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Original article

Circulating annexin A2 as a biomarker in gastric cancer patients: Correlation with clinical variables

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ABSTRACT

Annexin A2 (ANXA2) plays an important role in the pathogenesis of multiple malignancies and its expression strongly also affects the outcomes of cancer patients. The objective of this study was to determine the clinical significance of the serum levels of ANXA2 in patients with gastric cancer. A total of 63 patients with a pathologically confirmed diagnosis of gastric cancer were enrolled into this study. Serum ANXA2 concentrations were determined by the solid-phase sandwich ELISA method. Age- and sex-matched 30 healthy controls were included in the analysis. The median age at diagnosis was 62 years, range 28 to 82 years. The baseline serum ANXA2 levels of the gastric cancer patients were a significantly higher than those in the control group ($P < 0.001$). The known clinical variables including age of patient, gender, site of lesion, histology, histological grade, stage of disease, and serum levels of LDH, carcinoembryonic antigen (CEA), and carbohydrate antigen (CA) 19.9 were not found to be correlated with serum ANXA2 concentrations ($P > 0.05$). However, the chemotherapy-unresponsive patients had higher serum ANXA2 levels compared with chemotherapy-responsive ones ($P = 0.04$). Conversely, serum ANXA2 concentration was found no prognostic role on survival ($P = 0.53$). In conclusion, serum levels of ANXA2 may have a good diagnostic and predictive marker for response to chemotherapy in patients with gastric cancer and have not associated with prognosis.

1. Introduction

Annexins are a structurally related family of calcium and phospholipid-binding proteins that are involved in the regulation of a range of molecular and cellular process and, therefore, play complex roles during carcinogenesis [1–12]. The regulated expression of annexins has been shown to constitute a valuable marker of cancer progression.

Among them, annexin A2 (ANXA2) is the most extensively studied, especially with respect to mammalian biology and human disease [1–12]. ANXA2 has been associated with malignant progression including cell-cell adhesion, cell proliferation, cell surface fibrinolysis, cell growth regulation, angiogenesis, apoptosis [1–11] and resistance to chemotherapy [6–8]. Increased expression of ANXA2 is frequently observed in a broad spectrum of cancer including gastric cancer [1–11]. The up-regulation of ANXA2 in cancer may have several clinical applications including as a diagnostic marker for early detection, a predictive factor for prognosis, or a marker for drug resistance.

Gastric cancer displays multifactorial etiology and its genetic and immunological background has not yet been fully elucidated. In vitro trials showed that cultured gastric cancer cell lines produce excessive levels of cytokines and growth factors with pleiotropic biological activities [9,10]. Among them, ANXA2 functions as an autocrine and paracrine factor that drives many cellular processes such as tumor growth, invasion, angiogenesis and metastasis. However, a clinical association between up-regulated ANXA2 and carcinogenesis in gastric cancer was observed in only a few trials [9,10]. Therefore, there is an unsatisfactory understanding of the molecular function of ANXA2 and the possible clinical significance of ANXA2 has remained unclear in patients with gastric cancer.

Although all of available findings were provided from preclinical trials, so far, no clinical study to investigate the clinical significance of ANXA2 isoform in serum or plasma in gastric cancer patients [9,10]. Unsatisfactory data were provided by these tissue-scaled trials. Thus, the significance of the serological levels of ANXA2 in patients with gastric cancer is not known yet. Therefore, we evaluated the soluble serum levels of ANXA2 in gastric cancer patients, and assessed associations with the prognosis, various

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known clinical variables, and response to chemotherapy, in order to examine whether these are potential new biomarkers, for use in the treatment of gastric cancer in this study.

