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Immunolocalization of glycodelin in human adenocarcinoma of the lung, squamous cell carcinoma of the lung and lung metastases of colonic adenocarcinoma

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ABSTRACT

Glycodelin (Gd), which is localized in cells of bronchial epithelium, type II pneumocytes and alveolar macrophages in rats and humans, plays an important role in the pulmonary immune response in asthmatic inflammation. In this study, sections of paraffin-embedded tumor adjacent lung tissue and sections of adenocarcinoma of the lung, squamous cell carcinoma of the lung and metastases of colonic adenocarcinoma were investigated for the distribution and expression of Gd using a polyclonal anti-Gd antibody. Glycodelin protein is located in the cytoplasm of bronchial epithelial cells, pneumocytes and alveolar macrophages. Furthermore, Gd is expressed in adenocarcinoma and squamous cell carcinoma of the lung as well as in lung metastases of colonic adenocarcinoma. Densitometric analyses showed a significantly increased expression of glycodelin protein in cancer tissue compared to tumor adjacent lung tissue. The Gd protein level was 1.7–2.6-fold increased in lung carcinoma compared to tumor adjacent lung tissue. The Gd protein level did not differ from each other between the investigated types of cancer tissue. Because these data validate the recent findings of Gd mRNA expression, it may be concluded that glycodelin plays an important role in the pathogenesis of lung cancer and lung metastases.

Introduction

Glycodelin (Gd) has been mainly localized in organs of the female and male reproductive tract in rats, baboons and humans (Joshi et al., 1981; Mazurkiewicz et al., 1981; Fazleabas et al., 1997; Keil et al., 1999). Gd, previously known as placental protein 14 (PP14) (Bohn et al., 1982) and progesterone-associated endometrial protein (PAEP) (Kamarainen et al., 1991), is a member of the immunocalins (Logdberg and Wester, 2000) and under physiological conditions is synthesized by endometrial tissue and decidua during pregnancy (Julkunen et al., 1985, 1986; Julkunen, 1986; Kamarainen et al., 1998). Both Gd and its mRNA, have also been found in glandular tissues, e.g., in parabronchial and eccrine sweat glands (Kamarainen et al., 1997), and in erythroid precursors of human bone marrow cells (Kamarainen et al., 1994). Gd is also localized in bronchial and alveolar epithelium cells in the lung of rats (Kunert-Keil et al., 2009) and seems to be functionally relevant for these cells. Furthermore, Gd plays an important role in the pathogenesis of different neoplastic diseases such as endometrial, ovarian, breast and cervical carcinoma as well as in chronic inflammatory disorders such as bronchial asthma (Kamarainen et al., 1996; Horowitz et al., 2001; Shabani et al., 2005; Jeschke et al., 2005a,b; Richter et al., 2007; Toth et al., 2008; Kunert-Keil et al., 2009). Adenocarcinoma and squamous cell carcinoma of the lung are the two main sub-types of non-small cell lung carcinoma (NSCLC). Squamous cell lung carcinoma comprises 30–40% of NSCLCs, whereas adenocarcinoma represents 25–30% of invasive lesions (Haussinger and Kohlhaufl, 2005).

Recent studies showed that glycodelin transcript is localized in the cytoplasm of bronchial epithelial cells, pneumocytes and alveolar macrophages in human lung tissue. Gd is expressed in adenocarcinoma and squamous cell carcinoma of the lung, as well as in lung metastases originating from colonic adenocarcinoma. Densitometric analyses showed a significantly increased expression of glycodelin in cancer tissue compared to normal lung tissue. No differences in glycodelin transcript levels were found between the tested types of cancer tissue (Kunert-Keil et al., submitted for publication).

In order to acquire more information on Gd in human lung carcinoma, we investigated the distribution of Gd and quantified the expression of this protein in different types of NSCLCs, lung

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metastases of colonic adenocarcinoma and tumor adjacent lung tissue using a polyclonal anti-glycodelin antibody.

Materials and methods

Tissue samples

Formalin-fixed paraffin-embedded tissue samples were obtained from 43 patients who had undergone surgery for lung tumors and whose specimens were examined at the Institute of Pathology of the Ernst-Moritz-Arndt-University of Greifswald. Tissue samples were classified according to histological classification of cancer in the lung as follows: adenocarcinoma of the lung ($n = 15$, 13 male, 2 female, mean age 67.7 years $\pm$ 7.1), squamous cell carcinoma of the lung ($n = 15$, 14 male, 1 female, mean age 62.9 years $\pm$ 10.0), adenocarcinoma of the colon with lung metastasis ($n = 13$, 9 male, 4 female, mean age 62 years $\pm$ 13.8). Cancer tissue and tumor adjacent lung tissue were investigated for glycodelin protein expression by indirect immunohistochemistry using a polyclonal anti-glycodelin antibody.

