Expression of the Glycodelin A Gene and the Detection of its Protein in Tissues and Serum of Ovarian Carcinoma Patients

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Abstract. Background: Glycodelin A (GdA), also known as placental protein 14 (PP14), has been detected in endometrial, cervical and ovarian carcinoma cells. It is suspected to be a marker of human ovarian cancer tissues. Materials and Methods: We investigated serum, tissue and cyst fluid samples of patients with an ovarian carcinoma in contrast to patients with benign and malignant diseases such as uterine myoma, endometriosis, cervical, uterine and breast cancer, and metastases of bladder and colon carcinoma. Used methods were enzyme-immunoassay, immunohistochemistry (IHC) and polymerase chain reaction (PCR). Results: In 81% of the control group the GdA-expression was negative, which was confirmed by IHC and PCR. Of the ovarian carcinoma group only 52% showed correspondence between IHC and PCR. Conclusion: These results indicate that determination of GdA is not sensitive or specific enough for use as a tumour marker.

Glycodelin A (GdA), also known as placental protein 14 (PP14), is a glycoprotein of approximately 28 kDa (1, 2). As a major reproductive glycoprotein, it has several functions in cell differentiation and recognition (3). Unusually for mammals is the fact that GdA has an unique carbohydrate configuration consisting of sialylated LacdiNAc structures (2). GdA is mainly synthesized in secretory endometrial glands and gestational deciduas under normal physiological conditions (4-7). It is thought to act as an immunosuppressive factor, although its function is still unclear.

About 8,000 patients per year in Germany are hospitalized with ovarian carcinoma. In the majority of cases, the diagnosis is made in the terminal stage of cancer (stage III). Its pathogenesis is differentiated by genetic and sporadic diseases. Genetic ovarian carcinoma shows a lifetime-risk of 60% to 70%, while sporadic disease occurs in 0.9% of all cases. The peak of disease occurrence is between the ages of 55 and 60 years. When diagnosed at the terminal stage the survival rate is 20%. In contrast, about 80% survive when diagnosed at earlier stages. This demonstrates the relevance of reliable markers for the diagnosis of early stage ovarian carcinoma. In the present study, we investigated samples of serum, tissue and cyst fluids of patients with ovarian carcinoma in contrast to patients with leiomyomas; endometriosis; cervical, uterine and breast cancer, as well as metastases of bladder and colon carcinoma.

Materials and Methods

Tissue samples of 27 patients diagnosed with ovarian carcinoma were analyzed and compared with 48 patients with a clinical diagnosis of uterus myomatous; endometriosis; cervical, endometrial carcinoma; breast cancer; bladder carcinoma or ovarian metastases of a colon carcinoma. The individual samples were classified as ovary, tube, myometrium, endometrium, peritoneum, omentum and pelvic lymph nodes.

Immunohistochemistry (IHC). The samples were sectioned (thickness: 4-µm), fixed with formalin and incubated with antibody-containing supernatants (polyclonal N-20) glycodelin N-20: sc-12289) and Q-13 (glycodelin Q-13: sc-12290)) for one hour at room temperature. Cells were stained with the Vectastain® Elite ABC-Kit (Vector Laboratories, Burlingame, CA, USA). For visualization diaminobenzidine (DAB) was used.

Preparation of mRNA and reverse transcription-reaction. mRNA was prepared with the RNeasy® Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions. The samples were stored at –85°C. Reverse transcription reaction reagents were purchased from Invitrogen Life Technologies (Invitrogen GmbH, Karlsruhe, Germany) and the manufacturer’s protocol for reverse transcription reaction was used.

PCR. PCR was performed using a primer-mix from SuperArray, specific to the GdA sequence. It included an annealing step at 55°C and 40 cycles (on an Eppendorf Master Cycler, Eppendorf, Hamburg, Germany). The fragments were identified by agarose gel electrophoresis (2% agarose).
Enzyme-immunoassay. The self-developed enzyme-immunoassay was used as described elsewhere (12). Briefly, the assay is based on polyclonal rabbit-anti-glycodelin A antibodies and monoclonal mouse-anti-glycodelin A antibodies. We worked with an antibody concentration of 1 µg/ml at a dilution of 1:1000 and the optical density was measured at 450 nm.

Results and Discussion

The control group was GdA-negative in 81% of all cases, confirmed with GdA IHC and PCR. In 52% of the ovarian carcinomas, IHC and PCR confirmed the detection of GdA (Figure 1A-C). Figure 2 shows examples of positive and negative patient samples applied to an agarose gel. The results of the present study point to the fact that GdA is not specific for tumours, but it does correlate often with ovarian carcinoma. Moreover, the GdA expression of metastases is on a par with that of primary tumours. For an exact diagnosis and to verify tumour antigens it is recommended to combine the methods of immunohistochemistry and DNA detection.

The regulation of uterine immune responsiveness by GdA is known and it has been shown that decidual extracts containing GdA suppress thymidine uptake in normal and mitogen-stimulated human mixed lymphocytes cultures (8, 9). In addition, GdA reduced the synthesis of IL1 and IL2 and the expression of the IL2 receptor in cells stimulated by mitogens (8, 10, 11). Its role in ovarian carcinoma is not yet clear. Here we showed that GdA is not a reliable marker for the early disease stages, but it was found to correlate often with an ovarian carcinoma.

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References


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