Overview of the genetic basis toward early detection of breast cancer

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Abstract: Cancer is a socioeconomical burden in any nation. Out of that, breast cancer is identified as the most common malignancy worldwide among women irrespective of age. As women are an important segment in a community, the weakening of their strength toward the development of a nation is a critical problem in each nation. In this review, it was aimed to discuss the characteristics of cancer genome, cancer genetics, and cancer epigenetics in general and then focus on discussing both genetic and nongenetic factors responsible for the predisposition of breast cancer in humans. More emphasis was placed on genes responsible for the early onset of the disease and which can be used as genetic tools in the identification of the disease at an early stage. Then the context of genetic involvement toward the breast cancer occurrence before age of 40 years was highlighted accordingly. In addition to genetic testing, the review paid adequate attention to mention novel liquid biopsy techniques and other clinical, laboratory, and radiologic assessments. These techniques can be used in early detection and recurrence as well as the surveillance of the patients after primary therapies.

Keywords: breast cancer, genetic predisposition, early onset, recurrence

Introduction

Cancer can be defined as a complex human disease where growth of a group of abnormal cells occurs uncontrollably, disregarding the normal rules of cell division. With a few exceptions, cancers are derived from single somatic cells and their progeny. The cells in emerging neoplastic clone accumulate a series of genetic and epigenetic alterations that tend to modify gene activities of a number of genes and their products causing various phenotypic changes.1

Normal cells are subjected to signals that regulate whether the cell should divide, differentiate into another cell, or die. However, cancer cells develop a degree of autonomy for these signals and lead to uncontrolled cell growth and proliferation without regulation. As a result, six “hallmark features” of the cancer cell phenotype have been identified by Hanahan and Weinberg, namely self-sufficiency in growth, insensitivity to antigrowth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion, and metastasis.2 Due to theoretical progression in cancer field in the last decade, another two emerging hallmarks have been added to the list, namely reprogramming of energy metabolism and evading immune destruction.3 Apart from these, genomic instability and inflammation have been identified as two enabling characteristics of cancers. In hereditary cancers, genomic instability occurs as a result of mutations in DNA repair genes and leads to cancer development,
which is predicted by the mutator hypothesis.\textsuperscript{4} Inflammation promotes multiple hallmark functions by supplying bioactive molecules to the tumor microenvironment, including growth factors. Thus, inflammation is a critical component in tumor progression.\textsuperscript{3,5}

**Cancer genome**

Cancers are thought to share a common pathogenesis. Similar to Darwinian evolution of origins of species, cancer evolution and development are based on two constituent processes. These are continuous acquisition of heritable genetic variation (inherited mutation) in individual cells by more-or-less random mutation and natural selection acting on the resultant phenotypic diversity. The natural selection may promote cells carrying alterations that confer the capability to proliferate and survive more effectively than their neighboring cells or eradicate those cells that acquired the mutations. A single cell occasionally acquires a set of sufficiently advantageous mutations that allow a cell to proliferate autonomously, invade tissues, and metastasize during the selection.\textsuperscript{6}

The DNA sequence of a cancer cell genome as well as most normal cell genomes has acquired a set of differences from its progenitor fertilized egg. These are collectively termed somatic mutations to distinguish them from germline mutations that are inherited from parents and transmitted to offsprings.\textsuperscript{8} Somatic mutations namely driver and passenger mutations in a cancer cell genome are acquired from several different sources such as substitution of bases, deletions and insertions of DNA fragments, and rearrangement and amplification of DNA sequence. Furthermore from exogenous sources where completely new DNA sequences are acquired from viruses such as human papilloma virus, Epstein–Barr virus, and hepatitis virus.\textsuperscript{7,8}

Driver mutations are positively selected during the evolution of the cancer that gives growth advantage, tissue invasion and metastasis, angiogenesis, and evasion of apoptosis, whereas passenger mutations do not give growth advantage and therefore do not contribute to cancer development. By definition, driver mutations reside in a subset of genes known as “cancer genes”, whereas passenger mutations are mutations that were present in the progenitor cell of the final clonal expansion of the cancer and are biologically neutral.\textsuperscript{9} Thus, identification of driver mutations and the cancer genes is the main goal in cancer genome analysis. Systematic sequencing of more than 25,000 cancer genomes at the genomic, epigenomic, and transcriptomic level revealed the evolutionary diversity of cancers and implicated a larger range of cancer genes than previously anticipated.\textsuperscript{10} The Cancer Genome Project is utilizing the human genome sequence and high-throughput mutation detection methods to identify somatically acquired sequence variants and thereby identify critical genes in the development of cancers in humans.\textsuperscript{11}

