AN INTRONIC VARIANT IN PTEN GENE IS PROBABLY INVOLVED IN SPLICING ALTERATION EVENTS.

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ABSTRACT

PTEN is a gate-keeper tumor-suppressor gene involved in various cell cycle pathways. Germline mutations of one allele of PTEN were found associated with Cowden syndrome, which is an inherited disease characterized by multiple hamartomas. PTEN is frequently inactivated also by somatic mutations in various array of human tumors. In this study, a cohort of 50 Sicilian breast cancer patients, negative to screening for pathogenic mutations in BRCA1/2 genes, was analyzed. Variant c.492+46 G>A (rs775537003) in intron 5 of PTEN gene was identified. The possible role of this variant in splicing mechanisms was evaluated by in silico analysis. Alteration of an ESE and ESS site was found. This is a preliminary study with the aim to investigate about the involvement of PTEN gene in the breast cancer and the possible role of splicing alteration events into the risk of the disease.

1. Introduction

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor-suppressor gene which encodes a major lipid 3-phosphatase. This protein signals down the PI3 kinase/AKT proapoptotic pathway and various cell-survival pathways, such as the mitogen-activated kinase (MAPK) pathway. The role of PTEN mainly consists in the ability to dephosphorylate and degrade PIP3 phospholipid of membrane, and consequently inhibit serine/threonine kinase oncogenic AKT [1]. Furthermore, PTEN coordinates G1 arrest through up-regulation of p27, an inhibitor of cyclin-dependent kinases, and acts as a negative regulator of the cell cycle and concomitant down-regulation of cyclin D1 [2]. Originally germline mutations of one allele of PTEN were found associated with Cowden syndrome (CS), which is an inherited disease characterized also by hamartomatic gastrointestinal polyposis that can evolve into early-onset esophago gastric cancer. [3-5].

PTEN is frequently inactivated by somatic mutations in a wide array of human tumors. Selective inactivation of PTEN in the developing brain causes defects in the neuronal migration, synapses formation and stabilization, and progression of glioblastoma multiforme (GBM), one the most malignant astrocytic tumor [6]. The astrocytes play an important role in several different biologic activities and the PTEN loss can increase the proliferation of astrocytes in vivo [7].

Previous studies have shown that glial fibrillary acidic protein (GFAP) co-localizes with a wide range of proteins expressed in astrocytes, as sarcoglicans and distrophin complex. The loss of some elements of these complex could be cause of the loss of structural plasticity of the synapses resulting in a tumor progression.

Other neoplastic diseases in which PTEN takes part is prostate cancer; however, in this case, mechanism involves the glyoxalases (GLOs) [8], a class of enzymes involved in cellular detoxification from Reactive Oxygen Species (ROS); moreover, polymorphisms in GLO genes were associated to several pathological conditions affecting Central Nervous System and retina [9-11]. Their role in breast cancer is, instead, controversial [12-13]. Importantly, the reduced expression of PTEN is not only due to loss of heterozygozy (LOH) but also to epigenetic mechanisms, as methylation of promoter [14-15]. This phenomenon was also observed in endothelial cells derived from Cerebral Cavernous Malformations (CCMs) sporadic lesions [16]. CCMs are benign neo-formations derived from angiogenetic impairment due to mutations at three loci [17-19]. PTEN/VEGF signaling modulates the three CCM proteins activity [20].

In present study on a cohort of 50 Sicilian breast cancer patients, negative to screening for pathogenic mutations in BRCA1/2 genes, the entire coding sequence of PTEN gene was analyzed in order to evaluate the role of this gene in the disease.
2. Methods

Patients
The peripheral blood samples were collected from 50 women (over 50 years of age) living in Sicily. Before recruitment, each patient was asked to sign an informed consent form. No adjuvant chemotherapy was administered. We have no information on treatment with radiation and or endocrine therapy. The study followed the tenets of the Declaration of Helsinki and was approved by the Scientific Ethics Committee of the Azienda Ospedaliera Universitaria-Policlinico “G. Martino” Messina.

