

Antigenic Markers in Ovarian Carcinomas

by

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Abstract

Ovarian cancer is currently the fourth leading cause of death in women with cancer in the United States.² Each year, approximately 23,000 women are diagnosed with ovarian cancer in the United States alone and almost half will die from the disease.¹ Of the diagnosed cases, 75% will have advanced stage ovarian cancer¹, which is defined as having spread beyond the pelvic cavity.² The survival rate of ovarian cancer decreases depending on the staging of the disease.² Five year survival rates have been reported at 95% for stage I,¹ 25.1% for stage III and 11.1% for stage IV.² Of the 23,000 cases diagnosed each year, only 15% are diagnosed in stage I of the disease.

Early detection of ovarian carcinomas is paramount in diagnosing this disease in its most treatable stages. Fortunately, approximately 90% of all ovarian cancers arise from the epithelial lining of the ovary resulting in very specific targets for screening and therapeutic agents.² The purpose of this article is to discuss the possibilities of using antigenic markers of these epithelial cells to screen for early stages of ovarian cancer to diagnose and treat ovarian cancers with greater efficacy.

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Introduction

The cell membrane consists of many molecular components. The membrane itself is a lipid bilayer containing a multitude of cell surface receptors.⁹ Each receptor is specific to a binding ligand such as a hormone, enzyme or protein.⁹ These cell surface receptors are proteins comprised of amino acid sequences that specifically

recognize a substrate with a complimentary amino acid sequence.⁹ Internal receptors may also be present which bind to steroid hormones that easily traverse the lipid bilayer of the cell.⁹ It is at this molecular level that the future of ovarian cancer screening looks most promising.

Mutations in epithelial cells of the ovaries can occur in many different ways. Some

examples of this include altered gene expression resulting in abnormal protein production, gene amplification resulting in proliferation of protein product, and gene translocation resulting in mutations that have the capability to produce proteins that are not indigenous to the epithelial cell.³ In order for these changes to occur, cell signaling molecules, and their subsequent products, must be present to elicit changes, ultimately leading to mitogenesis in the cell. The goal of utilizing antigenic markers is to identify and isolate these specific signal proteins, their receptors and their unique products. This could produce a specific diagnostic test for ovarian carcinomas as well as generate antibodies that would identify errant or excessive cellular products upon production. This would ultimately target the destruction of the malignant cells while preventing the formation of malignant cells and simultaneously protecting healthy cells.

Proliferation is made possible in many tumors due to the suppression of the body's immune response to the mutations that have occurred at the DNA level or the inability of the immune system to detect these mutations.³ Cell specific targeted treatment of ovarian cancer has been difficult in part due to these mechanisms. The most popular treatment currently used for ovarian cancer is chemotherapy. While chemotherapy has been shown to be effective in 90 percent of stage I ovarian cancers, its mechanism of action is less than desirable in later stages of the disease due to the adverse effects felt by the greater proportion of normal cells involved with increased tumor size.⁴ During this article, theoretical possibilities for the future treatment of ovarian carcinomas will be discussed in an effort to encourage exploration regarding the development of potential biomarkers that could utilize the body's natural defense mechanisms.

Current Screening Test for Ovarian Cancers

Early diagnosis and treatment of ovarian cancer is dependent on the development of the disease. In most treatable cancers, a pre-malignant phase is present, followed by a long clinical phase (symptomatic phase) and a stage dependent outcome.² There has yet to be an identifiable pre-malignant phase for ovarian cancer and the pre-clinical phase is extremely short and asymptomatic; therefore, the majority of cases of ovarian cancer are discovered in the latter stages.² Antigenic markers could greatly increase the potential of detection of the disease in the asymptomatic, pre-malignant phase leading to much more effective treatment.

Cancer Antigen 125

Currently the most prominent antigen used is antigen 125 (CA-125). CA-125 is a glycoprotein that is expressed in many benign and malignant cells involving the ovaries, fallopian tubes and peritoneum.⁵ CA-125 is also routinely associated with endometriosis, pancreatitis, pelvic inflammatory disease, pregnancy and normal menstruation in premenopausal women.⁶ Because of this, CA-125 is a very sensitive, but non-specific testing agent in this patient category.