The cancer genome will also be able to acquire epigenetic changes that alter chromatin structure and gene expression when compared to the fertilized egg. Then it is manifested at DNA sequence level by changing the level of methylation of some cytosine residues.\textsuperscript{6} The epigenetic changes are stably heritable from the mother to the daughter cell and they generate phenotypic effects for selection to act on. Furthermore, somatic mitochondrial DNA mutations have been identified in primary human cancer types but their roles in the development and progression of cancer are not yet established by means of possible diagnostic and therapeutic implications.\textsuperscript{12}

Mutations in a cancer cell genome have accumulated over the lifetime of the cancer patient. Due to internal and external mutagens, a cell is continuously damaged but most of the damage is repaired. However, due to low intrinsic error rate in the DNA replication process, a small fraction of damage may be retained as fixed mutations. Mutation rates increase in the presence of exogenous mutagenic factors such as tobacco, some carcinogens, naturally occurring chemicals like aflatoxins from fungi, or harmful radiations like ultraviolet radiation.\textsuperscript{5}

**Cancer genetics**

Tumorigenesis in humans takes place in a stepwise manner, which is known as the multistep process of sequential alterations of several genes. In tumor cell, there may be dozens of different genes aberrant in structure or copy number and several genes may be differentially expressed. These genetic changes are usually somatic, while germline mutations can predispose heritable or familial cancer in an individual. A number of familial cancer genes with high-penetrance mutations have been identified but the contribution of low-penetrance genetic alterations for the development of sporadic cancers remains uncertain.\textsuperscript{13} Molecular genetic alterations such as chromosomal instability, dysfunction in cell cycle checkpoints, inherited defects in DNA repair, and possible defects in the regulation of epigenetic events cause abnormal DNA structures. All aspects affecting DNA integrity will increase the risk of cancer.\textsuperscript{14,15} Cancers are polygenetic disorders, as a result there are several groups of genes directly involved in the development of tumors in humans, namely oncogenes, tumor suppressor genes, DNA repair genes, as well as microRNA (miRNA) genes.
Cancer epigenetics

The term “epigenetic” refers to a heritable change in the pattern of gene expression that is mediated by mechanisms other than alterations in the primary nucleotide sequence of a gene. Epigenetic mechanisms are essential for normal development and maintenance of tissue-specific gene expression patterns. The best-known epigenetic marker is DNA methylation where gene expression is modulated by methylating DNA in the promoter region of the respective gene.

DNA methylation occurs in CpG-rich regions known as CpG islands, which span the 5’-end of the regulatory region (gene promoters) of many genes. These islands are usually not methylated in normal cells irrespective of the transcription of the gene. However, some of them (~6%) become methylated in a tissue-specific manner during early development or in differentiated tissues. More than 90% of methylated cytosines are located in repetitive sequences as well as in transposons and more vulnerable for modifications by exogenous and endogenous mutagens when compared to other bases on the DNA. The mutation rates of CpG-rich regions have been estimated to be about 40 times higher than other regions.

An important aspect of the mechanism of methylation is the inactivation of tumor suppressor genes as well as miRNA genes in the tumor cells. Methylation of CpG islands in gene promoter regions is associated with aberrant silencing of transcription and thereby inactivation of the tumor suppressor gene. The loss of gene function due to promoter hypermethylation and coding region mutations is similar. For example, both epigenetic and genetic changes in BRCA1 produce similar DNA-microarray pattern of gene expression in breast carcinoma. Human tumors are also characterized by an overall miRNA downregulation often caused by hypermethylation at the miRNA promoters. For example, miR-124a is repressed by hypermethylation, mediating CDK6 activation and Rb phosphorylation. Thus, inactivation of miRNA expression by hypermethylation is not only associated with cancer development but also with metastasis.

