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Loss of ANXA7 Expression is Associated with Poor Patient Survival in Ovarian Cancer

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Abstract

Epithelial ovarian cancer is morphologically heterogeneous being classified as serous, endometrioid, clear cell, or mucinous. Molecular genetic analysis has suggested a role for tumor suppressor genes located at chromosome 10q in epithelial ovarian cancer pathogenesis. Our objective is to evaluate the expression of ANXA7, a novel tumor suppressor gene located on 10q21, in these epithelial ovarian cancer subtypes, and to investigate its correlation with patient survival. ANXA7 is ubiquitously expressed in small amounts in nearly every normal cell and the *Anxa7* (+/-) knockout mouse has a cancer prone phenotype. Altered ANXA7 protein levels are associated with prognostically challenging aggressive forms of prostate and breast cancer. So far, information is not available regarding the association of ANXA7 expression in ovarian cancer and patient survival. Therefore, we used human tumor tissue microarray (TMA) technology in order to evaluate the ANXA7 immunoreactivity as possible diagnostic and/or prognostic marker of ovarian cancer by immunoperoxidase assay using ANXA7 monoclonal antibody. Using a 129 case diagnostic human tumor tissue microarray, we report that the expression of *ANXA7* is significantly reduced and is associated with disease progression. Furthermore, using a separate 301 case retrospective prognostic tumor tissue microarray, we find that loss of ANXA7 expression is also significantly associated with poor over-all patient survival. We conclude that ANXA7 may be a new prognostic marker or a target for improving the treatment efficiency of patients with ovarian cancers.

Keywords: ANXA7; Ovarian cancer; Survival; Prognosis

Introduction

Ovarian cancer is the fourth leading cause of cancer-related death in women in the U.S. and the leading cause of gynecologic cancer death [1]. Specifically, epithelial ovarian cancer (EOC) is characterized by few early symptoms, presentation at an advanced stage, and poor survival. Indeed, 80% of patients are diagnosed with advanced staged disease. Although advances in treatment have led to an improved 5-yr survival rate approaching 45%, overall survival has not been enhanced [2,3]. Therefore, finding a reliable biomarker or a target that could be used to individualize both patient prognosis and therapy is essential for the prevention and cure of ovarian cancers.

The finding of a novel tumor suppressor gene (*ANXA7*) in a chromosomal region (10q21) with frequent mutations/deletions in human cancers raises important questions as to its use as a prognostic factor for the ovarian cancer. Biochemically, we found that *ANXA7* codes for a membrane-associated, Ca^{2+}-activated GTPase and is involved in exocytotic secretion [4-7]. In our work with the *Anxa7* knockout mouse we found that the nullizygous *Anxa7* (-/-) mutant is embryonically lethal and the *Anxa7* (+/-) animals developed profoundly increased frequency of tumors compared to the *Anxa7* (+/+) normal littlermate controls. Tumor frequency is in the range of 20-50% of animals, becoming more accentuated with advancing age [8]. Consistently, using a human prostate and breast tissue microarray, we found that alterations of ANXA7 protein expression is associated with metastases and hormone insensitive local recurrent cancers. In addition, we found that allelic loss of the *ANXA7* gene occurs in over one third of primary carcinoma of the prostate and breast [9,10].

Further studies from our laboratory indicated that altered expression of ANXA7 was associated with metastatic breast cancer with poor patient survival [11]. We have therefore hypothesized that ANXA7 signaling might also play a role in ovarian cancer. To test this hypothesis and to gain more insight into the potential role of ANXA7 expression in ovarian cancer, we have used diagnostic human ovarian tissue microarrays containing approximately 129 biopsy specimens to ask whether the levels of expression of ANXA7 might have predictive value for early diagnosis of these patients. In addition, a prognostic tissue microarray containing 301 tumor samples with clinical follow-up data for survival was surveyed for ANXA7 expression. Our data suggest that the loss of ANXA7 is associated with disease progression and is linked to poor clinical outcome in ovarian cancer patients, which has potential therapeutic implications.