2. Material and methods

2.1. Patients and therapy

This study included 63 consecutive patients admitted to Institute of Oncology, Istanbul University. All patients had histologically confirmed gastric cancer and had not received chemotherapy or chemoradiation in the last 6 months. The staging was determined according to the American Joint Committee on Cancer (AJCC) and International Union against Cancer (UICC) staging systems. The pretreatment evaluation included assessment of detailed clinical history and physical examination with a series of biochemistry tests including lactate dehydrogenase (LDH), complete blood cell counts including thrombocytes (PLTs), leukocytes (WBCs), hemoglobin (Hb) and serum tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19.9. Those with Eastern Cooperative Oncology Group (ECOG) performance status 2 or less and appropriate blood chemistry tests received chemotherapy on an outpatient basis that included different combinations of fluorouracil, folinic acid, capecitabine, docetaxel, cisplatin, epirubicine, with/without radiotherapy depending on the stage of disease. Follow-up programs included clinical, laboratory, and radiological assessments performed at 8-week intervals during chemotherapy or every 12 weeks for no anticancer treatment. Response to treatment was determined according to the revised RECIST criteria version 1.1.

For comparison of serum levels of ANXA2, 30 age- and sex-matched healthy controls were included in the analysis. Informed consent was obtained from all patients and the study was reviewed and approved by our local ethical committee.

2.2. Measurement of serum ANXA2 levels

Serum samples were obtained on first admission before any adjuvant and metastatic treatment was given or follow-up patients. Blood samples were obtained from patients with gastric cancer and healthy controls by venipuncture and clotted at room temperature. The sera were collected following centrifugation (10 min 4000 rpm) at room temperature and frozen immediately at −20 °C until analysis.

Serum ANXA2 (USCN Life Science Inc, PR China) levels were determined by the solid-phase sandwich ELISA method. The ANXA2 ELISA used a double-antibody sandwich enzyme-linked immunosorbent assay to determine the level of human annexin A2 in samples. Serum samples and standards were added to the wells, which were pre-coated with human ANXA2 monoclonal antibody. Following incubation, ANXA2 antibodies labeled with biotin and combined with Streptavidin-HRP were added to form immune complex and allowed to incubate for 30 min. Unbound material was washed away and then Chromogen solution was added for the conversion of the colorless solution to a blue solution, the intensity of which was proportional to the amount of ANXA2 in the sample. As the effect of the acidic stop solution, the color has become yellow. The colored reaction product was measured using an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer, Atlanta GA, USA). The results were expressed as μg/mL.

2.3. Statistical analysis

Continuous variables were categorized using median values as cut-off point. Assessment of relationships, comparisons between various clinical/laboratory parameters and serum levels of ANXA2 assay were accomplished using Mann-Whitney *U* test. Survival was calculated from the date of first admission to hospital to death resulting from any cause or to last contact with the patient or any family member. Kaplan-Meier method was used for estimation of survival of patient and differences in survivals were assessed by the log-rank statistics. A P-value < 0.05 was considered significant. Statistical analysis was carried out using SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA).

3. Results

A total of 63 patients with diagnosis of gastric cancer were enrolled in this study. The baseline histopathological characteristics and the demographic characteristics of the patients are listed in Table 1. The median age at diagnosis was 62 years, range 28 to 82 years.

Table 1
Patient characteristics.

Variables	*n* (%)
Number of patients	63 (100)
Age, years	
≥60	35 (56)
<60	28 (44)
Gender	
Male	25 (40)
Female	38 (60)
Site of tumor	
Cardia	21 (33)
Antrum	27 (43)
Undetermined	15 (24)
Histology	
Adenocarcinoma	42 (67)
Signet ring cell	21 (33)
Grade	
I–II	10 (16)
III	44 (70)
Undetermined	9 (14)
Tumor (T) stage	
1–3	14 (22)
4	22 (35)
Unknown	27 (43)
Number of lymph node involvement	
0–2	12 (19)
≥3	13 (21)
Unknown	38 (60)
Stage of disease	
Nonmetastatic	32 (51)
Metastatic	31 (49)
Liver metastasis[a]	
Yes	14 (45)
No	17 (55)
Curative surgery[b]	
Yes	17 (53)
No	9 (28)
Unknown	6 (19)
Serum hemoglobin level	
Low (<12 g/dL)	35 (56)
Normal (≥12 g/dL)	28 (44)
Serum WBC count	
Normal (<10,000)	52 (83)
Elevated (≥10,000)	11 (17)
Serum platelet count	
Normal (<350,000)	54 (86)