



REVIEW ARTICLE

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Risk factors contributing to methylation shifts in BRCA1 and associated genes in African Americans with triple negative breast cancer

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Abstract

Triple negative breast cancer (TNBC) remains one of the most lethal breast cancers while only accounting for 10-20% of all breast cancers. Mortality rates are a staggering 50%, with high likelihood of metastasis to other tissues if left untreated. This is due to this cancer's heterogeneous nature and differentiation from other breast cancers, negatively staining for common mutations in estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2). The patient population is shifted more towards African Americans (AA) with increased incidence and mortality rates. To date the nature of this statistic remains multifaceted with no clear therapeutic regiment. Through the identification of methylation as viable cause for TNBC, the exploration of environmental, genetic, and socioeconomic risk factors serve as an important aspect of overall mortality rate. This review seeks to investigate the relationship between AA with TNBC and potentially important DNA methylation markers that change in response to multiple risk factors.

Keywords: Methylation, TNBC, African Americans, environment, BRCA1

Introduction

Cancer continues to be a disease epidemic in the United States. In 2017 cancer alone is responsible for more than 500,000 deaths nationwide. It is the second leading cause of health-related mortalities right behind heart disease. For women, breast cancer accounts for an estimated 250,000 new cases a year. This means that 12% of all women in the United States could be diagnosed sometime in their life and it continues to be the leading cancer diagnosis [1,2]. Due to the widespread nature of this disease, significant resources have been allocated to establish a gold standard for diagnosis. Traditionally breast cancer classification has been observed through genetic mutations in hormonal cell-surface receptor proteins but these are general functional classes that lack specificity in terms of the nature and invasiveness of different breast tumor types. This gene mutation based diagnoses focused on hormonal receptors that regulate growth and reproductive cycle changes in tissues are used to determine cancer subtypes and an appropriate course of treatment [3].

The American Society of Clinical Oncology and College of American Pathologists has agreed upon a consistent set of identifying factors for breast cancer focused around changes in ligand-activated transcription factors; estrogen receptors (ER), progesterone receptors (PR), and expression levels in human epidermal growth factor receptor 2 (HER2) [4,5].

When observing TNBC, the correlation between diagnosis and poor mortality rate has become increasingly more prevalent [5-7]. The definitive nature of this outcome continues to be the subject of debate. But what is well understood are the effects of genetics, environmental, and socioeconomic factors on changes in DNA methylation profiles [8,9] that can be considered risk factors. These changes cause hyper methylation leading to BRCA1 becoming silenced, which result in increased mortality rate [10]. Other genes have also been identified as potential markers including: RNF8 [11], CREB3L1 [12], and Wilms Tumor gene (WT1) [13]. But this does not take into account risk factors and ethnic backgrounds that increase the occurrence of these diagnostic markers. These studies show that through lifestyle choice and socioeconomic status, AA are predisposed to diabetes, hypertension and other conditions that lead to detrimental changes in methylation profiles [8,9,14]. This confirms the nature of increased epigenetic changes for the AA community versus other ethnicities. It is imperative to understand the relationship between methylation changes and risk factors amongst the AA community to improve the mortality rate of individuals with TNBC.

Subtyping Breast Cancer

Through the development of molecular breast cancer analysis, breast cancer classification has been observed through two different means, immunohistochemistry (IHC) testing and gene expression. This has the ability for the clinician to give a more targeted diagnosis. With the implementation of DNA microarrays, observing the fluctuation of hormone receptor gene expression with cDNA led to four main molecular subtypes; Luminal A, Luminal B, basal-like/Triple Negative Breast Cancer (TNBC), and HER2-enriched [3,15].

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Luminal A type breast cancer exhibits PR/ER-positive and HER2-negative while providing the best prognosis and high survival rate. Luminal B-type breast cancer exhibit PR/ER-positive with HER2 +/- representation and a poorer prognosis but the survival rate is relatively high. Basal-like/Triple Negative Breast Cancer (TNBC) type are ER-negative, PR-negative, and HER2-negative and are often aggressive with a poor prognosis [16]. The HER2-enriched classification is PR/ER-negative and HER2 positive and it tends to be more aggressive than Luminal cancers but a better prognosis is observed due to targeted hormone therapy of the HER2 protein [17]. The Basal-like/TNBC tumors are grouped together because they have very similar IHC and microarray expression signatures but this doesn't paint the full picture. Out of all of the breast cancer subtypes represented, the TNBC subclass has an abnormally high mortality rate while representing a fraction of all breast cancer types [18-21].

