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Differential expression of secretoglobins in normal ovary and in ovarian carcinoma – Overexpression of mammaglobin-1 is linked to tumor progression

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ABSTRACT

Secretoglobins (SCGB), such as mammaglobin 1 (MGB1, SCGB2A2), mammaglobin 2 (MGB2, SCGB2A1) and lipophilin B (LIPB, SCGB1D2), have been related to carcinogenesis. We profiled expression of MGB1, MGB2 and LIPB in human tissues and ovarian carcinoma and explored the impact of SCGB overexpression on cell proliferation. MGB1, MGB2 and LIPB mRNA are expressed at variable levels in most human tissues and we observed significant bilateral correlations between the different secretoglobins. Concerted overexpression of MGB1 and LIPB resulted in significant increase in cell proliferation. In clinical specimens of ovarian carcinoma we measured elevated concentrations of secretoglobin mRNA and for MGB1 this up-regulation was confirmed on the protein level. Overexpression of MGB1 positively correlated with the FIGO stage, the tumor grade and the mitotic index suggesting a patho-physiological role of the protein. Our data indicate that MGB1, MGB2 and LIPB mRNAs are expressed at low levels in human tissues but basal expression is upregulated in ovarian cancer. The in vivo correlation between nuclear MGB1 localization and the mitotic rate in ovarian cancer as well as the increased cell proliferation induced by secretoglobin overexpression in ovarian cancer cell lines suggest a pathophysiological role of these proteins in ovarian cancer.

Introduction

Secretoglobins (SCGBs)[2] form a heterogeneous family of low molecular weight secretory proteins and occur in vivo as either homo- or heterodimers [1]. More than 20 members of the secretoglobin family have been characterized in various animal species [2,3]. In humans, 11 SCGBs genes and five pseudogenes have been identified. Seven of these genes (uteroglobin, mammaglobin-1, mammaglobin-2, lipophilin-A, lipophilin-B, IFN-gamma inducible SCGB, RYD5) were mapped to a dense gene cluster on chromosome 11 [2,3]. The founding member of this protein family, uteroglobin (UGB), was discovered 30 years ago [4].

Belonging to the human SCGB family, mammaglobin 1 (MGB1, SCGB2A2), mammaglobin 2 (MGB2, SCGB2A1) and lipophilin B (LIPB, SCGB1D2) have been linked to multiple diseases [5]. MGB1 is the most widely studied SCGB and was first identified as mammary-specific protein, which is strongly overexpressed in human breast cancer [6,7]. In vivo, MGB1 forms a covalent complex with LIPB *via* redox sensitive disulfide bonds and may be present as dimer of covalent heterodimers [8]. A strong correlation between MGB1 and LIPB expression was observed in breast cancer suggesting a coordinated expression regulation of the two proteins [9,10]. A growing number of studies demonstrated that MGB1 and LIPB may not be restricted to normal and malignant breast tissue [11–13]. Both genes were abundantly expressed in tumors of the female genital tract, i.e. endometrial, ovarian and cervical cancer. Recently, it was shown that MGB1 is also expressed in normal human endometrium and that its expression is controlled by steroid hormones [14].

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[2] *Abbreviations used:* SCGBs, secretoglobins; UGB, uteroglobin; CDS, coding sequence; TMA, tissue microarrays; IRS, immunoreactivity scoring system; UTRs, untranslated regions; ORF, open reading frames.

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There are two distinct MGB-isoforms, which are referred to as MGB1 and MGB2. The isoforms are encoded for by two separate genes, which are highly homologous [15]. Expression of MGB-2 appears to be enhanced in ovarian carcinoma when compared with normal ovarian tissue [16–18]. MGB1 and MGB2 have been suggested as marker proteins for detection of breast cancer metastasis in sentinel lymph nodes [19]. In contrast to the MGB isoforms, little is known about expression regulation of LIPB. Gene expression profiles in ovarian carcinoma suggested that LIPB may be among the most strongly up-regulated gene products when compared with normal ovarian tissue [12,20] suggesting a patho-physiological role of the protein. In fact, several reports suggested that overexpression of MGB1 [21], LIPB [9] and MGB2 [18] may be associated with better prognosis in different carcinomas. Although the molecular function of SCGBs in cancer remains unclear, their overexpression in malignant tissue suggested these proteins as possible cancer markers. Large-scale analysis of 3924 micro-array data sets [22] suggested that MGB1, MGB2 and LIPB are frequently co-expressed and simultaneous presence of two isoform may be required for their biological functions [5].

In the present study we first explored co-expression of MGB1, MGB2 and LIPB in normal human tissues and examined the impact of artificial SCGB overexpression on cell growth and proliferation. Correlation analysis indicated a significant bilateral correlation across all tissues suggesting coupled in vivo co-expression of the proteins. In clinical specimens of ovarian cancer expression of MGB1, MGB2 and LIPB was significantly up-regulated and we observed a positive correlation between MGB1 immunoreactivity and clinico-pathological tumor characteristics (FIGO stage, tumor grade, mitotic index) suggesting a pathophysiological role of SCGB in ovarian cancer.

