

Original Research Paper

Relative Transcription Expression Level of SIRT1, SIRT2 and SIRT3 in Correlation to the Expression of a Set of Selected Cancer Related Genes in Human Breast Cancer

¹Hamdy Swelim, ²Omnia Mansour, ³Salah Abdel-Rahman, ²Mohamed Shwaireb and ⁴Amani Kazem

¹Department of Zoology, Faculty of Science, Ain Shams University, Egypt

²Department of Biological and Geological science, Faculty of Education, Alexandria University, Egypt

³Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt

⁴Department of Pathology, Medical Research Institute, University of Alexandria, Egypt

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Corresponding Author:

Omnia Mansour

Department of Biological and Geological Science, Faculty of Education, Alexandria University, Egypt

Email:

omnia_ahmed_mansour@hotmail.com

Abstract: Mammalian Sirtuins have been shown to perform distinct cellular functions and deregulated expression of these genes was reported to be involved in the development of various malignancies including breast cancer. An increasing number of evidence indicates that Sirtuins have both tumor promoter and tumor suppressor functions. However, the roles of Sirtuins have not been well-studied in breast cancer. In the present study, quantitative expression levels of Sirtuins (SIRT1-3) together with a set of cancer related genes (cMYC, P53, SOD and HIF-1 α genes) were assessed in malignant breast cancer and non-malignant control samples by using a high-throughput real-time PCR method. As a result, Sirtuins were found to be differentially expressed in breast cancer tissues and control samples, respectively. Particularly, expressions of SIRT1 ($p = 0.035$) and SIRT3 ($p = 0.033$) were found to be significantly up regulated, whereas SIRT2 ($p = 0.032$) gene was shown to be downregulated in breast cancer tissues compared to control samples in our study. Additionally, the expression levels of SIRT1-3 genes were correlated to both the selected cancer related genes and to clinicopathological parameters of breast cancer patients. In conclusion, SIRT1 and SIRT3 genes may act as oncogenes, whereas SIRT2 gene may operate as a tumor suppressor gene in human breast cancer.

Keywords: Breast Cancer, SIRT1, SIRT2 and SIRT3

Introduction

Breast cancer, as other cancers, has an unstable genome which generates the genetic diversity through deregulation of gene expression profiles and disruption of molecular networks (Hanahan and Weinberg, 2011). Intriguing evidence has recently emerged that genetic and epigenetic mechanisms are not separate events in cancer; they intertwine and take advantage of each other during tumorigenesis (Jones and Martienssen, 2005).

Histones acetylation is one of epigenetic marks that regulate gene expression, occurs on the ϵ -amino groups of lysine residues in the N-terminus of the histone proteins by the opposing action of Histone Acetyltransferases (HATs) and Histone Deacetylases (HDACs) (Dong and

Cui, 2016). The deacetylation of histone tails may underlie suppression of target genes expression and heterochromatin formation (Guarente, 2000). Sirtuins, Class III Histone Deacetylases (HDACs), are a family of proteins composed of 7 members, including SIRT1-7 which are evolutionary conserved enzymes homologous with the yeast Sir2 family of proteins (Landry *et al.*, 2000). They are Nicotinamide Adenine Dinucleotide (NAD⁺) dependent deacetylases and/or mono-Adenosine Diphosphate (ADP)-ribosyl transferases that have attracted tremendous attention as stress adaptors and post-translational modifiers and they have been linked to many diseases including cancer (Bosch-Presegué and Vaquero; 2011). SIRT1-3 are important members of Sirtuins family. They possess efficient deacetylase activity in vitro

and under certain extreme conditions such as chronic stress, SIRT1-3 can protect the organism by inducing cell senescence or apoptosis, they can also deacetylate a number of non-histone target proteins (Bosch-Presegué and Vaquero; 2011; He *et al.*, 2014). SIRT1 can, in one hand, regulate multiple target proteins involved in cell cycle progression, DNA repair machinery, cell-signaling and cell metabolism (Palacios *et al.*, 2010; Houtkooper *et al.*, 2012). On the other hand, SIRT2 can deacetylate the α -tubulin subunit of microtubules and it has been proposed that SIRT2 might function as a mitotic checkpoint protein in G2-M to prevent chromosomal instability, particularly in mitotic stress (North *et al.*, 2003; Vaquero *et al.*, 2006). While SIRT3 works as a major protein deacetylase within the mitochondrial matrix and is critical for maintaining mitochondrial integrity and function through regulation of proteins involved in metabolism, energy homeostasis, cell survival and death (Lombard *et al.*, 2007; Hallows *et al.*, 2011; Alhazzazi *et al.*, 2013). Moreover, SIRT1-3 are implicated in a variety of pathological conditions including cancer but their role in cancer as oncogenes or tumor suppressor genes is still controversial and more studies are needed to further delineate their precise functions (Alhazzazi *et al.*, 2011; Dan *et al.*, 2012; Chen *et al.*, 2014; Kulić *et al.*, 2014).

