Acta Histochemica 116 (2014) 688–693

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Matrix metalloproteinases and E-cadherin immunoreactivity in different basal cell carcinoma histological types

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ARTICLE INFO

Article history:
Received 4 November 2013
Received in revised form
10 December 2013
Accepted 12 December 2013

Keywords:
Basal cell carcinoma
MMP-1
MMP-2
MMP-9
MMP-13
E-cadherin

ABSTRACT

The immunohistochemical staining of matrix metalloproteinases (MMPs) and E-cadherin in tumor epithelial and stromal cells was analyzed in a group of solid, superficial spreading and cystic tumors and in a group of morpheaform and recurrent basal cell carcinomas (BCC) in order to determine whether any of these factors possibly contribute to tumor therapy resistance. Tumor tissues of 64 patients were obtained by complete excisional or curettage biopsy of BCC and these were immunohistochemically stained for MMP-1, MMP-2, MMP-9, MMP-13 and E-cadherin. In the morpheaform and recurrent BCC, MMP-9 expression significantly increased in the stroma, while E-cadherin expression was negative in epithelial cells. Odds ratio for development of morpheaform and recurrent BCC was 6.2 for positive MMP-1 immunostaining in epithelial tumor cells, 5.8 for positive MMP-9 immunostaining in tumor stroma, 3.2 for positive MMP-13 immunostaining in tumor stroma, and 4.5 for negative E-cadherin in epithelial tumor cells. Our results suggest that MMP-1 immunostaining in tumor cells, MMP-9 expression in stromal cells, and absence of E-cadherin expression are associated with morpheaform and recurrent BCC.

Introduction

Basal cell carcinoma (BCC) of the skin is the most common form of cutaneous tumors in Caucasians. BCC typically grows slowly, with minimal involvement of the surrounding tissues and has a high cure rate (Weedon, 2002; Pašić, 2008; Cockerell et al., 2011; O'Toole et al., 2012). However, morpheaform and recurrent forms of BCC, i.e. major tumor lesions located in the central part of the face, exhibit aggressive clinical behavior with deep tissue involvement and frequent recurrence after surgical removal of tumor tissue (Roudier-Pujol et al., 1999; Walling et al., 2004; Garcia et al., 2009).

Matrix metalloproteinases (MMPs) are enzymes that specifically degrade particular parts of the extracellular matrix and are expressed during various physiological and pathological conditions. The ability of MMPs to deal with molecules such as growth factors, adhesion molecules, other proteinases and proteinase inhibitors allows them to act as potent controllers of molecular processes within a microenvironment. Altered values of MMP expression seem to play an important role in tumor progression and surrounding tissue invasion, however large clinical trials have still not determined which of these molecules may be effective in the prevention or treatment of BCCs (Petrella and Margolin, 2012). The relationship between progression of the tumors and overproduction of MMPs has prompted the development of a variety of strategies aimed at blocking the proteolytic activities of these enzymes. However, so far most clinical trials using MMP inhibitors have yielded disappointing results (Boyd et al., 2008). These studies also revealed that specific MMPs can have dual effects on cancer development or even favor the host instead of the tumor (Folgueras et al., 2004; Monhiam et al., 2005). MMPs, in particular MMP-1, MMP-9 and MMP-13 expression have been shown to be involved in the degradation of the extracellular matrix and subsequent tumor invasion in BCCs of the head and neck. Moreover, they were upregulated in tumor cells and surrounding stroma to various degrees in all subtypes of BCC included in published studies (Varani et al., 2000; Brennan et al., 2004; Zlatarova et al., 2012).

E-cadherin mediates intercellular and cell to extracellular matrix interactions, and plays a role of suppressor upon tumor invasion and metastasis. Reduced or lost E-cadherin function has

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http://dx.doi.org/10.1016/j.acthis.2013.12.007

been associated with the development of poorly differentiated and advanced tumors, including BCC (Pizzaro et al., 1994; Haussinger et al., 2004).

The aim of the study was to assess the possible correlations between the immunohistochemical staining profiles of MMP-1, MMP-2, MMP-9, MMP-13 and intensity of the E-cadherin staining and the type of tumor within a group of patients with solid, superficial spreading and cystic BCC and a group of patients with morpheaform and recurrent BCC.

Materials and methods

Study materials were obtained by BCC complete excisional biopsy with clear margins or tumor curettage. Data on patient age and sex, tumor localization and histopathologic type were obtained from laboratory files. All BCCs were divided into two groups: group 1 including solid, cystic and superficial spreading BCC, and group 2 including morpheaform and recurrent BCC. BCC is a so-called "high-risk" tumor characterized by loss of the palisade arrangement and tumor cells with irregular sharp endings and desmoplastic stroma. The recurrent BCC samples were obtained after surgical excision and no other treatment for this type of BCC tumor was performed.

