]. PRMT5 has been overexpressed in many cancers, and its subcellular localisation depends on its cooperation with binding partners, such as transcriptional factor Snail [17]. Global effects of their interactions on epithelial cell gene expression result in the regulation of EMT-associated properties. Loss of adhesive desmosomal profile enables the transformation of migratory potential of epithelial cells in lesions progression, generally. Protein DSG2, transmembrane cadherin of the desmosomal cell-cell adhesion, acts as an oncogene or a tumour-suppressor. The deregulation of DSG2 protein expression was found in various human cancers, enhancing cell motility and invasiveness [18–21].

The presented study evaluated the PRMT5 and DSG2 expression patterns in cervical squamous intraepithelial neoplasia (SIL) to provide insight into its contribution to developing cervical cancer. We observed combined CN subcellular localisation of PRMT5 in the cervical epithelium. PRMT5 intensity of immunoreaction was considered separately for C and N positivity due to its heterogeneous expression in cervical tissue samples. Our results indicate increasing intensity of nuclear expression and decreasing intensity of cytoplasmic expression of PRMT5

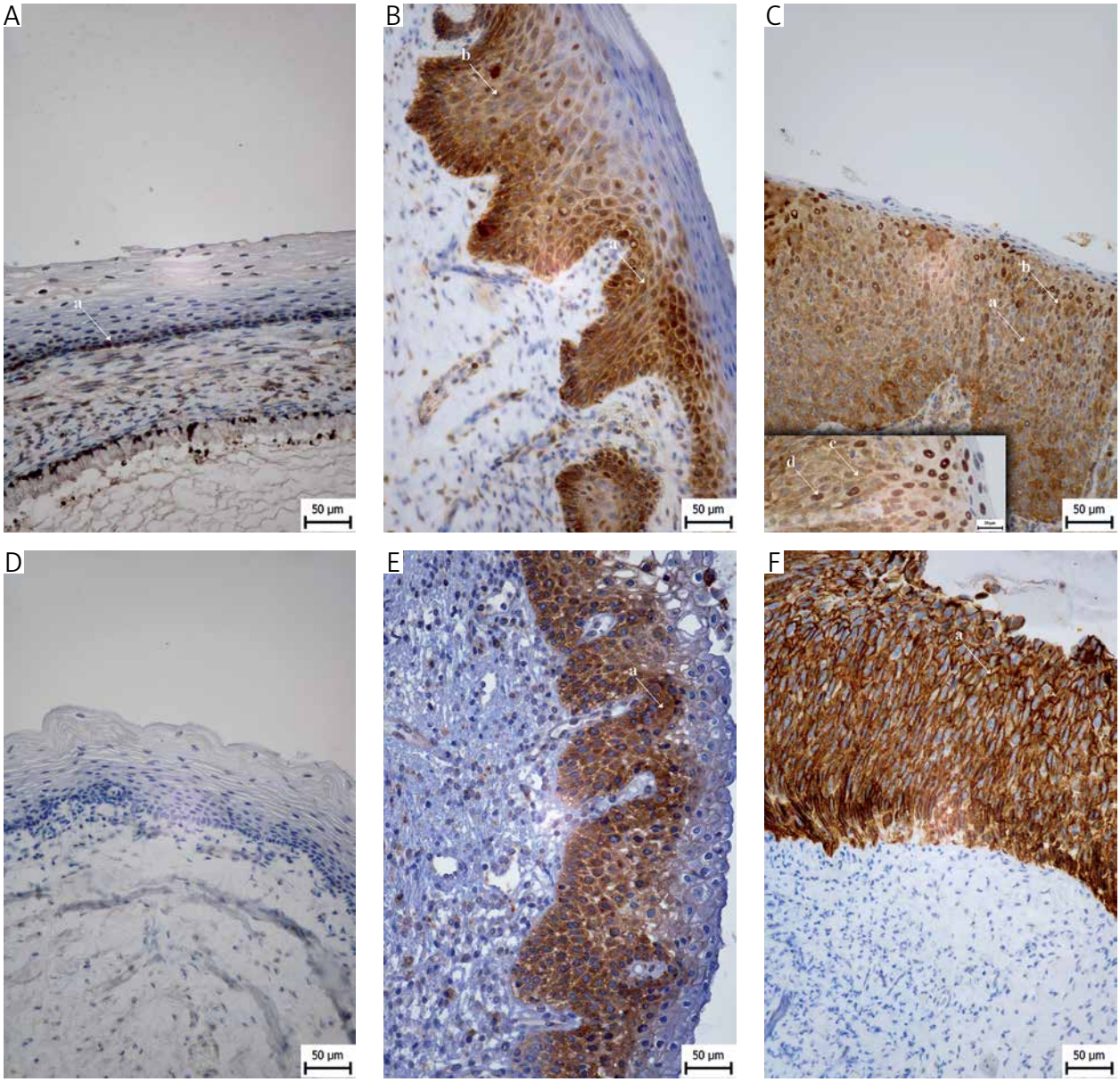

Fig. 6. Immunohistochemical staining of PRMT5 and DSG2 expression in normal cervical epithelium to compare within squamous intraepithelial lesions (SIL). A) PRMT5, normal cervical epithelium – cytoplasmic expression in the basal layer. B) PRMT5, low-grade SIL (LSIL) – moderate cytoplasmic expression (arrow-a), weak nuclear expression (arrow-b) showing basal and parabasal positivity. C) PRMT5, high-grade SIL (HSIL) – weak diffuse cytoplasmic expression (arrow-a; arrow-c;), moderate granular nuclear expression (arrow-b; arrow-d) with increasing trend from the basal to superficial layer. D) DSG2, normal cervical epithelium – no positivity. E) DSG2, LSIL – moderate cytoplasmic subcellular localisation (arrow-a) in the basal and parabasal layers of the epithelium. F) DSG2, HSIL - strong combined cytoplasmic/membrane subcellular localization (arrow-a) in the entire thickness of the epithelium

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions

with disease progression (Fig. 6B, C). By IHC method we also evaluated cytoplasmic, membrane, and combined cytoplasmic/membrane subcellular localisation of DSG2. LSIL showed moderate cytoplasmic positivity of DSG2 expression, compared with strong combined in HSIL (Fig. 6E, F).