Immunohistochemistry

Indirect immunohistochemistry was performed on paraffin sections (4 μm) using a polyclonal anti-glycodelin antibody and a biotinylated secondary anti-rabbit antibody (Vectastain® ABC Kit, Vector Laboratories, Burlingame, CA, USA) as described previously (Kunert-Keil et al., 2005, 2009; Jeschke et al., 2005a,b, 2009). Sections were incubated in methanol/H_2O_2 (30 min) to inhibit endogenous peroxidase activity, washed in phosphate buffered saline (PBS) and treated with goat serum (Vectastain® ABC kit, 20 min, 22 °C) to reduce non-specific background staining. Incubation with the polyclonal anti-glycodelin antibody (whole serum; 1:200 in PBS; Bioscience AG, Heidelberg, Germany) was performed overnight at 4 °C. Sections were then incubated with the biotinylated secondary goat-anti-rabbit antibody (Vectastain® ABC kit, 1 h, 22 °C) and avidin-biotinylated peroxidase (Vectastain® ABC kit, 45 min, 22 °C). Peroxidase staining reaction was done with diaminobenzidine/H_2O_2 (1 mg/ml; 5 min) and stopped in tap water. Sections were counterstained in hematoxylin and then cover-slipped. In controls, the primary antibody was replaced with pre-immune serum of the respective rabbit. The level of protein expression was determined in one run in a blinded manner using identical staff, equipment, and chemicals. From each section, five digital pictures were taken at random places of the tissue (200-fold magnification; 3CCD colour camera; Hitachi HV-C20 M; Hitachi Denshi Ltd., Japan, and Axiolab, Carl Zeiss, Göttingen, Germany), as described previously (Jeschke et al., 2005a,b, 2009; Kunert-Keil et al., 2009). For standardization of the measurements, the optical density of white background color was attuned to 250 in each picture. For all sections, we assessed the mean optical density and the quantity of pixels that had a positive reaction for glycodelin by use of KSRun software (imaging system KS400, release 3.0; Zeiss, Vision GmbH, Munich, Germany).

Statistical analysis

All statistical analyses were performed using the IBM SPSS Statistics 18 Software (SPSS GmbH Software, Munich, Germany). Statistical analyses were made using paired Wilcoxon's test. Data are given as means $\pm$ SEM. $P < 0.05$ was considered to be statistically significant.

Results

Localization of glycodelin in the lung

Experiments with the polyclonal anti-glycodelin antibody showed that tumor adjacent lung tissue gives a positive staining for Gd. Gd was preferentially found in cells of the bronchial epithelium, e.g., Clara cells and goblet cells. Furthermore, Gd could be detected in alveolar epithelial cells, particularly in pneumocytes and alveolar macrophages (Fig. 1A).

In tissue sections of all tested carcinoma in the lung we found a very strong immunostaining of Gd. Immunostaining of Gd was most intense in the cytoplasm of all tumor cells of adenocarcinoma and squamous cell carcinoma of the lung and lung metastases as well as immune cells and some fibroblasts (Fig. 1C–E). All sections incubated with the pre-immune serum were devoid of any reaction (Fig. 1B and F).

Computerized quantification of glycodelin immunostaining in lung cancer tissue

The densitometric quantification of the Gd staining intensity, without discrimination of the stained cell type, showed a highly significantly increased Gd expression in all tested lung carcinoma compared to the respective tumor adjacent lung tissues (Fig. 2).

77% of all tested lung metastases (10/13) showed on average a 1.7-fold increased amount of Gd compared to tumor adjacent lung tissue from the same patients. The Gd protein expression was significantly up-regulated in lung metastases [mean optical density (mod) metastases vs. tumor adjacent lung: 4399 $\pm$ 669 vs. 2617 $\pm$ 337, $P = 0.015$].

An increased protein level of Gd was found in 80% of all tissue sections of adenocarcinoma (12/15) compared to tumor adjacent lung tissue from the same patients. The immunostaining of Gd was on average 1.7-fold higher in adenocarcinoma of the lung compared to normal lung tissue [mod adenocarcinoma vs. tumor adjacent lung: 3655 $\pm$ 460 vs. 2188 $\pm$ 352; $P = 0.0022$]. The minimum level of Gd transcripts was 1.4-fold increased and the maximum level 44 times higher in adenocarcinoma compared to tumor adjacent lung tissue.

For the study of the Gd protein expression in squamous cell carcinoma of the lung we used sections from tumor adjacent and tumor tissue of 15 patients. We found that Gd was significantly up-regulated in squamous cell carcinoma in 11 out of 15 individuals [mod squamous cell carcinoma vs. tumor adjacent lung: 4143 $\pm$ 570 vs. 1567 $\pm$ 263; $P = 0.00073$]. The minimum level of Gd protein was 1.6-fold increased and the maximal level 125-fold higher in squamous cell carcinoma compared to tumor adjacent lung tissue.

Discussion

We demonstrated for the first time Gd immunolocalization in formalin-fixed paraffin-embedded tissue from patients who had undergone surgical removal of non-small cell lung cancers such as adenocarcinoma and squamous cell carcinoma of the lung as well as lung metastases of colonic carcinoma. In this study, we found that bronchial and alveolar epithelial cells, e.g., Clara and goblet cells, pneumocytes and macrophages, express glycodelin. The results are in agreement with earlier findings on the localization of Gd in lung tissue of rats (Kunert-Keil et al., 2009). The localization of Gd in highly differentiated epithelia was also shown in ductal and lobular epithelium of breast tissues (Kamarainen et al., 1999). A possible physiological function of Gd is the promotion of epithelial differentiation. A former study has shown that epithelial cells are capable of producing and responding to a number of