Materials and methods

Chemicals

The chemicals used were from the following sources: Superscript II reverse transcriptase and RNaseOUT from Invitrogen (Karlsruhe, Germany); BD Advantage 2 Polymerase Mix from BD Biosciences (Pharmingen, Germany); M-MLV reverse transcriptase and agarose from Promega (Mannheim, Germany); dNTPs from Carl Roth GmbH (Karlsruhe, Germany); DNA molecular weight markers (100 bp, 1 kb) from New England Biolabs GmbH (Schwabach, Germany); QuantiTect SYBR Green PCR Kit from Qiagen (Hilden, Germany); polyclonal rabbit anti-human MGB-1 antibody from AgriSera (Vännäs, Sweden), peroxidase-labeled anti-rabbit IgG from Sigma (Deisenhofen, Germany). PCR primers were custom-synthesized by BIOTEZ (Berlin, Germany). Total RNA from different normal, human tissues was purchased from Ambion (Huntingdon, UK).

Cell lines and cell culture

MDAH-2774 ovarian carcinoma cell lines was obtained from American Type Culture Collection. COH-BR1 cells, an epithelial line derived from pleural effusion of a patient diagnosed with breast cancer [23] were obtained from Dr. James Doroshow, City of Hope Cancer Center (Duarte, CA, USA). MDAH-2774, COH-BR1 and HOSE cells [24], an immortalized human ovarian surface epithelium cell line, were cultivated in DMEM/F12 medium (Sigma, Deisenhofen, Germany) supplemented with 10% fetal bovine serum, 2 mM glutamine and antibiotics – 50 μg/ml penicillin, 50 μg/ml streptomycin (PAA Laboratories GmbH). All cell lines were maintained at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

Preparation of expression plasmids

The coding sequence (CDS) of the different SCGB (MGB1: SCGB2A2, NM_002411; MGB2: SCGB2A1, NM_002407; LIPB: SCGB1D2, NM_006551) was amplified by reverse transcriptase PCR using the primers given below. The resulting PCR fragment was digested with HindIII and EcoR1 and cloned into the linearized mammalian expression vectors pcDNA3.1(−) (Invitrogen, Darmstadt, Germany) or pCMV-3Tag-1A (Agilent Technologies, Waldbronn, Germany).

MGB1: forward 5′-CCCGAATTCATGAAGTTGCTGATGGTCCTCATGC-3′,
reverse 5′-CCCAAGCTTTTAAAATAAATCACAAAGACTGCTGTCAT-3′,
MGB2: forward 5′-CCCGAATTCATGAAGCTGCTGATGGTCCTCATG -3′,
reverse 5′-CCCAAGCTTTTAATTACTCTTCATATTACACCAAATG-3′
LIPB: forward 5′-CCCGAATTCATGAAGCTGTCGGTGTGTCTCCTG-3′,
reverse 5′-CCCAAGCTTTCACACACTACATTTCTTCAATATTTTC-3′;

The full length cDNA of the different SCGB was amplified by reverse transcriptase PCR using the primers given below. The resulting PCR fragment was also digested with HindIII and EcoR1 and subsequently cloned into the linearized mammalian expression vector pcDNA3.1(−). Empty vector was used for control transfections.

MGB1: forward 5′-GAATTCGACAGCGGCTTCCTTGATCCTTG-3′,
reverse 5′-AAGCTTTGCCATCAATTTATTAAAATAAACATGTAT-3′,
MGB2: forward 5′-GAATTCCCTCCACAGCAACTTCCTTGATCC-3′,
reverse 5′-AAGCTTAGTGATTTGCAGTTAGTTATTGAAATGG-3′
LIPB: forward 5′-GAATTCGGAGTCCAAATCACTCATTGTTTGTG-3′,
reverse 5′-AAGCTTGGAGAGCAAGTGATTTATTAAAGCAAG-3′;

Clinical human tissue specimens

To quantify expression of secretoglobin mRNA clinical specimens of malignant and normal ovarian tissue were removed during surgery at the Department of Gynecology and Obstetrics, University of Schleswig–Holstein, Campus Lübeck. The local ethics committee had approved this study. Clinical and paraclinical data on histology, tumor size and FIGO stage were extracted from the patient reports. After removal the tissue samples were immediately shock-frozen in liquid nitrogen and stored for further workup at −80 °C. For this study ten samples of women with ovarian cancer [FIGO I [1], FIGOII [1], FIGOIII [7], FIGOIV [3]] were collected. The age of the patients varied between 35 and 81 years (57 ± 13 years). In addition, two metastases from women with ovarian cancer (ages 62 and 86 years) were analyzed. For comparison, 10 samples of different normal ovaries were obtained from women in the age range of 27–59 years (47 ± 10 years).

Immunohistochemical analysis was performed retrospectively on tissue samples collected from patients who underwent surgery for diagnostic or therapeutic purpose at the Charité University Hospital Berlin, Germany between 1989 and 2005. This study has also been approved by the local ethics committee. Data on histology, tumor size, nodal status and FIGO stage were extracted from the pathological report at primary diagnosis. The tissue specimens comprised 67 primary invasive ovarian carcinomas, 9 borderline tumors, and 7 samples of normal ovarian tissue. In our cohort, the majority of tumors were serous carcinomas (63.7%) and the majority (60 out of 67) were high grade (G2-3). Among the non-serous carcinomas, the main histology was endometriod (11 cases). However, these subgroups are to small for a detailed subgroup analysis. For additional statistical evaluation and survival analysis, only patients with invasive ovarian carcinomas were included. Tumor grading was carried out according to the Silverberg