Over 30 000 genetic aberrations have been detected in tumor cells when compared with normal cells and according to the Cancer Gene Data Curation Project, about 4 700 genes have been identified as being related to cancer (Kumar *et al.*, 2009; Pleasance *et al.*, 2010). The latter include tumor suppressor genes mainly P53 gene, oncogenes like Myelocytomatosis (cMYC) gene or stress adaptation genes as Superoxide Dismutase (SOD) and Hypoxia Inducible Factor-1 (HIF-1 α) genes (Feinberg *et al.*, 2006).

The c-Myc oncogene is the most frequent amplified gene in human cancers and is associated with tumor aggression and poor clinical outcome. In normal cells, it promotes cell replication in response to extracellular signals, by driving quiescent cells into the cell cycle (Lin *et al.*, 2012). In tumor cells that express high levels of c-Myc, cellular proliferation is no longer dependent on growth-factor stimulation and this uncoupling from growth factor regulation leads to the uncontrolled proliferation characteristic of cancer cells, protection against programmed cell death, loss of respect for normal tissue boundaries and metastases (Dunning *et al.*, 1999; Lin *et al.*, 2012). The effect of high levels of c-Myc on global gene regulation is poorly understood but is widely thought to involve newly activated or repressed "Myc target genes" (Lin *et al.*, 2012).

The P53 gene is the most frequently mutated tumor suppressor gene in cancer and P53 loss may be required for maintenance of aggressive carcinoma (Xue *et al.*, 2007). It exists in non-stressed cells at a very low concentration due to its rapid ubiquitin-mediated

degradation in proteasomes. Under stress conditions, P53 induces the transcription of various genes that are involved in cell-cycle control, apoptosis, DNA repair, differentiation and senescence (Slee *et al.*, 2004). In cancer, its activities are inactivated and result in the loss of normal functions, especially in accurate DNA replication that leads to the proliferation of cells that are under stress conditions and tumor development (Dunning *et al.*, 1999). An increasing number of studies indicate that a subset of mutated P53 are oncogenic and actively participate in neoplastic transformation (Weisz *et al.*, 2007).

SOD is essential enzyme which acts as antioxidant scavenger that catalyzes the dismutation of highly reactive O₂ \cdot and H₂O₂ to O₂ and less reactive H₂O₂ respectively (Halliwell, 1994) and protect cells from oxidative damage which can cause lipid peroxidation, mutagenesis and carcinogenesis. Huang *et al.* (2000) showed that malignant cells are highly dependent on SOD for survival that inhibition of SOD causes accumulation of cellular O₂ \cdot that cause free-radical-mediated damage to mitochondrial membranes, the release of cytochrome c from mitochondria and apoptosis of the cancer cells.

HIF-1 is an essential transcription factor induced by reduced O₂ availability in the cellular environment (hypoxia) and can activate over 60 direct genes, that have roles in many critical aspects of cancer biology including glycolytic metabolism, immune avoidance, angiogenesis, metastasis and therapeutic resistance (Papadakis *et al.*, 2010; Semenza, 2010; Zhang *et al.*, 2010). Increased HIF-1 α levels in diagnostic tumor biopsies are associated with increased risk of mortality in many types of tumors including breast cancer (Semenza, 2010). Recent studies have provided evidence indicating that HIF-1 mediates resistance to chemotherapy and radiation (Aebbersold *et al.*, 2001).

Cancer cell transformation has a multitude of different intertumoral genetic and epigenetic alterations (Hutchinson, 2010). The objective of the current study was therefore to elucidate in human breast cancer the role of SIRT1, SIRT2 and SIRT3 in association with the selected cancer related genes: cMYC, P53, SOD and HIF-1 α genes. Herein, we aimed to correlate the expression of these genes with the clinicopathological parameters of breast cancer patients.

Materials and Methods

Patients and Specimens

In total, 30 breast cancer patients have been enrolled from those admitted to the Medical Research Institute, Alexandria University, Egypt. All subjects were recruited according to the ethical rules approved by the ethical committee of the Medical Research Institute based on Belmont report. The clinicopathological prognostic characters of breast cancer patients were obtained and reviewed and reported in Table 1.

Specimens included 30 malignant breast cancer as well as 20 adjacent non-malignant control samples freshly collected from breast cancer patient's mastectomy after surgical removal and clinical examination at the Clinical Pathology Department, Medical Research Institute, Alexandria University, Egypt. Samples were stored in RNA-later (Ambion, UK) at -80°C until they were used.