PRMT5 overexpression in the cervical cancer cells compared to normal tissue was observed only in a few published studies, e.g. Gao *et al.* [7] demonstrated that PRMT5 overexpression drives the EMT through decreasing expression of epithelial protein markers including E-cadherin, α-catenin, and γ-catenin and the onset of expression of mesenchymal markers such as N-cadherin, vimentin, and fibronectin. They confirmed the positive correlation between the nuclear expression of PRMT5 and the tumour grade. Similarly to our study, nuclear PRMT5 expression increased from LSIL to HSIL with predom-

Table IV. Spearman´s correlation analysis of PRMT5 and DSG2 with other parameters in total number of samples

Parameters	LSIL + HSIL		
	N	*R*	*P*
PRMT5			
Cytoplasmic/nuclear intensity of immunoreaction	129	– 0.46	< 0.001
Percentage of positive cells/nuclear intensity of immunoreaction	129	0.18	< 0.001
$p16^{Ink4a}$ expression/nuclear intensity of immunoreaction	117	0.45	< 0.001
$p16^{Ink4a}$ expression/cytoplasmic intensity of immunoreaction	117	– 0.25	< 0.001
Ki-67 expression/nuclear intensity of immunoreaction	96	0.56	< 0.001
DSG2			
Percentage of positive cells/intensity of immunoreaction	118	0.25	< 0.05
Age of the patients/percentage of positive cells	118	– 0.23	< 0.05
$p16^{Ink4a}$ expression/intensity of immunoreaction	109	0.37	< 0.05
Ki-67 expression/intensity of immunoreaction	89	0.53	< 0.05
Ki-67 expression/percentage of positive cells	87	0.28	< 0.05

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions, n – number of samples, r – Spearman´s correlation coefficient

Table V. Spearman´s correlation analysis of PRMT5 and DSG2 expression between low-grade squamous intraepithelial lesions and high-grade squamous intraepithelial lesions groups and in total number of samples

Parameters	LSIL			HSIL		
	N	*R*	*P*	*N*	*R*	*P*
PRMT5/DSG2						
Percentage of positive cells/percentage of positive cells	44	0.20	>0.05	70	0.26	< 0.05
LSIL + HSIL						
Percentage of positive cells/percentage of positive cells	114		0.27	< 0.05		

H – high-grade squamous intraepithelial lesions, LSIL – low-grade squamous intraepithelial lesions, n – number of samples, r – Spearman´s correlation coefficient

inance in the upper third of the cervical epithelium (Fig. 4B, C). Moderate nuclear positivity was observed in 52.50% (42/80) of the HSIL samples (Table I, Fig. 3B). The study of Ma *et al.* [22] was supplemented with bioinformatic analysis. Their observations indicated the knock-down of PRMT5 expression with decreased levels of AKT phosphorylation so that PRMT5 could affect the proliferation of cervical cancer cells by regulating the AKT signalling pathway. Dong *et al.* [23] clarified the potential inhibition effect of arginine methyltransferase inhibitor 1(AMI-1) on the PRMT5 overexpression in cervical cancer cell lines by inducing cancer cell apoptosis.