Material and Methods

Patient characteristics

Samples from 129 ovarian carcinomas were included into a set of diagnostic progression ovarian tissue microarray. The patient group consisted of 34 patients with endometriod carcinoma, 12 patients with mucinous carcinoma, 44 patients with serous cystadenocarcinoma, 4 patients with Brenner tumor, 28 patients with ovarian cancer not otherwise specified and 8 normal ovarian tissues. The carcinomas of 301 different set of patients for whom follow up data (tumor specific survival and treatment information) could retrospectively be evaluated, were

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included in a prognostic tissue microarray. The patient group consisted of 119 patients with serous-papillary carcinoma, 40 patients with mucinous carcinoma, 67 patients with endometriod carcinoma and 75 patients with not otherwise specified. Formalin-fixed (buffered neutral aqueous 4% solution), paraffin-embedded archival ovarian carcinomas were available from the Institute of Pathology, Basel University Clinics, the Institute of Clinical Pathology in Basel, and the Triemli Hospital in Zurich. The Ethics Committee of the Basel University Clinics approved the use of these specimens and the data in this research. Raw survival data were either obtained from the cancer registry of Basel or collected from the patient's attending physicians. The mean follow-up time was 42 months [range 1–200]. The pathologic stage, tumor grading, and histological tumor type were obtained from the primary pathology reports. The slides from all tumors were reviewed by one pathologist (H.M.) to define the histological grade and the histological tumor type.

Tissue microarray construction

Tissue microarray (TMA) construction was as described previously [12,13]. Briefly, tissue cylinders with a diameter of 1.3 mm were punched from representative non-necrotic tumor areas of a "donor" tissue block using a homemade semiautomatic robotic precision instrument and brought into two different recipient paraffin blocks each containing 129 and 301 individual samples, respectively. The tissue microarray blocks were constructed in four replicas each containing samples from different regions of the donor tissues. One of these four samples was taken from the central part of the tumor and three from different peripherial areas. Four µm sections of the recipient blocks were then cut using an adhesive coated slide system supporting the cohesion of the 0.6mm array elements on glass. One section from each of the four replica arrays was used for immunohistochemical analysis.

Immunohistochemistry

Three conventional "large" sections from all tumors and three sections from each of the four different replica tumor tissue microarray blocks were used for immunostaining. The guidelines from the package insert were followed for ANXA7 antibody. Standard indirect immunoperoxidase procedures (ABC-Elite, Vector Laboratories) for monoclonal antibodies were used for detection of ANXA7 (1:1000, DAKO). Tumors with known positivity were used as positive controls. The primary antibody was omitted for negative controls. These arrays have previously been tested for lack of interaction with irrelevant monoclonal antibodies. Scoring of the immunohistochemical staining followed the guidelines in the package insert using an objective at 10x magnification.

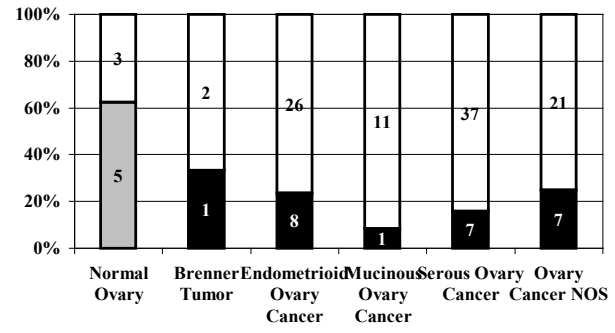

Figure 1: Disease progression relative to immunological ANXA7 expression (129 patients). The percent of ANXA7 positive samples are plotted against different stages of ovarian cancer starting from normal ovary (8 specimens); Brenner tumor (3 specimens); endometriod carcinoma (34 specimens); mucinous ovarian cancer (12 specimens); serous cystadenocarcinoma (44 specimens) and ovarian cancer not otherwise specified (28 specimens). The ANX7 positivity decreases with disease progression.

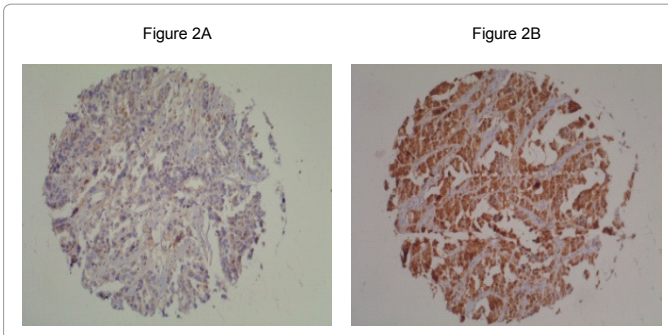

Figure 2: Immunohistochemistry on tumor tissue microarray. Analysis of ANXA7 protein in the representative clinical specimens of mucinous ovarian cancer (a) and normal ovary (b) (X100). Intense staining is observed in normal ovary compared to very weak staining in mucinous ovarian cancer.