Triple Negative Breast Cancer

Under the breast cancer spectrum TNBC accounts for around 50% of patient deaths, while only representing 10-20% of all cancer types [5,6,7]. This unusual mortality rate is due to its aggressive behavior and high metastatic nature that spreads to other parts of the body such as the lung, brain, and liver [22]. It is also well known that African American women struggle with high mortality rate associated with this cancer compared to women of European descent [23,24]. The highly aggressive nature of this cancer subtype can be characterized by high levels of cytokeratin 5, and cytokeratin 6 with high expression levels of epidermal growth factor receptor (EGFR) which pertain to basal-like carcinomas [19]. TNBC also holds a unique characteristic making it hard to correctly treat. Without the particular receptor sites, (ER, PR, and HER2) present this cancer requires a drug cocktail for an improved prognosis, but there is still not an effective target for therapy [25,26].

TNBC compares to other breast cancers as having unique changes in gene expression as well as having a heterogeneous composition. With TNBC being on the poorer side of a prognosis, understanding the genetic makeup is crucial for a better-targeted therapy. This can come in multiple forms such as mutation, standard genetic subtyping, and epigenetic modifications that all impact changes in gene expression.

Mutational Risk of TNBC

One particular cause of TNBC can be derived from germ-line mutations, specifically BRCA1, representing 10%-20% of all TNBCs. While only representing 10%-20% of TNBC patients its mutation causes drastic outcomes [27]. BRCA1 is an anti-oncogene and it is responsible for repairing double-strand breaks. If it undergoes mutation and/or silencing, TNBC progression has a greater probability of occurring by 68%-80% [26,28]. This was a very important finding but the correlation between this and other BRCA1 deficient tumors give rise to a broad differential diagnosis. By identifying TNBC through more detailed gene expression the patient can receive a specialized and unique diagnosis negating false positives or negatives [28,29].

A more promising and efficient diagnosis can come from genetic and epigenetic characteristics that aid in the early expression of TNBC and prevent early metastasis [30,31]. The difference here is that epigenetic changes alter gene expression without altering the genome DNA sequence directly. Chemical modifications

of DNA (cytosine methylation, histone modifications) in the epigenetic makeup of each patient will give a stronger -pinpoint-classification system because there is a larger distinct difference between different tumor stages [32].

Genetic subtyping of TNBC

Through gene expression profiling, various genes have been identified that are responsible for TNBC and help subtype them into more genetically relatable categories. Lehmann and colleagues have compiled 21 gene expression datasets with 3,247 human breast cancers and analyzed them to identify 6 different subtypes to help give an accurate prognostic outcome. These subtypes include basal-like 1 (BL1) which heavily involves genes in cell cycle and cell-cycle checkpoint pathways, as well as DNA damage response pathways. Basal-like 2 (BL2) involves genes that contribute to growth factor signaling pathways and glycolysis/gluconeogenesis. The immunomodulatory (IM) subtype involves genes that are incorporated into immune cell signaling and immune signal transduction pathway processes. The Mesenchymal (M) subtype involves genes that correspond to pathways in cell motility and cell differentiation pathways. The Mesenchymal stem-like (MSL) subtype has similar genetic pathways as the Mesenchymal subtype except MSL has a unique involvement in growth factor signaling pathways such as EGFR, PDGF, and G-protein coupled receptor pathways. Lastly, the luminal androgen receptor (LAR) subtype which is heavily involved in hormonal regulation including steroid synthesis and the androgen/estrogen metabolism pathways [10]. The limitation with this subtyping is that they are undetectable when cross-referencing for tumors with ER, PR, and Her2 IHC data.

Burstein and colleagues then modified this subtyping method in 2015 when they sought to increase the understanding and methodology of TNBC subtyping. Their results led to 4 distinct subtypes that compile mRNA and DNA expression and combine the original 6 subtypes. The first one is the Luminal AR (LAR) subtype, which characterizes a percentage of TNBC tumors as AR, ER, prolactin, and ERBB4 signaling as well as Estrogen Receptor alpha1 (ESR1) gene expression that leads to molecular evidence of ER activation [33]. Subtype 2 is a mesenchymal (MES) subtype involves pathways that regulate the cell cycle, mismatch repair, and DNA damage networks, as well as hereditary breast cancer signaling pathways. Genes involved in this subtype include osteocyte related (OGN), adipocyte-related (ADIPOQ, PLIN1), and growth factor (IGF1) related. Subtype 3 is Basal-like immune suppressed (BLIS) which involves down regulation of pathways relating to the function of immune cell regulatory pathways. Lastly, subtype 4 Basal-like immune activated (BLIA) shows the up regulation of pathways relating to immune cell function [34]. The (LAR) and (MES) subtypes overlap with Lehmann and colleagues while the (BLIS) and (BLIA) take from the other four types. Due to the nature of the BLIS, an individual with this TNBC subtype will end up with the worst prognosis. Although all of these subtypes have been identified, this indicates that TNBC is of heterogeneous nature making accurate therapeutic intervention difficult. This also only show shows part or the overall picture without taking into account epigenetic makeup. Epigenetic alterations can show an alternative pathway for abnormal gene expression [35].