Breast cancer

Breast cancer is the main emphasis of this review, which is the common malignancy and the leading cause of cancer death among females worldwide, with an estimated 1.7 million cases and 521,900 deaths in 2012. According to Global Cancer Statistics, 2012, breast cancer accounts for 25% of all cancer occurrences and 15% of all cancer deaths among females, where more developed countries account for about one-half of all breast cancer cases and 38% of deaths.

Risk factors of breast cancer

As breast cancer is a multifactorial disease, several genetic as well as nongenetic factors predispose to malignancy. According to the epidemiologic studies done on breast cancer, several risk factors that predispose to the disease have been identified. Only about 10% of all breast cancer cases are due to the involvement of genetic factors, whereas other 90% of breast cancers are due to nongenetic factors. A complex interplay between environmental and genetic factors affects the development of breast cancer.

Nongenetic risk factors

Female breast cancer risk is affected by the reproductive history. The hormonal background also influences the course of the disease. The female reproductive hormones such as estrogens, progesterone, and prolactin have a major impact on breast cancer and control postnatal mammary gland development.

Most of the hormonal risk factors are associated with estrogen hormone. Prolonged exposure to estrogen is known to be associated with elevated levels of breast cancer risk. Factors such as early age at menarche, late onset of menopause, long menstrual history, nulliparity, recent use of postmenopausal hormone therapy or oral contraceptives, late age at first birth, and obesity are considered as hormonal risk factors.

There are a number of nonhormonal risk factors associated with the development of breast cancer, which are directly attached to modulate the estrogen exposure, such as age at exposure to ionizing radiation, alcohol consumption, and dietary factors.

Genetic risk factors

Breast cancer attributable to family history of the disease has been reported to account for 5%–10% of all breast cancer cases. Family history of the disease is the important genetic risk factor related to breast cancer. The most established model of breast cancer susceptibility is the cancer due to several number of high-penetration mutations, such as in BRCA1, BRCA2, p53, PTEN, STK11, and CDH1, and a much larger number of moderate penetrance variants in CHK2, ATM, RAD51C, BRIP1, and PALB2 predisposing the disease. High-penetrance genes, BRCA1 and BRCA2, are conferred as main predisposing genes to breast cancer and recommended for genetic testing. Alternatively, recent studies have identified PALB2 gene as a bona fide breast cancer susceptibility gene and recommended for genetic testing in patients with hereditary breast cancer along with BRCA status.
BRCA1 and BRCA2 genes

According to epidemiologic studies, only 15%–20% of familial breast cancer carries strongly predisposing BRCA1 and BRCA2 mutations, whereas the remaining 80%–85% of familial risk is from other known and unknown familial predisposing genes.13 However, individuals carrying mutations in either BRCA1 or BRCA2 genes have a 47%–87% risk of developing breast cancer and 17%–44% risk of developing ovarian cancer by 70 years of age. BRCA1 carriers have a lifetime risk of 65%–80% as well as 37%–62% of developing breast cancer and ovarian cancer, respectively, whereas BRCA2 mutation carriers have a lifetime risk of 45%–85% for breast cancer and 11%–23% for ovarian cancer.37–39 Approximately 52% of the families with four or more breast cancer cases have inherited mutations in BRCA1, and about 32% possess BRCA2 mutations. In contrast, somatic mutations in BRCA1 and BRCA2 are rare in sporadic cases of breast cancer.40 According to a study done in sporadic breast cancer patients, several somatic mutations in BRCA2 gene were found, harboring in BRC domains of exon 11, which are critical for BRCA2 function.41 A few studies have been done to characterize somatic mutations in BRCA1 gene in sporadic breast cancers comparatively to familial breast cancer. From those studies done on BRCA1 somatic mutation, a few mutations were detected in different populations.42,43

Women with breast carcinoma diagnosed before 40 years of age have a greater prevalence of germline BRCA1 or BRCA2 mutations than women with breast carcinoma diagnosed at older ages. Several recognizable histologic characteristics have been identified in breast carcinoma from studies of BRCA1/2 mutation carriers who belong to multiple-case families.44 Prevalence of BRCA4 mutations is higher in women with an early onset of the disease as founder mutations in the respective population. In Ashkenazi Jewish women, 13%–43% carry BRCA4 mutations and age of onset of breast cancer is below 40 years.45,46

