Expression of p300 and p300/CBP associated factor (PCAF) in actinic keratosis and squamous cell carcinoma of the skin

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ABSTRACT

p300 and p300/CBP-associated factor (PCAF) are histone modifiers and transcriptional co-factors involved in a number of cell processes. We investigated their expression patterns in 79 actinic keratoses (AK), 45 cases of Bowen's disease (BD), and 168 invasive squamous cell carcinomas of the skin (SCC). Using tissue microarray and immunohistochemistry, we evaluated p300 and PCAF expression in relation to the type of the lesion and SCC prognostic parameters (grade, diameter, thickness and level of invasion). High nuclear expression of p300 (>60% of positive cells) ($p = 0.001$) and absent cytoplasmic expression ($p = 0.026$) were more frequent in SCC compared to AK and BD, respectively. Cytoplasmic expression of p300 was associated with the SCC invasion of subcutaneous fat and deeper tissues ($p = 0.049$). Diffuse distribution of cells with p300 nuclear expression was more commonly seen in BD and SCC compared to AK ($p < 0.001$), in moderately- and poorly-differentiated SCC compared to well-differentiated SCC ($p < 0.001$), in tumors thicker than 6 mm ($p < 0.001$), and in deeply invading tumors ($p = 0.001$). More frequent loss of PCAF nuclear expression was observed in SCC than in AK and BD ($p < 0.001$). Diffuse distribution of cells with PCAF cytoplasmic expression was more common in BD and SCC compared to AK ($p < 0.001$), and in poorly-differentiated SCC compared to well- and moderately-differentiated SCC ($p < 0.001$). Our results suggest that increase in nuclear expression of p300, as well as the presence of cytoplasmic but loss of nuclear expression of PCAF, could play an important role in the development and progression of cutaneous SCC.

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1. Introduction

In different parts of the world incidence of non-melanoma skin cancer (NMSC) has been rising in the past three decades (Birch-Johansen et al., 2010; Perera et al., 2015; Rogers et al., 2010). Squamous cell carcinoma (SCC) is the second most common malignant NMSC in Caucasian population (after basal cell carcinoma) (Diepgen and Mahler, 2002), and exposure to ultraviolet radiation is the greatest risk factor for its development (Kallini et al., 2015). Actinic keratosis (AK) and in situ squamous cell carcinoma, also known as Bowen's disease (BD), represent dysplastic/neoplastic proliferation of keratinocytes in one part or within whole thickness of epidermis, respectively (Smoller, 2006). The risk of progression of an AK to invasive SCC is small, and it ranges from 0.075% to 0.89% per year, depending on the history of previous NMSC (Werner et al., 2013). A study by Ra and coworkers revealed that evolution of AK and SCC from normal skin involves disturbances in expression of hundreds of genes and affects several molecular pathways. Nevertheless, the same gene expression study identified differences in only nine genes between AK and SCC (Ra et al., 2011). The precise effects these changes have on molecular pathways during cancerogenesis and the effect on biological behavior of AK and SCC still remain to be solved.

p300 and p300/CBP-associated factor (PCAF) are lysine acetyltransferases (K-acetyltransferases, KAT) that were first known as histone modifiers. p300 acts on multiple lysine residues of all four core histones (H2A, H2B, H3, H4), while PCAF is histone H3 specific (Bedford et al., 2010; Howe et al., 2001; Iyer et al., 2004; Ogryzko et al., 1996). They acetylate histones, neutralize their positive charge and reduce histone binding to DNA, which leads promotion of transcription of target genes (Bedford et al., 2010). Both p300 and PCAF act on histone H3, but it seems that p300 is indispensable for histone H3 mediated gene activation (Jin et al., 2011). Numerous other proteins, including various transcription factors, are identified as their substrates. Thus, p300 and PCAF act as transcriptional co-factors, and are involved in regulation of cell cycle, apoptosis, and cell differentiation (Bedford et al., 2010; Grossman, 2001; Iyer et al., 2004). These two proteins play an important

role in the differentiation of normal keratinocytes and in the preservation of epithelial characteristics in cancer cell lines (Pickard et al., 2010). During differentiation of normal keratinocytes, p300 maintains its nuclear position. On the other hand, PCAF changes from a cytoplasmic location (in early phases of differentiation induced in cell cultures and in the basal layers of the epidermis) to a nuclear location (in later phases in cell cultures and in the suprabasal layers of the epidermis) (Pickard et al., 2010). PCAF has a positive role in regulation of E-cadherin expression and in suppression of epithelial-mesenchymal transition (EMT) in cancer cell lines (Mizuguchi et al., 2012). The role of p300 in EMT is not entirely understood, as it has been shown to be either positive or negative, depending on the type of cancer cell lines used (Krubasik et al., 2006; Mizuguchi et al., 2012; Peña et al., 2006; Yokomizo et al., 2011). p300 and PCAF have been examined in several types of human carcinomas. High expression of p300 was found in invasive breast carcinoma, oral, laryngeal, and esophageal SCC, prostatic, nasopharyngeal, colorectal, hepatocellular, and non-small cell lung carcinoma (Chen et al., 2013; Cho et al., 2015; Debes et al., 2003; Hou et al., 2012; Huh et al., 2013; Ishihama et al., 2007; Li et al., 2011; Liao et al., 2012; Syrjänen et al., 2010; Vleugel et al., 2006; Xiao et al., 2011; Yokomizo et al., 2011). An increase in p300 expression has been shown to correlate with progression of cervical intraepithelial neoplasia (CIN) from CIN1 to CIN3 (Syrjänen et al., 2010). p300 was examined previously in cutaneous SCC. Its expression was higher in SCC than in normal skin, but AK or BD were not included in the expression analysis (Chen et al., 2014). Unlike p300, immunohistochemical studies of PCAF expression in human carcinomas have been conducted only in esophageal SCC and intestinal-type of gastric carcinoma, and in both cancers it was found to be decreased compared to normal tissues (Ying et al., 2010; Zhu et al., 2009).