Few published studies showed different CN subcellular localisation of PRMT5 expression also in various types of head and neck cancers. Due to the same type of epithelium in the oral and cervical regions with the common HPV risk factor, we assume the expression of PRMT5 might be comparable.

Kumar *et al.* [24] found an association between the nuclear PRMT5 expression and $p16^{Ink4a}$ positivity in oropharyngeal squamous cell carcinoma (OPSCC). Protein $p16^{Ink4a}$ is a cell-cycle inhibitor overexpressed in high-risk HPV (HR-HPV)-associated lesions of oral and cervical cancer. Its tumour-suppressor activity in most cancer cells is abolished by the expression of E7 viral oncogene and the degradation of retinoblastoma protein. High nuclear PRMT5 expression was observed in $p16^{Ink4a}$ negative oropharyngeal tumour tissues, associated with poor prognosis. They also demonstrated that IL-6 probably promotes the localisation of PRMT5 to the nucleus by a complex PRMT5-MEP50 with a binding factor Snail. In contrast, we observed a positive/negative correlation between the nuclear/cytoplasmic expression of PRMT5, respectively, and $p16^{Ink4a}$ in cervical precancerous tissues (Table IV). Our results could be related to the findings explaining the role of $p16^{Ink4a}$ in a tumour-suppressor switch to its oncogenic activity in HPV-associated cervical tissue samples [25]. In addition, upregulated nuclear PRMT5 positivity expressed an exclusive correlation with the progressing

tumour stage and lymph nodal status in nasopharyngeal carcinoma and head and neck squamous cell carcinoma (HNSCC) [26, 27]. Together with our results showing elevated nuclear PRMT5 expression with a lesion progression, the abovementioned outcomes could indicate the oncogenic potential of the examined protein, particularly in the acquisition of aggressive biological behaviour (Fig. 6B, C). On the one hand, Amano *et al.* [28] showed weak PRMT5 expression only in the cytoplasm of basal cells in the normal oral squamous epithelium, supporting our observations in the normal cervical epithelium (Fig. 6A). The authors also confirmed uniform cytoplasmic PRMT5 positivity in all the examined oral cancer diagnoses, e.g. oral epithelial dysplasia (OED), oral intraepithelial neoplasia, and oral squamous cell carcinoma (OSCC). On the other hand, the intensity of cytoplasmic PRMT5 expression was not proven to be significant. The parabasal layer of OED with non-dysplastic epithelial cells showed weak nuclear PRMT5 expression with an even stronger intensity in the invasive front of OSCC. Similarly, the presented results confirmed weak nuclear intensity of PRMT5 expression in the basal and parabasal layer of the epithelium in 77.55% (38/49) of LSIL (Table I, Fig. 3A). The research group of Amano *et al.* [28] also described the association between the simultaneous CN PRMT5 expression and EMT induction. The PRMT5 overexpression probably downregulated levels of E-cadherin and cytokeratin 17 and upregulated the expression of vimentin in SCC – signs typical for the loss of epithelial features and gain of mesenchymal characteristics. Similarly, Wang *et al*. [29] proved the induction of EMT by PRMT5 also in laryngeal carcinoma. The nuclear PRMT5 expression was predominantly associated with high-grade laryngeal tissue samples. The overexpression of PRMT5 in laryngeal carcinoma probably promotes proliferation, invasiveness of tumour cells, and lymph-node metastasis by activating the Wnt/β-catenin signalling pathway. The described observations showed overlapping similarities with PRMT5-associated EMT induction in cervical cancer.

Wu *et al.* [30] assessed the expression of PRMT5 in breast cancer, as one of the leading causes of mortality in women worldwide. They observed CN subcellular localisation of PRMT5 expression in primary and metastatic breast tumours compared to normal breast tissue. Correlation analysis confirmed a positive significant correlation between the expression of PRMT5 and the widely used proliferative marker Ki-67 in primary breast tumours, which is consistent with our observations (Table IV). These results indicate the function of PRMT5 in promoting cancer cell proliferation by interaction with the Wnt/β-catenin pathway [31]. Yang *et al.* [32] examined the positive correlation between tumour necrosis factor receptor-associated 4 and predominant nuclear PRMT5 expression, which favours poor prognosis for patients with breast cancer. The overexpression of PRMT5 in breast cancer cell lines observed by Wang *et al.* [33] contributes to the resistance to the doxorubicin chemotherapeutic treatment by influencing the cancer cells' stemness, triggering the disease progression. Based on the rising incidence of breast and cervical cancer among women all over the world, these findings could open new research approaches to perceive the complex and often antagonistic mechanism of PRMT5 in cancer.