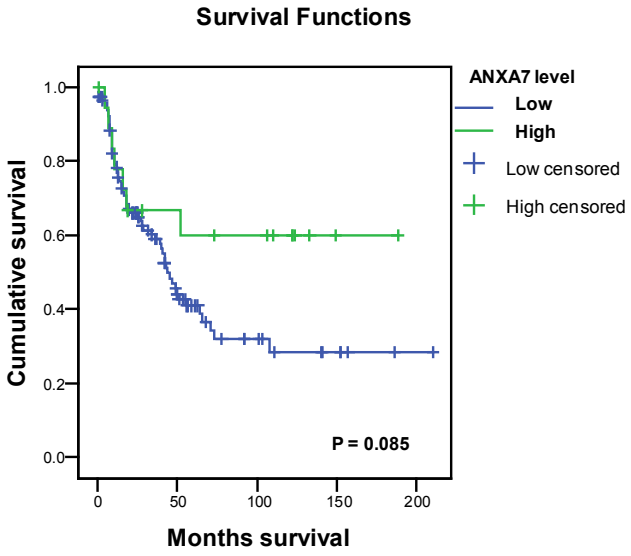

Figure 3: Kaplan-Meier survival analysis of overall survival related to the expression of ANXA7 (301 patients). The patients whose tumors had low ANXA7 expression had significantly shorter survival than patients whose tumors had weak or moderate ANXA7 expression. The 5-year survival is 30% for low group and 72% for high group.

Immunohistochemical Evaluation of ANXA7 Expression

The ANXA7 monoclonal antibody has been shown to recognize specifically ANXA7 and proved to be a useful reagent for immunohistochemical studies [11]. Human ovarian carcinomas with 129 specimens diagnosed as ovarian cancer and 301 specimens with follow-up data were examined for the expression of ANXA7 and their reactivity compared with normal human ovary tissues. Three types of ANXA7 expression were detected in ovarian cancer specimens. The first group showed weak ANXA7 expression (designated 1), the second group showed moderate ANXA7 expression (designated 2), and the third group showed strong ANXA7 expression (designated 3). The staining was nuclear and cytoplasmic as expected for a protein localized to the nucleus and cytoplasm. The specificity of tissue staining was determined by the demonstration of negative staining by omitting primary antibody and with an irrelevant antibody.

Statistical analysis

All data were analyzed by statistics software (SPSS 13.0 for Windows; SPSS, Inc). Survival time was measured in months from date of surgery until date of death or last follow-up. Survival analysis was performed using the Kaplan-Meier method and compared by the log-rank test. $P < 0.05$ was considered as significant.

Results

Loss of ANXA7 expression is associated with ovarian disease progression

We used 129 tumor specimens from all stages of human ovarian tumor progression as well as normal human ovary tissues to investigate whether there is a relationship between ANXA7 expression and disease progression in patients with ovarian cancer. We found that ANXA7 expression is almost lost in ovarian patients with mucinous carcinoma. For example, in mucinous carcinoma, the proportion of ANXA7 positives are only 4%. However, ANXA7 positives are 33%, 22%, 18%, and 23% respectively in Brenner tumor, endometriod carcinoma, serous cystadenocarcinoma, and ovarian cancer not otherwise specified (Figure 1), Figure 2a and 2b shows representative examples of mucinous ovarian cancer with loss of ANXA7 expression and strong ANXA7 expression in normal ovary tissue. These results suggest that loss of ANXA7 is associated with ovarian disease progression.