Epigenetic Nature of TNBC

Research in epigenetic modifications has been a relatively new genetic phenomenon with a plethora of new findings. For TNBC

there have been genetic alterations attributed to epigenetic changes. So far these changes have occurred through DNA methylation or histone acetylation to name a few, even though there are many other mechanisms for regulation.

DNA methylation can affect the gene of interest is by adding a methyl group to the CpG sites of the promoter region preventing associated transcription factors from binding [36] This can have adverse effects on gene expression by either hypermethylation or hypomethylation. In recent research, there has been unique methylation patterns found that differ greatly from hormone receptor positive breast cancer. This can be seen directly with BRCA1 and has a similar effect as if it was mutated. Although BRCA1 mutations only account for 10-20% of all TNBCs, they do show strikingly similar pathological features to breast cancer caused by germ-line BRCA1 mutations. What can be deduced from this is that BRCA1 promoter region is being inactivated through epigenetic hypermethylation [37,38,10]. One study emphasized the important role hypermethylation played versus mutation where they compared TNBC to non-TNBC cases. The results revealed that the BRCA1 promoter region was inactivated in 16% of the TNBC cases and not at all represented in non-TNBC patients [10].

Then in 2013, Watanabe and colleagues found BRCA1 and RNF8 methylation association with TNBC [39]. RNF8 has been known to play a role in the protein damage regulation through interacting with ubiquitin-conjugating enzymes and is also involved in the DNA damage response pathway [11]. Another study by Ward and colleagues found a methylation pattern of CREB3L1 expression in high-grade tumors but with the greatest association with TNBC [12]. On the other end of the methylation spectrum, one group observed promoter hypomethylation signatures in CD44, CD133, and Musashi-1 genes. They also hypothesized the same correlation with CD24 but there was no correlation [13]. But when observing changes in histone acetylation there have been multiple findings recently that point to this having an effect as well. Kwon and colleagues discovered a correlation between histone acetylation and CD24, which leads to an unfavorable prognosis in TNBC through hypoacetylation [40]. Although these are only a few genes responsible for TNBC this paints a picture on what role methylation and acetylation play in altering TNBC probability.

Moving forward, one team discovered methylation clusters littered throughout the genome that targeted 208 genes by hypermethylation. Some of these genes include glycoproteins that have the functional significance in the immune response. Based on Burstein's subtyping method this form of gene silencing would fall under BLIS. They also observed methylation patterns in the Wilms Tumor (WT1) gene, where if the gene body is methylated the prognosis is poor. But if the promoter region is methylated the prognosis improves, providing vital information for stratifying prognostic outcome in TNBC [37]. What should also be discussed are the risks that influence changes in methylation frequency to stay ahead of the cancer risk.

Ethnicity and Environmental Factors on Methylation Frequency

Epigenetics responds to a number of variables that influence gene expression. These variables can include various environmental, genetic, and socioeconomic factors [8,9]. Such disparities are evident because there is a prognostic difference between African

American women and Non-Hispanic White Women, and this holds true for TNBC [41,20].

Socioeconomic factors work in tandem with environmental factors that lead to changes in diet, exercise and stress levels to name a few. Research has already shown that AA have the predisposition for obesity, hypertension and diabetes [14]. Recent studies have shown higher levels glucose in one's diet can lead to increased epigenetic changes [42]. While alcohol consumption impairs methylation frequency [43]. These forms of environmental factors accelerate inflammation and along with a poor diet lead to obesity. Obesity has recently been identified to work conjunction with methylation to promote mortality amongst breast cancer patients [14,44]. There was also a genome wide DNA methylation profile on the effects of diabetes that showed a number of differentially methylated regions in response to the onset of diabetes. The genes affected were associated with the immune system and signal transduction that can also be associated with MES, BLIS and BLIA subtypes [45].