RNA Isolation

Total RNA was isolated from 30 mg of frozen tumor and control tissues using ISOLATE II RNA Mini Column Kit (Bioline, UK) according to the manufacturer's instructions. The purity and concentration of RNA samples were determined using NanoDrop spectrophotometer. Only the RNA samples that have A260/A280 ratio range from 1.8 to 2.00 were used, otherwise the RNA samples were considered contaminated. RNA samples were then stored at -80°C until be used.

Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction (qRT-PCR)

The reaction and data analysis were performed according to the instructions of the thermo PikoReal™ Real-Time PCR system, using SensiFAST™ SYBR® No-ROX One-Step Kit (Bioline, UK) and specific primers. Forward and reverse primers (Qiagen, Germany) were used to target gene expressions of the following: SIRT1: Forward 5'-AAA TGC TGG CCT AAT AGA GTG G-3', reverse 5'-TGG TGG CAA AAA CAG ATA CTG A-3'; SIRT2: Forward 5'-GAA CGC TGT CGC AGA GTC ATC-3', reverse 5'-GGT TGG CTT GAA CTG CCC AG-3'; SIRT3: Forward 5'-GCA TTC CAG ACT TCA GAT CGC-3', reverse 5'-GTG GCA GAG GCA AAG GTT CC-3'; P53: Forward 5'-GTT CCG AGA GCT GAA TGA GG-3', reverse 5'-TCT GAG TCA GGC CCT TCT GT-3'; cMYC: Forward 5'-CTT CTC TCC GTC CTC GGA TTC T-3', reverse 5'-GAA GGT GAT CCA GAC TCT GAC CTT-3'; HIF-1 α : forward 5'-TGG CCT TGT GAA AAA GGG T-3', reverse 5'-TTG ATG GGT GAG GAA TGG GT-3'; SOD: Forward 5'-TGT GGG GAA GCA TTA AAG G-3', reverse 5'-CCG TGT TTT CTG GAT AGA GG-3'; B-Actin: Forward 5'-AGA AAA TCT GGC ACC ACA CC-3', reverse 5'-AGA GGC GTA CAG GGA TAG CA-3'. QRT-PCR program was applied as one cycle of cDNA synthesis at 50°C for 15 min, one qRT-PCR was performed in a .reaction mixture of 20 μL using 10 μL SensiFAST™ SYBR® No-ROX One-Step Mix(1X), 0.2 μL Reverse transcriptase, 0.4 μL RiboSafe RNase Inhibitor, 1.6 μL forward and reverse primers (10 pm), 4 μL RNA template (10 ng) and up to 16 μL sterile water. The thermal cycling program included reverse transcription at 45°C for 10 min, then polymerase activation at 95°C for 2 min followed by 40 cycles of denaturation at 95°C for 5 s, annealing at 60°C

for 10 s and extension at 72°C for 5 s. Duplicate samples were used for data accuracy. The data for relative gene expression were analyzed by the comparative Ct method ($2^{-\Delta\Delta\text{CT}}$) using β -actin as an endogenous control and gene expression in non-malignant samples as calibrator.

Statistical Analysis

All statistical analyses were done using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp). Experiments were carried out in triplicate. The Kolmogorov-Smirnov test was used to verify the normality of distribution. The comparisons between groups were determined by Mann-Whitney test. The correlations between groups were determined by Spearman's rank correlation coefficient test. Values of $p < 0.05$ were considered significant.

Results

Clinicopathological Characteristics of Breast Cancer Patients

The clinicopathological features of our series of Egyptian breast cancer patients are described in Table 1. All individuals underwent curative surgery at the Medical Research Institute, Alexandria University of Egypt. The mean age at diagnosis was 53.5 years, ranging from 30 to 76 years. Among these, nearly quarter of cases (26.7%) were premenopausal while the remaining 22 cases (73.3%) were postmenopausal. The majority of patients (28 cases out of 30, 93.3%) were suffering from Invasive Ductal Carcinoma (IDC) whereas only 2 cases (6.7%) showed Invasive Lobular Carcinoma (ILC). Tumor size (the largest diameter) ranged from 1 to 9 cm, patients showing tumor size < 2 cm are only 3 cases (10%); while those having tumor size included between 2-4 cm are 19 cases (63.3%) and those with tumor size > 4 cm are 8 cases (26.7%). Most patients had tumor grade II (20 out of 30, 66.7%) where only 10 patients (33.3%) had tumor grade III. The frequency of positive lymph node metastases was 63.3% (19 patients out of 30). The profile of ER α and HER2 as determined by immunohistochemistry was positive in 24 and 18 patients (80% and 60%) respectively based on the 10% cut off level for expression analysis.