One study claimed that mutations were detected in 5.9% of women diagnosed with breast cancer before 36 years of age (3.5% in BRCA1 and 2.4% in BRCA2) and in 4.1% of women diagnosed from ages 36–45 years (1.9% in BRCA1 and 2.2% in BRCA2). Eleven percent of patients with a first-degree relative who developed ovarian cancer or breast cancer by 60 years of age were mutation carriers, compared to 45% of patients with two or more affected first- or second-degree relatives. Recent penetrance estimates indicate that the proportions of BRCA1 and BRCA2 mutation carriers are 3.1% and 3.0%, respectively, among patients younger than 50 years, 0.49% and 0.84%, respectively, in patients who are 50 years or older, and 0.11% and 0.12%, respectively, in women in the general population.47 The presence of multiple primary cancers (such as prostate, colon, and pancreas) of any kind may increase the likelihood of finding a BRCA1 or BRCA2 mutation and supports previous studies suggesting that BRCA1 and BRCA2 mutations may be associated with an increased susceptibility to cancers other than breast and ovarian cancers.48

Hereditary breast and ovarian cancer syndrome

Hereditary breast and ovarian cancer syndrome (HBOC) occurs due to pathogenic germline mutations in BRCA1 or BRCA2, which is associated with an increased risk of early onset breast cancer as well as ovarian, prostate, and pancreatic cancers in all ethnic and racial populations and inherited in an autosomal dominant pattern.49,50 When one copy of either BRCA1 or BRCA2 is mutated in germline, this will result in HBOC syndrome.50 This syndrome accounts about 5%–7% of all breast cancer cases as well as 10%–15% of ovarian cancers. There is a 50%–80% lifetime risk of developing breast cancer, 30%–50% risk of ovarian cancer, and 1%–10% risk of male breast cancer for individuals with HBOC syndrome.49,50 Presence of HBOC in a family can be identified by the presence of close relatives diagnosed with breast, ovarian, or other related cancers, premenopausal breast cancer diagnoses (diagnosed before the age of 50), multiple related cancers in an individual (such as breast and ovarian cancer in a single individual), presence of male breast cancer, and having Ashkenazi Jewish ancestry.51

PALB2 gene

PALB2 gene encodes for PALB2 protein (partner and localizer of BRCA2), which binds to BRCA2 as a functional partner and facilitates the colocalization of both BRCA1 and BRCA2 to DNA damage sites.52 Biallelic mutations in PALB2 gene were recognized to be present in Fanconi anemia subtype FA-N and later on it was also shown that pathogenic mutations in PALB2 predisposed to hereditary breast cancer.53 Recent studies done on PALB2 mutation carriers showed that they have a risk of breast cancer 9.47 times higher than average. Risk of developing breast cancer for women with an abnormal PALB2 gene is 14% by 50 years and 35% by 70 years. The risk of developing breast cancer in PALB2 carriers is dependent on her age and family history. Relative risk of developing breast cancer in PALB2 mutation carriers is 8–9 times higher than average in 20–39-year age group, 6–8 times higher in 40–60-year age group, and 5 times higher in...
women older than 60 years. In contrast, women with PALB2 mutation at the age of 70 years with no family history of breast cancer have a 33% risk of getting the disease while the presence of first-degree relatives increases the risk to 58%. With respect to the occurrence of early onset breast cancer, it was identified that 25% of contribution is from BRCA1 and BRCA2 pathogenic mutations, whereas the contribution from loss-of-function PALB2 mutations is 2% in these young breast cancer patients. As PALB2 activates in the same pathway where BRCA1 and BRCA2 are involved in DNA damage repairing, the mutations of PALB2 may have similar effects on other cancers as BRCA proteins. Many studies identified that PALB2 involvement is similar to BRCA2 in the predisposition to male breast cancer, pancreatic cancer, and also to ovarian cancer. Hence, screening for PALB2 gene mutations was recommended as a useful step for BRCA1- and BRCA2-negative hereditary breast cancers, risk individuals, as well as male breast cancer patients.