For immunohistochemistry, the following protocol was used: paraffin embedded tumor tissues were cut into 4-μm thick sections, mounted on silanized slides, dried at 37 °C, deparaffinized in xylene and rehydrated in graded concentration of ethanol. Endogenous peroxidase was blocked using 3% H_2O_2 for 30 min. After washing in distilled water and heating in a microwave oven for 17 min at 95 °C with epitope retrieval solution pH 9. Primary mouse monoclonal antibody MMP-1 (sc-21731; (SB) (3B6): Santa Cruz Biotechnology, Santa Cruz, CA, USA) previously diluted 1:10 with antibody diluent (S2022 antibody diluents; Dako, Glostrup, Denmark) was applied for 45 min and then washed in PBS solution. LSAB method (LINK biotinylated secondary antibody, Dako) was used as secondary antibody applied for 15 min, followed by washing in PBS and streptavidin peroxidase application (HRP, K5001; Dako) for 15 min. Diaminobenzidine-hydrogen (DAB, K3468; Dako) was applied as a chromogen for 10 min and then washed in distilled water. The sections were counterstained in hematoxylin, dehydrated in increasing concentration of alcohol, cleared in xylene, mounted in Canada balsam and coverslipped.

The same immunohistochemical protocol was used for primary monoclonal mouse antibodies MMP-2 (5K162; Santa Cruz: sc-71595); MMP-9 (Santa Cruz, 2C3: sc-21733) and MMP-13 (Santa Cruz; MM0019-12E10): Santa Cruz-101564 as described above, previously diluted 1:20, 1:75 and 1:20, respectively. Primary antibody for detection of E-cadherin was monoclonal mouse antibody (M3612, Dako) previously diluted 1:100, processed in BenchMark Ultra IHC/ISH Staining Module (Ventana Medical Systems, Tucson, AZ, USA), and incubated for 32 min.

A placenta section was used as positive control for MMP-1 and MMP-2, colorectal carcinoma sample for MMP-9, and breast carcinoma tissue for MMP-13 and E-cadherin. Any cytoplasmic and membrane brown staining of tumor cells and surrounding tissue was considered positive. Positive immunohistochemical reaction to MMP was indicated by display of brown coloration. The result of staining in these tissues was analyzed, and immunohistochemical reaction was indicated as 0 (negative) or 1 (positive). All slides were examined using a Zeiss Axiostar plus microscope (Zeiss, Oberkochen, Germany).

Fisher exact test was used for statistical processing of the results. The level of correlation of study variables was assessed by calculating odds ratio (OR) with 95% confidence interval (95% CI) for the variables for which χ^2-test yielded statistical correlation with the histopathologic type of BCC. Multivariate logistic regression included all risk factors that proved statistically significant (MMP-1, MMP-9, MMP-13 and E-cadherin) and their simultaneous effect on the development of morpheaform and recurrent BCC.

Results

The study included 64 BCC patients; median age was 68.5 (range 35–89) years. There were 31 (48%) men, median age 70 (range 35–85) years, and 33 (52%) women, median age 65 (range 43–89) years. According to histological types, out of 64 samples examined, there were 15 (23.4%) micronodular BCC, 12 (18.8%) superficial spreading BCC, 13 (20.3%) cystic BCC, 15 (23.4%) morpheaform BCC and 9 (14.1%) recurrent BCC.

Sites of the tumors: 41 (64%) tumors were found on the face, 15 (23%) on the back and 8 (13%) at other sites of the body (neck, presternal region and legs). Tumor localization on the face was 2.7-fold greater compared to the back and 5 times greater compared to other body sites (neck, neckline and legs) ($\chi^2 = 27.5$; $P < 0.001$).

For statistical processing of data obtained by MMP and E-cadherin determination, study patients were divided into two groups according to BCC histopathological types: group 1 (micronodular, superficial spreading and cystic BCC) and group 2 (morpheaform and recurrent BCC).

MMP-1

In group 2, the rate of patients with positive tumor epithelium MMP-1 was 5-fold greater than recorded in group 1, yielding a statistically significant difference (Fisher exact test; $P = 0.045$). Between the 2 groups, difference in MMP-1 expression in tumor stroma did not reach statistical significance (Fisher exact test; $P = 0.41$).

MMP-2

There was no statistically significant difference between the two groups in either tumor epithelium or tumor stroma MMP-2 expression ($\chi^2 = 0.030$; $P = 0.863$ and $\chi^2 = 0.029$; $P = 0.865$, respectively).

MMP-9

All samples in group 2 showed intensive tumor epithelium staining for MMP-9, which was statistically significant ($\chi^2 = 22.9$; $P = 0.001$). In group 1 samples, tumor epithelium staining for MMP-9 was three times less intense than in group 2. In group 1, the rate of patients with negative stromal MMP-9 was threefold greater that in group 2. The rate of patients with positive stromal MMP-9 was 1.8-fold greater in group 2 as compared with group 1, yielding a statistically significant difference ($\chi^2 = 7.1$; $P = 0.008$).

MMP-13

There was no statistically significant difference between the two groups according to the intensity of MMP-13 expression in BCC epithelium and stroma ($\chi^2 = 0.027$; $P = 0.870$). In group 1, the rate of tumor stroma samples negative for MMP-13 was 2.4-fold greater that in group 2, yielding a difference at 94% level of significance ($\chi^2 = 2.58$; $P = 0.108$).

E-cadherin

In group 2, the rate of patients with negative tumor epithelial E-cadherin was threefold greater that in group 1; the difference was statistically significant ($\chi^2 = 3.94$; $P = 0.047$). There was no statistically significant between-group difference according to E-cadherin expression in tumor stroma (Table 1).