Molecular analysis
Primers and polymerase chain reaction (PCR). The heparinized peripheral blood samples were collected from breast cancer patients. Genomic DNA was extracted from lymphocytes by kit QIAamp DNA Blood Mini Kit (QIAGEN®). The nucleotide sequence of primers employed for PCR reaction is shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Primer sequence (bp)</th>
<th>Ta (°C)</th>
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<td>1</td>
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<td>50.5</td>
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<tr>
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<td>2</td>
<td>5′-CAGTCTGAGTACTGTATATG-3′</td>
<td>50.5</td>
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<tr>
<td>PTEN</td>
<td>3</td>
<td>5′-GCTGAGTACTGTATATG-3′</td>
<td>50.5</td>
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<tr>
<td>PTEN</td>
<td>4</td>
<td>5′-GCTGAGTACTGTATATG-3′</td>
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<tr>
<td>PTEN</td>
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<td>PTEN</td>
<td>9</td>
<td>5′-GCTGAGTACTGTATATG-3′</td>
<td>50.5</td>
</tr>
</tbody>
</table>

Table 1. Primer sequences used for PCR and sequencing reaction


In silico analysis
Intron variant c.492+46 G>A was found and its possible role in splicing mechanisms was evaluated. The possible creation of exonic splicing silencer (ESS) and the abolition of exonic splicing enhancer (ESE) sites, as well as the abolition and creation of canonical sites and cryptic splice sites respectively, were analyzed through an in silico analysis using Human Splicing Finder software (http://www.umd.be/HSF3/HSF.html) [21].

3. Results
The mutation analysis was performed in the patients negative for pathogenic mutations in BRCA1/2 genes. The analysis of the entire coding sequence of PTEN gene, performed on 9 exons and partial intron sequences upstream and downstream of each exon (+/- nucleotides) identified the variant c.492+46 G>A (rs775537003) (http://www.ensembl.org/Homo_sapiens/Gene/Sequence?db=core;g=ENS G00000171862;r=10:87863113-87971930) in intron 5, a few dozen nucleotides upstream of exon 6.

Although this variant does not alter the structure of the protein, it could be involved in splicing mechanism. To evaluate the possible role of the variant in the splicing mechanism, in silico analysis was performed by Human Splicing Finder. The software revealed the alteration of an exonic splicing enhancer (ESE) site and an exonic splicing silencer (ESS) site. (Figure 1).

Figure 1. Possible alteration of the splicing process due to intronic c.492+46 G>A variant.

In addition, a reduction in SF2/ASF pattern score may be correlated with an exon-skipping phenotype associated with various disease-causing mutations, such as breast cancer (doi: 10.1093/hmg/ddl171) (Figure 2).
Figure 2 - Reduction in SF2/ASF pattern score due to c.492+46 G>A variant.

4. Discussion

More than 50 genes are involved in the progression of breast cancer. Over 1500 variants in two principal genes (BRCA1 and BRCA2) were listed in the Breast Cancer Information Core (BIC) [22-23]. In this study, the choice about PTEN is first linked to the important pathway largely controlled by this gene, to its role as gate-keeper tumor-suppressor gene, but above all because it is an haploinsufficient tumor-suppressor gene. Therefore, a single mutation can determine the production of a dysfunctional phosphatase.

Furthermore, the variant identified in PTEN gene is a polymorphism. To establish potential pathogenic or protective role of polymorphisms in diseases’ development is very complex; while, for rare disease, few cases may be sufficient [24-27], for breast cancer, having a wide incidence, a very large cohort of patients is required. Moreover, this study was limited to PTEN coding regions and intron/exon boundaries and did not consider possibility of presence of variants in the promoter region that was shown may be interfere with normal gene expression [28-30].

Certainly, further functional studies will be necessary to confirm the lack of activity of the aberrant protein form. Other unidentified genes should be screened in order to complete the framework in the field of the breast cancer.

References