**TP53 gene and Li–Fraumeni syndrome**

TP53 is defined as the guardian of a cell where it is involved in many regulatory mechanisms including as a decision maker in stress conditions such as DNA damage, metabolic deprivation, or telomere erosions. Functional alterations of TP53 protein occur in nearly 50% of tumor types including breast cancer. Inactivation of TP53 can be due to mutations in the DNA-binding domain or deletion of the carboxy-terminal domain of the protein. A classic autosomal dominant hereditary tumor predisposing disorder called Li–Fraumeni syndrome is associated with germline mutations in TP53 gene and shows an early onset of the disease. Germline mutations in TP53 account for <1% of breast cancer incidences comparative to the occurrence of somatic mutations of 19%–57% in breast cancers. p53- or TP53-mediated breast cancer shows an early onset in women with onset at about 29 years of age, whereas in men, onset of cancer is about 40 years of age.

**PTEN gene and Cowden syndrome**

PTEN is a tumor suppressor gene that encodes for phosphatase and tensin homolog where one of the key functions is inhibition of the oncogenic AKT/PI3K signaling pathway. Germline mutations in this gene cause the Cowden syndrome, which is inherited in an autosomal dominant pattern and characterized by multiple hamartomas and benign and malignant tumors. Such individuals with Cowden syndrome are at an increased risk for developing breast, thyroid, endometrial, and renal cancers. Females with Cowden syndrome have a 30%–50% of lifetime risk of developing malignant breast cancer and a 67% lifetime risk for developing benign breast disease apart from the other cancer types.

**STK11/LKB1 gene and Peutz–Jeghers syndrome**

STK11/LKB1 gene encodes for serine/threonine kinase 11, which acts as a tumor suppressor gene that mediates apoptosis and cell cycle regulation. Germline mutations in this gene cause Peutz–Jeghers syndrome, which is inherited in an autosomal dominant pattern and characterized by mucocutaneous melanin pigmentation and gastrointestinal polyposis. Apart from the occurrence of gastrointestinal cancers, those patients with Peutz–Jeghers syndrome also have an increased risk of the predisposition to extraintestinal cancers such as in the breast and the cervix. Breast cancer risk for females with Peutz–Jeghers syndrome was estimated to be 8% at the age of 40 years, which dramatically increases up to 45% at the age of 70 years. Somatic mutations in STK11/LKB1 are rare in breast cancer, where it maintains a low breast cancer risk in such individuals.

**ATM gene and ataxia telangiectasia (AT)**

ATM gene encodes for a serine-threonine protein kinase, which plays an important role in activating checkpoint signaling as a response to DNA damage (double-strand breaks), through phosphorylating proteins such as BRCA, p53, and Chk2 involved in DNA repair pathways. Inactivating mutations in the ATM gene caused a complex, autosomal recessive cancer syndrome known as AT, which is characterized by typical cerebellar AT, immunodeficiency, as well as cancer predisposition. Germline mutations in the ATM gene are rare in breast cancer families, whereas there is a twofold higher breast cancer risk in heterozygous carriers of AT-causing mutations compared to the general population. Somatic ATM mutations are more prevalent in a number of sporadic human cancers, especially in leukemias as well as in breast and lung cancers.

**Breast cancer before 40 years**

Breast cancer is the most common cancer type diagnosed among younger women between 15 and 39 years of age accounting for 14% of all young cancer incidence and 7% of all breast cancer cases. One of the emerging risk factors for breast cancers before age of 40 years is the personal factor of the patient compared to postmenopausal breast cancer patient. Strong family history of breast cancer is a major indicator for the early onset of the disease. Risk for breast cancer occurrence will be...
expression.93,94 Patients with triple-negative tumors are associated with aberrant EGFR expression and higher proliferation and genomic instability. Majority of breast tumors with younger onset and associated to hormone receptor expression. EGFR expression is more common in 15%–45% of breast tumors, which is inversely associated with the disease status and age of onset.87,88

Women with breast carcinoma diagnosed before 40 years of age with a strong familial risk have a greater prevalence of germline BRCA1 or BRCA2 mutations than women with breast carcinoma diagnosed at older ages.44,48,83 Multiple cases of breast and/or ovarian cancers are often in the same family.84 Most of the early onset breast cancers differ to some extent clinically and pathologically from other breast cancers, sharing more aggressive phenotypes, carrying invasive features with higher pathologic grade tumors with more lymph node positivity, especially in BRCA1-related tumors.85,86 Due to aggressive behavior of breast cancer in women <40 years, they have a poor prognosis and more vascular invasion than in older patients.87,88

