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How Can We Accelerate the Early Identification of Biomarker Glycoprotein NMB for The Early Detection of Breast Cancer

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Abstract

The current methods for screening for any type of breast cancer involve the use of radiation levels or some form of waves. Widely used methods are CT scans, mammograms, MRIs, and ultrasounds, majority requiring the use of harmful radiation. A mammogram uses low energy x-rays to examine the human breast, a CT scan involves the usage of ionizing radiation, and an ultrasound transmits sound waves into the body. By identifying specific biomarkers like Glycoprotein NMB which are overexpressed in conditions like TNBC, we can find a more efficient and less harmful method of diagnosis. Usage and identification of these biomarkers to diagnose TNBC would also help with quicker and earlier detection compared to existing screening measures. The results of clinical trials experimenting with Glycoprotein NMB and various cancers point in a positive direction.

Keywords: Glycoprotein NMB, Triple Negative Breast Cancer, Radiation

1. Introduction

Triple negative breast cancer (TNBC) is regarded as one of the most aggressive and rarest cancers. The lack of hormone receptors, including estrogen and progesterone receptors, on breast cells renders it particularly difficult to treat with hormone or HER2 therapy. TNBC affects approximately 13 in 100,000 women each year in the USA and has an overall mortality rate of 77% (Chen. L, et. al, 2016). TNBC accounts for approximately 15% of all breast cancers diagnosed all over the world (Sheng. J., 2023). Given its rapid course of progression, there also tends to be a short window for timely intervention. Currently screening and detection methods rely heavily on mammography's, ultrasounds, and MRIs. These forms of testing sometimes result in false negatives or fail to detect a cancer that's present, making them a suboptimal form of testing. The sensitivity of the current forms of testing ranges from 80%-95% and the specificity ranges from 90%-98%. A new protein of interest in recent studies is Glycoprotein NMB (GPNMB) due to its expression in specific types of cancers. GPNMB is a protein in humans encoded by the GPNMB gene (McCarthy, A. M., et al., 2021). This glycoprotein has been found to be overexpressed in many cancers, including triple negative breast cancer. Given the aforementioned limitations in current screening and detections measures, I believe, it could be beneficial to investigate targeting GPNMB as a potential biomarker for TNBC. Blood tests can be used to identify the presence of the biomarker GPNMB in the patient's blood. GPNMB is a form of testing for various types of cancer, not just TNBC. Currently there has been limited research performed that analyzes the relationship between GPNMB and TNBC in patients. My aim is to identify whether identification of GPNMB is a potential method for detecting early-stage TNBC. Through blood tests testing for GPNMB we can find a more accurate and efficient way of detecting TNBC.

Triple negative breast cancer is a subtype of breast cancer known for its rarity and difficulty to treat. The reason the term triple negative breast cancer was coined for this type of cancer was due to the lack of estrogenic or progesterone receptors (ER or PR) as well as limited production of the protein known as HER2. It does not have crucial receptors which are commonly found in other types of breast cancer (Scott et al., 2019). This makes it particularly

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difficult to identify and treat. The route of metastasis in TNBC correlates with the survival of TNBC patients which have brain metastases (the poorest survival indicator), followed by liver metastases, pleura, bone, and lastly lung (Prakash O., et al., 2020). The cause of TNBC is yet to be identified, but researchers seem to correlate the BRCA 1 genetic mutation as an indicator. As a tumour suppressor gene BRCA1 plays an important role in DNA repair mechanisms and preventing aberrant growth, but an oncogenic hit could reverse the course and cause cells to be more vulnerable to cancer (Stewart, 2019). Along with this specific reproductive and lifestyle-related factors link to an increased risk of TNBC (Cho, B., et al., 2021).