Further studies observed CN expression of PRMT5 in a variety of lung tumours. Shilo *et al.* [34] showed diffuse cytoplasmic PRMT5 expression in a variety of lung tumours, such as non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). The nuclear positivity pattern varied not only within one tumour section but also between the diagnoses. A granular pattern of nuclear positivity was observed in SCLC, while in NSCLC it was in the form of intranuclear globules. In our study, we assessed different staining patterns of PRMT5 subcellular localisation. Cytoplasmic and nuclear positivity proved diffuse and granular patterns, respectively, with heterogeneous distribution within the tumour grade of SIL (Fig. 6C). Supporting the previous statements of PRMT5 protein expression in cervical lesions, Zhang *et al.* [35] reported the role of PRMT5-associated upregulation of Akt signalling, thus increasing proliferative potential of human lung cancer cells [22]. For the first time, the expression of protein DSG2 in cervical cancer was described by Alazawi *et al*. [36]. Significant inhibition of DSG2 expression from normal cervical epithelium to LSIL and HSIL was observed, assigned to diminution of DSG2 function with the tumour progression. In contrast, our observations proved the moderate expression of DSG2 in 53.19% (25/47) of LSIL and strong DSG2 expression in 56.16% (41/73) of HSIL samples (Table III, Fig. 5). These findings support the assumption of heterogeneous roles of DSG2, as both oncogene or/and tumour-suppressor protein, in cancer diseases.

Interestingly, the high DSG2 expression and HPV-associated cervical cancer samples showed a positive correlation, as presented by Zhao *et al*. [37]. HPV infection is probably more closely related to HSIL neoplastic epithelial transformation. Therefore, we believe strong combined DSG2 expression might reflect the transforming stage of HPV infection with increased susceptibility to HPV-related cancer development in the HSIL group (Table III, Fig. 5, 6). Our hypothesis is supported by Qin *et al.* [38], who observed higher DSG2 expression in cervical cancer tissue compared to normal epithelia. The authors also found a significant positive correlation between the high DSG2 expression in early-stage cervical lesions and lymphatic microvessel density.

De novo lymphangiogenesis influences the presence of pelvic lymph node dissemination. Increased DSG2 expression in cervical lesions was associated with an elevated prevalence of circulating tumour clusters after intravasation [39]. DSG2-positivity might be considered a biological predisposition of precancerous lesions to stimulate the potential of colonisation and growth of disseminated cancer cells with disease progression. Considering the abovementioned observations, different DSG2 expression levels related to the strength of adhesive cell junctions seem to support tumour development and metastatic processes in different cancer stages. HPV infection might drive various signalling pathways related to tumourigenesis *via* divergent regulation of the proteins involved [40].

Conclusions

Ongoing studies showed different PRMT5 and DSG2 expression in a variety of human cancers, including cervical cancer, cancers of the head and neck, breast, and lung. Our results confirmed an increasing trend of PRMT5 and DSG2 expression with different subcellular localisation from LSIL to HSIL. The expression of examined proteins was correlated with clinico-morphological parameters including patient age, tumour grade, and the expression of $p16^{Ink4a}$ and Ki-67. In terms of the correlation between the PRMT5 and DSG2 nuclear expression and the expression of $p16^{Ink4a}$ and Ki-67, studied proteins indicate their predictive and prognostic potential by participation in the regulation of cancer cell survival and cancer progress.

The centre of interest is to shed light on the regulation of PRMT5 and DSG2 involvement in the determination of poor prognosis and higher recurrence risk in patients with cervical cancer. Our findings might serve as preliminary indicators of PRMT5 and DSG2 to become potential plausible predictive biomarkers of cervical cancer progression, patient prognosis, and medical treatment.

Disclosures

1. The presented study was approved by the Independent Ethics Committee of JFMED CU, registered at the US Office for Human Research Protection, US Department of Health and Human Services (approval number: EK 59/2022, date: 7.12.2022). The scientific research was conducted ethically, with all study procedures being performed following the requirements of the World Medical Association´s Declaration of Helsinki. All patients included in the study signed a detailed informed consent form, as well as a consent form for scientific purposes, preoperatively. The presented retrospective study does not require any additional informed consent due to archival material usage only. Data were examined in compliance with the privacy and sensitive data concepts valid in the EU.
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4. Conflicts of interest: None.

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