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EXPRESSION OF PAR1 IN SQUAMOUS CELL SKIN

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ABSTRACT:
PAR_1 is proposed to be involved in variety of invasive and metastatic cancers. Its expression correlates with the degree of invasiveness in some tissues and cancer cell lines. The aim of this study was to analyze the expression of PAR_1 in human squamous cell carcinomas with different grade of differentiation.
Immunohistochemical analysis in human SCCs and healthy skin was performed to evaluate the expression of PAR_1. The quantitative analysis demonstrated that the expression of PAR_1 was significant up-regulated in well- and moderate to poorly differentiate SCCs compared to normal skin. A significant correlation emerged between an expression of PAR_1 and differentiation of SCC. Our results could clearly show that PAR_1 expression in keratinocytes is significantly up-regulated with a decrease of tumor differentiation.

Key words: proteinase-activated receptor, squamous cell carcinoma, epidermis, skin, keratinocyte, immunohistochemistry.

INTRODUCTION

Protease-activated receptors (PARs) are a novel subfamily of seven transmembrane G protein-coupled receptors activated by serine proteinases. To date, four PARs have been cloned and characterized.

PAR_1 is proposed to be involved in variety of invasive and metastatic cancers, such as breast, colon, lung, pancreas and prostate cancer as well as malign melanoma (1-8). It was demonstrated that PAR_1 expression correlated with the degree of invasiveness in both primary breast tissues and cancer cell lines, as well as in colon cancer (5, 9, 10). This receptor is absent in normal human colon epithelial cells, but aberrantly expressed during colonic carcinogenesis (9). PAR_1 was overexpressed in tumor cells and in cells of the tumor microenviroment while low expression or absence was found in benign or normal tissues (11). Interestingly, it was shown that thrombin has a bimodal effect on PAR_1 dependent growth of melanoma, colon, and prostate carcinomas. Low concentrations of thrombin (0,1-0,5 U/mL) enhance tumor cell growth, in contrast to higher concentrations (0,5-1 U/mL), which impair growth and induce apoptosis (7). The non-serine protease MMP-1 is also implicated in invasion and tumorogenesis of breast cancer and melanoma cells (12, 13).

Together, results from different tissues strongly support the idea that PAR_1 is involved in carcinogenesis. Although PAR_1 is highly expressed by human keratinocytes, its possible functions in the biology of malign skin tumors, however, is still unknown.

Therefore the aim of this study was to analyze the expression of PAR_1 in human squamous cell carcinomas with different grade of differentiation.

MATERIALS AND METHODS

- **Antibodies:** The primary antibody directed against human *PAR_1* (clone ATAP2-mouse monoclonal) was purchased from Santa Cruz™, CA, USA) and used in **1:150** dilution.
- **Patients:** Permission for human studies was given by the Ethical Committee of the University of Münster, Germany. The study included 12 initial SCCs (10 males and 2 females, range: 42-87 years, mean age 71,0 years), 10 well differentiated SCCs (5 males and 5 females, range: 31-82

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years, mean age 62,6 years) and 9 moderate to undifferentiated SCCs (5 males and 4 females, range: 64-90 years, mean age 81,0 years). Normal skin was obtained from post-operative material (4 males and 8 females, range: 20-86 years, mean age 59,2 years). Patients did not receive topical medications within two weeks before obtaining biopsies.

- **Immunochistochemistry:** 5-6 μm paraffin sections from human skin carcinomas were heated in oven for 3h. Sections were rehydrated and heated in a steamer. Endogenous peroxydase activity was quenched with 100 mM NaN3/0.1% H_2O_2 in PBS for 20 minutes at room temperature. Sections were further incubated with first antibody diluted in 1% BSA overnight at 4°C in a humid chamber. After rinsing with PBS, slides were incubated with second antibodies for 1 hour (DAKO En-Vision+ System Labelled Polymer-HRP™®, anti-mouse). The immunoreactivity was detected with Liquid DAB+ Substrate Chromogen System® (Dako Cytomation, Glostrup, Denmark) Sections were counterstained with Vector Hematoxylin® (Vector Laboratories, Burlingame, CA, USA, and mounted with Aguamount ® (BDH, Solms, Germany).

  The staining of the tumor was scored as follows: 0: no cells positive; 1: 0-25 % positive tumor cells (weak); 2: 25-50% positive cells (moderate); 3: 50-75 % positive cells; 4: more than 75% positive tumor cells (strong).

RESULTS

Immunohistochemical analysis in human SCCs and healthy skin was performed to evaluate the expression of PAR_1. We examined 31 SCCs of different histological stages.