A glycoprotein is a protein consisting of oligosaccharide chains which are covalently attached to an amino acid side chain. Glycans can attach to either lipids or amino acids, through a bond or process known as glycosylation (Raas, 2023). Glycosylation is a process where secreted extracellular proteins are glycosylated. Glycoproteins play an important role in cell-cell interactions as well as in integral membrane proteins. Another essential role played by glycoproteins is their role in primary and secondary immune responses allowing for white blood cell migration around the body (Dent R., et al., 2007). The synthesis of a glycoprotein occurs between two organelles: the endoplasmic reticulum and the Golgi apparatus. Glycoprotein NMB is a transmembrane glycoprotein which is encoded in humans by the GPNMB gene. GPNMB is characterized by two transcript variants which encode 560 and 572 amino acids. GPNMB is usually expressed in different cell types like melanocytes, osteoclasts, osteoblasts, dendritic cells, along with being overexpressed in different types of cancer. Previously GPNMB was a gene which did not express or was expressed poorly in highly metastatic cell lines and in metastatic human melanoma cell lines. But recent studies have identified high GPNMB expressions in aggressive melanoma, glioma, as well as breast cancer specimens.

Triple negative breast cancer is a rare cancer which affects approximately 13 in 100,000 women each year (Sussman D., et al., 2014). Around 91% of all women with TNBC are still alive after 5 years of diagnosis. Although, if the cancer spreads to the lymph nodes near the breast the 5 year survival rate reduces to 65%. Lastly, if the cancer spreads to distant areas in the body, the 5 year survival rate further reduces to (Birdia A., et al., 2019). TNBC being more aggressive than other cancers, causes it to be harder to treat and also increases the chances of recurrence. TNBC is usually treated with surgery, radiation, chemotherapy, or a combination of all three. Chemotherapy, a medicine which kills cancer, is usually the first treatment therapy used for TNBC. This is often followed by radiation therapy.

The current method to identify TNBC is through ultrasounds, MRIs, and mammograms. These methods are used to first detect breast cancer in a patient. Once an ultrasound, MRI, or mammogram indicates that the patient has breast cancer, a biopsy is conducted. The biopsy is used to assess cells of a patient and further check for estrogen, progesterone, and HER2 receptors to determine the person's breast cancer subtype. This process is lengthy, and also includes the patient being exposed to a lot of radiation, which is unhealthy. TNBC tends to double in size every 180 days, or every 6 months, so it is crucial for its identification to be quick. By using GPNMB as an indicator for TNBC, the diagnosis of this subtype of cancer can be much faster, and avoid using unhealthy radiation.

As mentioned previously, GPNMB is highly expressed in cancers or tumour's like melanoma, glioma, breast cancer, and cholangiocarcinoma. Currently researchers are exploring the potential of targeting GPNMB in osteosarcoma. Using human osteosarcoma samples, researchers are identifying the expression of GPNMB (Weterman, 2020). A similar research is being conducted for glioblastoma tissues and the mediation of glioma progression (Zhang, 2017). In both of these studies there has been high expression of GPNMB and clinical trials are being conducted to assess the efficiency of targeting GPNMB in patients.

My aim is to identify a method of detecting triple negative breast cancer which does not include radiation or biopsies. TNBC is one of the most difficult breast cancers to detect, so finding a way to detect it fast and accurately is crucial. Using GPNMB is a relatively new method as clinical trials and research regarding it are still being pursued. I would like to research more regarding the identification of TNBC through GPNMB, a new and upcoming method.

Based on preliminary studies showing that GPNMB has been effective in diagnosing other conditions like Alzheimer's disease, Parkinson's disease , Non-Alcoholic Fatty Liver Disease, etc (Budge, et al., 2017). Various clinical studies and trials have been conducted, experimenting with GPNMB and neurological diseases, but very few experimenting with cancer, especially TNBC.

I hypothesize that using glycoprotein NMB to target or identify patients with triple negative breast cancer will be a possible, effective, and efficient method of diagnosis.

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2. Methods

Based on my interest in TNBC I analysed recent studies that have conducted experiments on GPNMB. I looked through various trials that had been conducted so far about this topic. Since the use of GPNMB is still upcoming and fairly new, research on it is limited. Additionally, research with the use of GPNMB as a biomarker of TNBC is further specialized.