Clinical correlation of ANXA7 expression with ovarian cancer patient survival

We used a human prognostic ovarian cancer array containing 301 ovarian cancer patient specimens with the retrospective follow-up. ANXA7 expression was detected by immunohistochemistry and the presence of ANXA7 in each of these patients was correlated to survival parameters. Kaplan-Meier curves of cumulative survival in patients with low (1 and 2) versus high (3) ANXA7 expression shows a significant separation within 5 years of follow-up. Figure 3 illustrates the cumulative survival of 2 groups from the diagnosis of ovarian cancer. The duration of survival was significantly shorter in patients with weak ANXA7 expression (groups 1 and 2) compared with patients with strong ANXA7 expression (group 3) [30% versus 72% in 5 years]. When considered in a cox- regression analysis, the patient group with strong ANXA7 have greater probability of survival, and low staining of ANXA7 is associated with lower probability of survival which is in accordance with its tumor suppressor role in many cancers ($p = 0.085$, Figure 3). These results indicate that ANXA7 levels have considerable potential to be of practical use in routine assessment of ovarian cancer patients.

Discussion

There are more than 190,000 new cases of epithelial ovarian cancer (EOC) each year worldwide and this malignancy represents the leading cause of death from gynaecological cancers [14,15]. It is a complex disease, largely asymptomatic, and over 70% of patients present with advanced stage disease at initial diagnosis. EOC is a heterogeneous disease, and each EOC subtype exhibits distinct clinical characteristics, morphology, biological behaviour, and chemotherapeutic response [16]. Indeed, molecular studies support the notion that the different histological types likely represent distinct disease states [17,18]. Nonetheless, it is current practice to treat all subtypes with the same platinum/taxane chemotherapy, although some do not respond well to this regimen [19–21]. As a consequence subtype-specific therapeutic trials have been recommended for clear cell and mucinous EOCs in particular [22]. The molecular and morphological differences of these EOC subtypes are also reflected in the efficiency for detection using the only approved serum biomarker for ovarian cancer, CA125; while 60–80% of patients with endometrioid and serous EOC show high levels of serum CA125, only 20–30% of patients with clear cell and mucinous are positive for this serum biomarker [23]. Thus, the discovery of novel molecular targets for its diagnosis and treatment has the potential to improve the clinical strategy and outcome of patients with this disease.

Very little is known about the role of ANXA7 in the development of ovarian carcinomas. Therefore, our study focused on the relationship between ANXA7 expression and human ovarian cancer. This study's findings include the downregulation of the expression of ANXA7 from the normal ovarian tissue to the advanced tumor tissue. This protein has previously been identified as a protein involved in secretion and exocytosis belonging to the annexins, a multigene family of phospholipids binding proteins [6]. Several other biological properties have since been described for the ANXA7 protein, including Ca^{2+}-channel activity [6]. Consistent with its location on the tumor suppressor gene site 10q21, ANXA7 tumor suppressor function was derived from the cancer-prone phenotype in *Anxa7*(+/-) mice and ANXA7 prognostic role in human cancers including tumors from the hormone-responsive tissues such as prostate and breast [8,9,11]. Most importantly, the *Anxa7* deficiency was associated with the inhibition of PTEN (a lipid phosphatase which downregulates the phosphatidylinositol 3-kinase, PI3K and a gene implicated in ovarian cancer) which is also located on chromosome 10q23 as *ANXA7* gene [24]. Given the well-established roles of the PTEN-AKT pathway in the development of ovarian carcinoma, it will be important to determine whether the ANXA7 pathway cross talks with these signaling pathways and to assess how inactivation of ANXA7 contributes to tumor initiation and progression in ovarian carcinoma. In the present work, we show that ANXA7 expression is reduced in serous, endometriod and mucinous EOC tissue samples, but not in normal ovaries (Figure 1). No studies have closely investigated the expression of ANXA7 in individual EOC subtypes. Our results in this study indicate that low ANXA7 expression is associated with ovarian tumor progression. In addition, our studies with a 200 months follow-up demonstrate that low expression of ANXA7 is associated with poor prognosis (Figure 3).

In summary, we demonstrate that ANXA7 is differentially expressed in morphologically distinct EOC subtypes and its level negatively correlated with the malignancy of ovarian cancer. Thus ANXA7 playing a tumor suppressor role in ovarian cancer which are consistent with previous reports, may be a potential candidate for therapeutic investigation in ovarian cancer. The present study shows the possibility of using ANXA7 as both a clinically relevant indicator of disease progression and a prognostic biomarker for survival in the patients with ovarian cancer. We conclude that if these data can be validated in a larger population of patients and in prospective studies with extensive follow-up, low ANXA7 expression could become an important bio-marker for identifying ovarian cancer patients at high risk, and is worthy of further exploration as a prognostic factor in survival.

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