Immunohistochemistry revealed that in normal epidermis surrounding the tumors, PAR_1 was observed both in basal and suprabasal layers except the squamous and corneal layers. In the stroma, PAR_1 immunoreactivity could be detected in fibroblasts, endothelial cells, mast cells and neutrophils. PAR_1 expression was mainly located in the cytoplasm of the tumor cells. Immunoreactivity for PAR_1 was weak or even absent in initial SCCs **(Figure 1a)**. In well and moderate to poorly differentiated SCCs, positive immunolabeling for PAR_1 was noted in 17 (89.5 %) of the tumors **(Figure1b, c)**.

In fact, quantitative analysis demonstrated that the expression of PAR_1 was significant up-regulated in well- and moderate to poorly differentiate SCCs compared to normal skin. Moreover, we found difference in PAR_1 expression between well- and poorly differentiated SCCs. A significant correlation emerged between an expression of PAR_1 and differentiation of SCC. Our results could clearly show that PAR_1 expression in keratinocytes is significantly up-regulated with a decrease of tumor differentiation. This is in line with several other observations in the literature confirming a relationship between PAR_1 expression and SCC differentiation (14).

DISSCUSION

Our knowledge about the pathogenesis of skin-derived epithelial tumors is still incomplete. Beside classical growth factors, PARs have been implicated with the control of proliferation and differentiation events in several normal and tumor tissues (5, 15-17). These effects may be at least in part mediated by activated PARs.

PAR_1 modulates keratinocyte growth and differentiation in cultured human and murine keratinocytes (18-21) and induces growth and proliferation in primary human keratinocytes (18, 20).

However, the possible function of PAR_1 in cutaneous cancerogenesis is still unknown. There is still no published data about a role of PAR_1 in non-melanoma skin cancer. Our investigation is the first to examine PAR_1 expression in squamous cell skin carcinomas (SCCs). A significant correlation emerged between an expression of PAR_1 and differentiation of SCC.

PAR_1 is well recognized as a regulator of a variety of extracellular and adhesion molecules and proposed to be involved in the invasion and metastasis of various cancers. On the other hand, PAR_1 also inhibits invasion and migration in certain tumor cells (22-24). These observations promote PAR_1 to be capable of inducing diverse intracellular signaling pathways leading to tumor progression or even tumor growth inhibition under certain circumstances. Therefore, diverse pathways of PAR_1-induced intracellular signaling pathways seem to be crucial for the invasiveness of tumor cells (25).

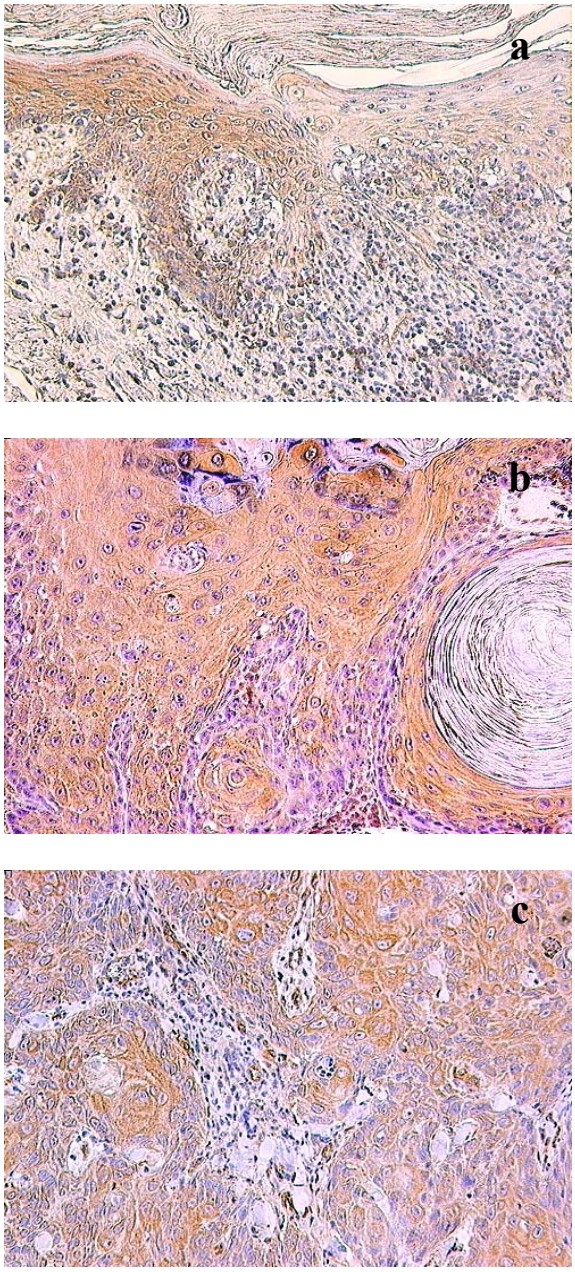

Figure 1. Expression of PAR1 in human squamocellular skin carcinomas: a) initial SCC; b) well differentiated SSC; c) moderate differentiated SCC.

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