There are multiple ways experiments with GPNMB are carried out. Each experiment uses a tumor sample and tests it to find expression of GPNMB. Most studies or clinical trials use immunohistochemistry which is a laboratory method that uses antibodies to identify whether certain antigens are present (Horwitz S, et al., 2019).

Some of the criteria I used while picking the clinical trials to use for my research were ensuring that the data was focused specifically on TNBC. I also tried to use sources which were acclaimed or reliable like Nature and PubMed by the National Library of Medicine. I also focused on ensuring the experimental design used either immunohistochemistry or gene expression data. This is so that it would give me results which were specific to what I was looking for.

The data for the first clinical trial was collected through testing with immunochemistry, in-silico survival analysis, cell culture reagents and transfection, invasion assay as well as western blot analysis. 759 patients who tested positive for TNBC and underwent primary surgery were enrolled into the clinical trial. Various clinico- pathological characteristics like TNM staging, histological grade, as well as tumor type were all matched through the WHO classification system. The clinical parameters collected by oncologists and past medical records. The representative areas of each tumor were carefully selected and constructed into tissue microarrays (TMA). The histology score through a semi-quantitative assessment of the percentage of positive-stained carcinoma cells as well as the staining intensity was assessed. The range of H scores, which ranged from 0 to 300 were evaluated by pathologists and categorized into high and low subgroups.

The immunostainings were performed on 4 micrometer paraffin-embedded tissue sections. The slides were then placed in a 10mM citrate buffer for 20 minutes in a pressurized heating chamber. The tissues were incubated with the antibodies against GPNMB like E-cadherin and vimentin. After the slides were taken out and washed with phosphate-buffer saline, bound antibodies were detected. Tissues were identified as ER or PR positive breast cancer based on the amount of nuclei stained. If the percentage was higher than 10 it was classified as ER or PR positive breast cancer, and as HER-2 positive breast cancer if 3+HER2 expression was identified. After identifying this the Kaplan-Meier analysis was done and western blot analysis was done to quantify protein levels. Invasion assay was conducted to identify the number of invaded cells, and research was analyzed and concluded through statistical analysis.

The second clinical trial was conducted with a lower number of patients. The significance of GPNMB expression was addressed by analyzing GPNMB levels in various gene expression data sets. The clinical trial involved using two independent tissue arrays from human breast tumors. To identify the GPNMB expression IHC stainings were used. The IHC stainings were analyzed to identify the intensity of GPNMB expression. The sample was analyzed and the percentage of positively stained carcinoma cells were analyzed. The GPNMB expressing breast cancer cells that were identified were further analyzed to understand the significance of using GPNMB as a prognostic biomarker.

Both the clinical trials used women of ages between 20-70. The women were from different ethnicities and different backgrounds, but age was a variable that remained constant. The patients enrolled in both trials consisted of women ranging from different severities of TNBC. In the first trial majority of the women had undergone primary surgery, whereas in the second trial many women hadn’t.

3. Results

There have been primarily positive results with identifying GPNMB as a marker for TNBC. Various clinical trials have been done which test the expression of GPNMB in different cancers.

In one clinical trial, 759 specimens were collected, and in immunohistochemistry it was found that GPNMB was expressed in different subtypes of cancer but was significantly higher in TNBC. The data was collected from 759

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patients with primary breast cancer with a median follow-up of 74 months. Among the patients with TNBC, the median age was 55 years. The Kaplan-Meier analysis revealed that the overexpression of GPNMB in TNBC was associated with an advanced or worse prognosis, in specific distant metastasis, especially visceral metastasis which includes the lungs, liver, and brain. The trial results showed that in TNBC the mean and medium of GPNMB expression were 102.9 and 87.5 with the interquartile ranges of 45.0 to 165.0 (Huang, et al, 2021). The average H-scores of GPNMB in TNBC subtypes were also significantly higher compared to those of non-TNBC subtypes. These results demonstrated that GPNMB might be overexpressed in TNBC preferentially. In silico analysis there was high expression of the mRNA of GPNMB and it can be correlated with distant metastasis. Additionally, GPNMB was overexpressed in TNBC in the silico analysis. The protein levels of Twist and MMP2 were also upregulated by GPNMB overexpression in TNBC cells. These graphs and diagrams from the study in the Figure 1 indicate that GPNMB expression does correlate with breast cancer. The graph on the bottom right indicates that low GPNMB expression results in a long survival time compared to high GPNMB expression. The pictures help us identify the GPNMB expression intensity in different samples.