There is also a correlation between stress levels and epigenetic aging, where more long-term stress can aid in the acceleration of epigenetic changes [8,46]. This mechanism of action looks at the response of stress to glucocorticoids that then alter lasting changes in DNA methylation patterns. What is also hypothesized is that cumulative stress throughout life increased the probability of this type of methylation [8,46]. When taking environmental factors out of the equation the amount of global methylation hits its lowest point with the non-Hispanic blacks compared to non-Hispanic Whites. This is an interesting finding due to the understanding of BRCA1 hypermethylation in association with TNBC.

Factors for methylation frequencies in TNBC

Ethnicity and race have played a strong role in the response and mortality rate of breast cancer. When examining breast cancer between Caucasian women and AA women, the mortality rate has widened to 42% [1]. There is also a difference in DNA methylation frequencies in breast tumors amongst AA and Women of European Descent [47]. This could show a strong correlation with the increased prevalence of TNBC in the AA community due to differentially methylated loci. Although research shows the frequency of BRCA1 mutations in AA women remains low compared to women of other descents, there are other possible risk factors could come into play that affect genes of interest [15].

Through the exposure of particular environmental factors the induction of methylation changes on genes already heavily influencing TNBC. This is evident in one study where obese individuals receive a 1.89 fold change in hypermethylation of BRCA1 based off of a BMI of 30kg/m² versus healthy individuals [44]. This is a particular environmental factor that can be altered with a change in diet and exercise [48,49]. Even when proper BMI is achieved, there is an increase in global methylation, which indicates a healthy individual. If the methylated site correlates to a tumor suppressor gene, the incidence of cancer increases. One recent study looked at the role of resveratrol, a natural plant polyphenol, on methylation changes in TNBC [50]. They observed the effects of this bioactive polyphenol at 24 and 48 hours on TNBC cells in a genome wide survey. This compound can be found naturally in someone's diet in the form of grapes, and blueberries, and peanuts. What the data revealed was that with at

24 and 48 hour exposure there was a change in gene expression due to influence of methylation frequency [50,51]. This is important because it only took 24 hours to see a change in epigenetic nature of TNBC. What is also important is how long exposure of other environmental factors as well as ethnic background can lead to change in methylation frequency. Resveratrol is already a strong example of the effects of even a brief exposure.

These are just a few examples of the factors that contribute to TNBC progression. The other disparities include co-morbid disease, socioeconomic factors, access to healthcare that aid in the aggressive and fatal nature of TNBC in African American Woman [1]. A study has yet to be performed that looks at the direct relationship between changes in methylation of TNBC in AA and how that can aid in the understanding of high mortality rate. This has been done for breast tissue in healthy women of different backgrounds [52] and breast cancer tumor methylation frequencies for African-Americans (AA) and European American (EA) Women [53]. What Song and colleagues discovered was higher differentially methylated CpG sites in promoter regions in EA but higher in gene body with AA. What can be hypothesized from this is that different methylation CpG sites play different roles. The next step that needs to be taken is a global methylation analysis on the changes in CpG sites for AA versus other ethnic backgrounds through environmental changes and how that can play a role in understanding such a poor prognosis.

Concluding Remarks

TNBC is a disease diagnosis with aggressive heterozygosity that requires much more research to come up with an effective targeted treatment. So far there is an understanding over the increased mortality rate for AA compared to other ethnicities, but the origin of this result remains unclear. What is understood is that less than 25% of AA women with TNBC have the BRCA1 mutation but it still remains a good place to start. Based off of the vast understanding of BRCA1 gene, Myriad Genetics has utilized its resources to develop a targeted treatment for BRCA1/2 mutations called BRCAAnalysis. This treatment detects BRCA1/2 mutations through a simple blood test. This minimally invasive procedure can help prevent or delay the onset of cancer [54]. But with the ability of BRCA1/2 silencing from hypermethylation origin, the BRCA1 influence can exceed much higher than 25%.

Although there are novel treatments being developed the nature of TNBC leaves patients with the high rate of incidence, while still not having a definitive therapeutic target [55,56]. What is known about TNBC today should help pave the way for decreased cancer diagnosis. The objective should be set on preventive measures for TNBC due to its poorer overall mortality rate and higher rate of relapse [55]. With the known outcome of obesity and other disparities on hypermethylation, the implementation of diet and exercise can be the starting point for slowing down progression and improving prevention of AA with TNBC.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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