Epithelial-mesenchymal transition (EMT) plays an important role in gaining invasive and metastatic capacity in carcinomas. Dismantling of epithelial cell-to-cell adhesions is mediated, in part, by decrease in E-cadherin expression, and this is considered as a hallmark feature of EMT (Lamouille et al., 2014). Cytoskeletal changes during EMT are most easily seen with de novo expression of vimentin within neoplastic epithelial cells; most epithelial cells normally have minimal or no expression of vimentin (Lamouille et al., 2014). The role of EMT in progression of cutaneous SCC has been examined earlier (Barrette et al., 2014; Jang, 2012; Leong et al., 2010), but not in correlation with p300 or PCAF.

We report here the results of immunohistochemical evaluation of p300 and PCAF expression in actinic keratosis, Bowen's disease, and invasive squamous cell carcinoma of the skin. We also analyzed the results of immunostaining in relation to SCC prognostic parameters (grade, diameter, thickness, level of invasion). Finally, we evaluated expression of E-cadherin and vimentin in correlation to nuclear and cytoplasmic immunostaining of p300 and PCAF.

2. Material and methods

2.1. Patients and specimens

Tissue microarrays (TMAs) were constructed, containing samples from 292 patients whose tissue specimens were analyzed at the Institute of Pathology, Faculty of Medicine, University of Belgrade, in the period between 2002 and 2012. The study was approved by the Ethical Committee of Faculty of Medicine, University of Belgrade. Clinical characteristics of patients included in the study are presented in Table 1. Actinic keratosis (AK, n = 79), Bowen's disease (BD, n = 45) and cutaneous squamous cell carcinoma (SCC, n = 168) were included in the study. Among SCC analyzed, there were 58 well differentiated (SCC-WD), 58 moderately- (SCC-MD) and 52 poorly differentiated (SCC-PD). SCC diameter was ≤20 mm in 106 patients, and >20 mm in 62 patients. SCC thickness was ≤2 mm in 4 cases, >2 mm and ≤6 mm in 116 cases, and >6 mm in 48 cases. Due to tissue loss during serial

Table 1
Clinical characteristics of patients.

Clinical characteristics		Total	Histological diagnosis		
			AK	BD	SCC
Gender (n)	Male	183	51	23	109
	Female	109	28	22	59
Age (years)	Mean	72.9	72.0	72.7	73.7
	Range	31–99	44–91	36–100	31–99
Site (n)	Head and neck	213	67	23	123
	Trunk	26	5	11	10
	Extremities	53	7	11	35

Abbreviations: n, number of cases; AK, actinic keratosis; BD, Bowen's disease; SCC, squamous cell carcinoma.

sectioning and immunohistochemical staining, analysis of EMT markers was only done in 69 AK, 42 BD, and 153 SCC.

2.2. Tissue microarray construction

After reviewing the original hematoxylin- and eosin-stained slides, 1.2 mm diameter tissue cores from corresponding donor blocks were transferred to a recipient tissue microarray (TMA) block. Three representative samples from every case of AK, BD and SCC were taken from the donor blocks. All recipient blocks also contained cores from lymph nodes, appendix, placenta, normal skin and testis, for orientation of samples in the TMA block and as positive controls for immunohistochemical staining.

2.3. Immunohistochemical staining

Serial 5 μm-thick sections were cut from each TMA block for immunohistochemical analysis. Briefly, tissue slides were deparaffinized in xylene and hydrated through graded ethanol to water. Antigen retrieval was carried out in a water bath at 95 °C for 30 min in citraconic acid (1:2000) for p300 staining, in ethylenediaminetetraacetic acid (EDTA) (pH 8.0) for PCAF staining, and incitrate buffer (10 mM, pH 6.0) for the E-cadherin and vimentin staining. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 min. Non-specific binding was blocked with 1% bovine serum albumin (BSA) at room temperature for 20 min. Slides were incubated with primary antibodies against p300 (sc-584, rabbit polyclonal, 1:800 dilution; Santa Cruz Biotechnology, CA, USA), PCAF (sc-13124, mouse monoclonal, 1:200 dilution; Santa Cruz Biotechnology), E-cadherin (NCH- 38, mouse monoclonal, 1:50 dilution; DAKO, Glostrup, Denmark), and vimentin (3B4, mouse monoclonal, 1:50 dilution; DAKO) at room temperature for 1 h. The reactions were visualized using Ultravision LP detection system (Lab Vision, Thermo Scientific, Fremont, CA, USA) with 3,3′-diaminobenzidine (DAB) as a chromogen and Mayer's hematoxylin as a counterstain. Normal lymph node, skin, appendix, placenta, and testis were used as positive controls for immunostaining. Testicular germ cells served as positive control of immunostaining for p300, cytotrophoblasts for PCAF, epidermis and epithelium of the appendix for E-cadherin, and fibroblastic stroma and smooth muscle cells in blood vessel walls for vimentin. Normal epidermis served as negative control of immunostaining for vimentin, and interfollicular lymphocytes were negative controls for p300, PCAF, and E-cadherin.

2.4. Evaluation of immunostaining

The microscopic analysis of the slides was performed on light microscope, at 100× magnification. Immunohistochemical interpretation for the specimens was performed in blinded mode by two pathologists (MBB and DCB). The cases, in which there was disagreement, were re-evaluated in the presence of both pathologists. The extent of nuclear expression for p300 was scored as high (immunoreactivity in >60% of cells) and low (≤60%), while the cytoplasmic expression in each case