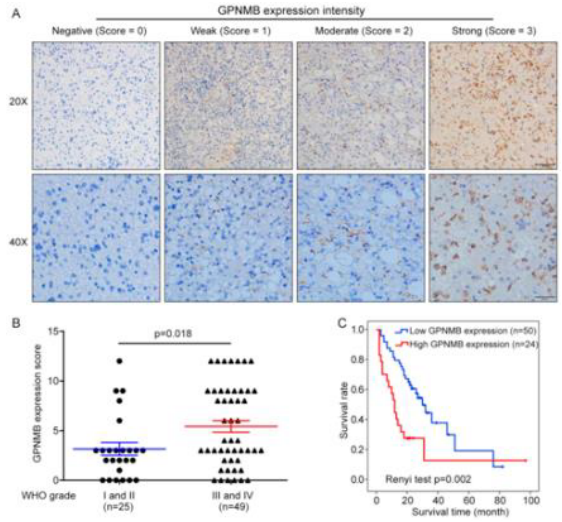

Figure 1. GPNMB expression correlated to triple negative breast cancer

In another study the expression of GPNMB was studied specifically in TNBC. Through immunohistochemical analysis, findings show that GPNMB is commonly expressed in breast tumors. These results were found in two studies specifically. In the first study, it was found that GPNMB was detected in approximately 71% of breast tumors. Whereas, in the second study GPNMB was detected in 64% of breast tumors, and additionally 10% of tumors expressed this gene in the tumor epithelium (Burris, et al., 2009). Through this study it was found that GPNMB expression in the tumor epithelium could be seen as an independent prognostic indicator of breast cancer recurrence. Epithelial GPNMB expression was highest in triple negative breast cancer and found to be a prognostic marker for this type of breast cancer subtype. In addition to this, GPNMB expression in breast cancer cells is also capable of promoting cell migration, invasion, as well as metastasis in both in-vitro and in-vivo.

Both clinical trials resulted in positive results proving that GPNMB is a potential and effective indicator of TNBC. From the first clinical trial the results indicated that GPNMB might be overexpressed in TNBC preferentially and that it may be an indication of worse prognosis. Even so, it does indicate the presence of TNBC. The second clinical trial the results also pointed in a positive direction. GPNMB was found in 71% of the breast tumors tested, which is a relatively high percentage. Along with this there was a finding of another independent prognostic indicator: GPNMB expression in the tumor epithelium.

4. Discussion

Overall I believe that GPNMB is a promising indicator for TNBC. A lot more research is yet to be conducted, but according to clinical trials which are currently being undergone, results seem to be pointing in a positive direction.

More research and clinical trials need to be conducted to ensure that GPNMB can be used to identify TNBC. Many factors like distant metastasis and recurrence of TNBC need to be looked into. Along with this more detailed trials need to be conducted which specifically look into GPNMB's effects at the protein and gene levels. Antibodies against GPNMB like glembatumumab vedotin need to also be considered.

Though results seem promising, there are many limitations regarding identifying GPNMB as a breast tumor. In both the clinical trials the follow up procedures with the patients were of different lengths and did not take into

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consideration each one's unique course of medication and treatment.

New information which has been found through the clinical trials above also could be potential indicators. GPNMB expression specifically in tumor epitheliums has not been looked at, but it could be another solution to the current problem we are facing.

5. Conclusion

TNBC is an extremely difficult form of breast cancer to detect, and overall using GPNMB to accelerate the identification seems to prove useful. According to the clinical trials currently being conducted, the results look promising and effective. Though much more research still needs to be conducted, usage of biomarker GPNMB has the potential to accelerate the identification of TNBC.

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