Triple-negative breast cancers, the most lethal type of breast cancer, tested negative for estrogen receptor, progesterone receptor, and HER2 with low clinically significant levels. It occurs more frequently in young breast cancer patients carrying BRCA1 mutation. In 20–34-year-old young African American women, prevalence rate was 56%, whereas the rate was 42% in white women.89,90 These types of tumors have a relatively poorer prognosis than other breast cancer subtypes, are more advanced in disease stage and grade at diagnosis, and account for 10%–17% of all breast cancers.91,92 Recent studies done on hormone receptors status and EGFR expression identified that aberrant expression of EGFR is present in 15%–45% of breast tumors, which is inversely associated to hormone receptor expression. EGFR expression is more common in breast tumors with younger onset and associated with higher proliferation and genomic instability. Majority of patients with triple-negative tumors are with aberrant EGFR expression.93,94

Although previous studies were aimed at identification of BRCA1/BRCA2 mutations in breast cancer patients in order to identify unaffected family members and thereby preventing the disease in them, recent prospective data have shown that patients with unilateral breast cancer carrying BRCA1/BRCA2 mutations are at an elevated risk (16%–35%) of developing cancer in the contralateral breast.95 Risk for occurrence of contralateral second primary breast cancer in a BRCA carrier is 30% at 10 years postdiagnosis.96

Population-based studies identified the association between the early age onset of breast cancer and mutations of BRCA1 gene as an indicator of genetic susceptibility to breast cancer.97 Some studies done on different populations have identified that mutations in the BRCA1 and BRCA2 genes make approximately equal contributions to early onset breast cancer.97 Inherited syndromes, specifically BRCA1 and BRCA2 as well as p53 status, must be considered when developing treatment protocols for younger women. Other treatments such as chemotherapy, endocrine, and local therapies have the potential to create a significant impact on both the physiologic health including future fertility, premature menopause, and bone health as well as psychological health of young women as they face a diagnosis of breast cancer.79 Prevalence of BRCA1/BRCA2 mutations in early onset breast/ovarian cancer patients with a family history appears to be similar across race/ethnicity, but there is evidence of important racial and/or geographic differences in the spectrum of BRCA1/BRCA2 genetic variation, including pathogenic variants as well as variants of uncertain significance. These differences may reflect population history and genetic drifts and could have a significant impact on genetic counseling, genetic testing, and follow-up care.98,99

Early detections of recurrent breast cancer

Genetic testing is proven to be the early detection method especially for individuals with a strong family history of breast cancer and having relatives with early onset of the disease. Other than that, several assessment methods have been introduced to the patients and most of them can be used to monitor for disease recurrence after primary therapy as long-term surveillance programs. There are three clinical assessments considered for early screening of breast cancer, namely mammography, clinical breast examination (CBE), and breast self-examination, but these methods have their own limitations, as an example, estimations done by CBE on women aged 40–49 are approximately lower by 10% at first instance compared to women aged 50–59 due to heterogeneity of breast tissue.100,101 In addition to clinical assessments, there are several laboratory assessments such as detecting circulating serum tumor markers such as CA 15-3 and CA...
show multifactorial occurrence. Thus, identification and treatment of cancers is a challenging task. In this review, breast cancer has been discussed in detail along with main predisposing genetic factors for hereditary type. Breast cancer has been identified as the most common malignancy among women worldwide irrespective of age. Evaluating breast cancer risk at an early stage is important for women especially with a high risk of developing the disease thereby able to take preventive measures to reduce risk in the future. Risk factors, such as family history and genetics, are inherited and nonmodifiable, whereas lifestyle, diet, exercise, and alcohol consumption are modifiable risk factors that can be modified to reduce the risk. Women with a strong genetic predisposition are recommended to have personalized medical management plans such as frequent clinical assessment, risk-reducing drug therapy, and risk-reducing surgeries. Thus, breast cancer causes severe health and financial impact in each country because breast cancer incidences are rising drastically each year worldwide. When the onset of the breast cancer is early, then the burden for the local economy is much higher by means of decreasing productive young labor strength, financial cost of disease management, and so on, thereby having an adverse socioeconomic impact on the nation.

Disclosure

The authors report no conflicts of interest in this work.

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