CA-125 has demonstrated a positive predictive value of 98% for ovarian cancer in postmenopausal women with palpable asymptomatic pelvic masses; however, ovarian cancer is usually not detected before the patient has become symptomatic in the latter stages of the disease in 50% of diagnoses.⁶ CA-125 has shown its greatest significance in patients in remission of ovarian carcinomas. During remission of ovarian carcinoma, elevated CA-125 levels is almost always correlated with recurrence.⁶

Alternative Screening Methods

Other screening tools that are currently used are trans-vaginal ultrasound and routine pelvic exams. While these methods are currently accepted, they have a very high false positive rate and are not very sensitive or specific for ovarian cancer.² New methods must be identified that have a high sensitivity and specificity to ovarian cancer and are present in early development of the disease. To be a trusted predictor of ovarian carcinoma, it is recommended that a potential marker have a sensitivity greater than 80 percent, a specificity of 95 to 99 percent and a positive predictive value of greater than 90 percent.⁸

Current Research in Antigenic Markers for Ovarian Cancers

Many antigens are currently being explored as a mechanism for early detection of ovarian cancer. Some of the most promising of these agents and their mechanisms of action will be discussed including FBP-GP38, HER-2/neu, MAGE 1, MUC 1, and P53 Tumor Suppressor Protein. Developments in proteomics could potentially lead to a greater efficacy in antigen identification in ovarian cancer and shall be discussed as well.

FBP-GP38

One of the most prominent antigens undergoing research is that of Folate Binding Protein GP38. Folate is a cofactor used by cells to generate energy and the necessary building blocks for cell proliferation.⁷ FBP-GP38 is a receptor protein specific to non-mucinous ovarian carcinoma cells that facilitates the transport of folate and its derivatives into the mitochondrial matrix of the cell for processing.⁷ A recent study at the National Cancer Institute in Naples, Italy studied the presence of FBP-GP38 in carcinogenic ovarian cells. The study compared 37

specimens of carcinogenic ovarian cells with 13 healthy “normal” ovarian cells. These cells were treated with a radioactive labeled monoclonal antibody (125I-Mov18) raised against FBP-GP38. Antibody binding to FBP-GP38 in the cells was then measured. Carcinogenic cells showed an 80-90% increase in the expression of FBP-GP38 in ovarian carcinoma cells when compared to normal ovarian cells.⁷ This suggests that carcinoma cells increased the load of FBP-GP38 in support of excess cell growth and tumor proliferation.

HER-2/neu

HER-2/neu is another promising antigenic marker currently under research. HER-2/neu is a member of the epidermal growth factor receptor family and normally present in squamous epithelial cells, which plays a fundamental role in cell proliferation in fetal development.³ In normal mature cells, HER-2/neu is present as a single copy; however, it has been shown to proliferate in the recurrence of carcinogenic cells not limited to the ovaries.³ This antigen has been especially useful in detecting relapse of ovarian cancers after chemotherapy.³ Subjects studied after chemotherapy for ovarian carcinomas were shown to be HER-2/neu negative, but upon relapse of ovarian cancer, histological review showed that subjects had become HER-2/neu positive in all 20 stage III and IV cell lines studied.¹⁰ This suggests that this antigen may become more abundant as ovarian carcinomas advance.³ Further study must be done to determine the specificity of HER-2/neu to ovarian carcinoma.

MAGE-1 Gene

The MAGE-1 gene has also been correlated with ovarian carcinomas. This gene is thought to be responsible for production of proteins responsible for cell cycle regulation, although the exact function is yet

to be determined.³ The MAGE-1 proteins are specific to ovarian carcinomas of serous origin and are recognized by cytotoxic T lymphocytes in the body's immune system; therefore, they are a prime target for immunologic treatment.³ The MAGE-1 gene expression has also been correlated with early stage development of serous tumors making it a promising candidate for early detection of ovarian carcinomas.³ Less specificity has been shown for carcinomas of epithelial origin and the mechanism of action of the MAGE-1 proteins requires further investigation before more specific conclusions can be made.

MUC-1 Gene

The MUC-1 gene is a high-molecular-weight cell surface glycoprotein that is thought to be associated with cell signal transduction.³ It has shown a high specificity to epithelial carcinomas in ovarian, colorectal and breast tissues. It is thought to be an important modulator in cell adhesion and metastasis. Cytotoxic T lymphocytes (CTL's) and CD4 positive T helper cells have been identified as being specific to the MUC-1 glycoprotein, thus there is reason to believe that targeted immune response treatment could be promising.³ More research is necessary to determine the exact mechanism and function of these glycoproteins; however, this molecule does seem to provide a real possibility for immunotherapy in ovarian cancer.

P53 Tumor Suppressor Protein

Many malignant cells produce an anti-immune response or immunosuppression. Ovarian carcinomas are known to be one of the most prominent of these malignancies.³ As part of the body's immune response, the p53 tumor suppressor protein can be used to destroy malignant cells by promoting cellular apoptosis. Ovarian carcinomas are

notorious for their ability to mask themselves from the p53 tumor suppressor protein.³ The carcinogenic cells induce an anti-p53 suppressor protein that recognizes and destroys or mutates the natural p53 suppressor protein rendering it ineffective.³ Levels of anti-p53 suppressor can be measured in ovarian carcinomas.³ Most importantly, elevated levels of anti-p53 suppressor antibodies have been found to correlate with tumor stage, grade and survival rate of patient.³ Naturally expressed peptide epitopes have been identified and are being studied as a possible strategy to bypass the anti-p53 suppressor proteins and target malignant cells.³

Future Treatment of Ovarian Carcinomas

One avenue of study for investigating ovarian carcinomas is largely based on immunologic response. Such an avenue utilizes the body's own defense mechanisms to target only cells specific to the carcinoma. Such mechanisms could potentially be exploited to develop potential vaccines that would provide the body with new antibodies directed towards specific mitogenic antigens. Several theoretical treatment mechanisms that could potentially be explored in the future will be discussed.

The research previously discussed regarding FBP-GP38 as an antigenic marker has a high correlation to the current treatment of ovarian cancer using methotrexate. Methotrexate inhibits the conversion of dihydrofolate to tetrahydrofolate and its subsequent conversion to many precursors to cell proliferation. A major detriment of methotrexate is that it affects all cells' ability to regenerate reduced folates, thus retarding growth and inducing cell death in otherwise healthy cells. FBP-GP38 would allow for early detection of ovarian carcinoma and the potential for development of an antibody to FBP-GP38 that could

possibly block the specific FBP-GP38 protein, disabling its ability to deliver folate to the carcinogenic cell. This therapy would favor folate deprivation in carcinogenic cells, thus retarding their ability to proliferate.

Many of the above mentioned antigens could have the possibility to be used as a potential means for vaccination. Once the antigen and its mechanism of action are identified, it could be introduced into the body much like a vaccination in order for the host to develop antibodies to the marker before malignant cells ever form. Specificity would have to be absolute prior to attempted vaccination. Failure to do so would illicit an immune response to healthy cells containing the known antigen that would be much like that of methotrexate with the exception that in this instance a therapeutic agent(methotrexate) could not be withdrawn and the immune response would most likely prove fatal.

These potential therapeutic methods are strictly theoretical in nature; however, they are designed to provoke discussion regarding the possibilities of future treatments of ovarian carcinomas. The process of achieving such therapeutic agents is a long and difficult one. Most ovarian carcinomas are notorious for defending themselves against the body's immune system. Methods must be found to counter this defense mechanism in order for treatment to be effective.

Advances in Research

Research in the field of ovarian carcinoma is continually evolving. The use of techniques such as proteomics may provide an avenue by which significant progress could be made. Proteomics is a recent development in which a blood or serum sample is added to a protein chip. The protein chip has the ability to be pre-loaded with a variety of substances to illicit multiple

chromatographic properties of serum proteins. The specimens are allowed to incubate and unbound products are then washed away. The protein chip is then analyzed in a computer using mass spectrometry, and multiple chromatographic properties can be reviewed for each blood or serum sample. This method allows for a review of the chromatographic properties of hundreds of serum samples within days, drastically reducing the amount of time needed to determine whether a protein has predictive properties of a potential biomarker of ovarian carcinoma. Since such a marker is extraordinarily rare, proteomics could provide a way to enhance the speed at which millions of proteins can be screened and analyzed.

Conclusion

Of the many known carcinomas, ovarian carcinomas are one of the leading causes of death in women. The exploration of antigenic markers as a biomarker has shown great promise in providing a more specific diagnosis of early stage ovarian cancers. Understanding these antigens and their mechanisms will also provide researchers with a clearer picture of how tumors are formed and proliferated. This understanding will reveal many new treatment possibilities and potentially even aid in the search for a vaccine to ovarian and other cancers. The use of antigenic markers will undoubtedly enable physicians to isolate ovarian carcinomas in their earliest and most treatable stage greatly impacting the prognosis for patients diagnosed with the disease.

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