The Relative Expression Level of SIRT1, SIRT2 and SIRT3 and Selected Cancer Related Genes: cMYC, P53, SOD and HIF-1 α , in Human Breast Cancer Samples

The qRT-PCR technique was used to quantify the relative transcription expression level of SIRT1, SIRT2, SIRT3, cMYC, P53, SOD and HIF-1 α genes in malignant and non-malignant samples of human breast cancer (Fig. 1).

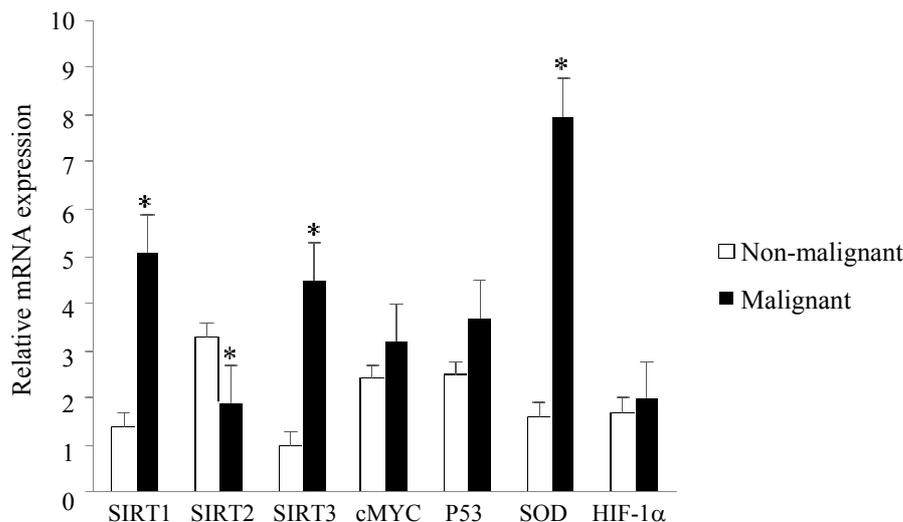


Fig. 1: Real-time PCR experiments showing differential gene expression associated with human breast samples, as compared to their corresponding controls (* $p < 0.05$)

Table 1: Clinicopathological prognostic data of breast cancer patients

Parameters	Number (%)
Age (years)	
Min-Max	30-76
Mean \pm SD	53.5 \pm 11.5
Median age	52
Menopausal status	
Pre	8 (26.7)
Post	22 (73.3)
Histological type	
Invasive lobular carcinoma (ILC)	2 (6.7)
Invasive ductal carcinoma (IDC)	28 (93.3)
Tumor size (T) (cm)	
Min – Max	1-9
<2	3 (10)
2-4	19 (63.3)
>4	8 (26.7)
Tumor grade	
II	20 (66.7)
III	10 (33.3)
Lymph node metastasis	
Negative	11 (36.7)
Positive	
1(1-3)	7 (23.3)
2(4-9)	5 (16.7)
3(\geq 10)	7 (23.3)
ER α	
Negative	6 (20)
Positive	
+1	2 (6.7)
+2	12 (40)
+3	10 (33.3)
HER2	
Negative	12 (40)
Positive	
+1	10 (33.3)
+2	6 (20)
+3	2 (6.7)

In our experiment, SIRT1 and SIRT3 genes were found to be upregulated in 76.7 and 63.6% of malignant samples, respectively; whereas, SIRT2 gene was found to be downregulated in 62.1% of malignant tissues. The upregulation of both SIRT1 (5.1 \pm 1.5, mean \pm SEM, $p = 0.035$) and SIRT3 genes (4.5 \pm 1, $p = 0.033$) as well as the downregulation of SIRT2 gene (1.9 \pm 0.48, mean \pm SEM, $p = 0.032$) were found to be significant in malignant samples compared to non-malignant samples of human breast cancer.

On the other hand, 33.3, 55.6, 66.6 and 42.3% of malignant samples had over-expression of cMYC, P53, SOD and HIF-1 α genes, respectively. Malignant tissues had non-significant high level of mRNA expression of cMYC (3.2 \pm 1.3, mean \pm SEM, $p = 0.228$), P53(3.7 \pm 1.1, $p = 0.690$) and HIF-1 α (1.7 \pm 0.57, $p = 0.428$) genes than those in non-malignant samples (2.4 \pm 0.64, 2.5 \pm 0.8, 2.0 \pm 0.68 respectively). However, there was significant high level of SOD gene expression in malignant tissues (7.97 \pm 3.9, $p = 0.046$), compared to non-malignant samples (1.6 \pm 0.4) of human breast cancer.

The correlation between SIRT1, SIRT2 and SIRT3 and a set of selected cancer related genes: cMYC, P53, SOD and HIF-1 α , in human breast cancer samples

In regard to the correlations between the relative transcription expression levels of genes included in this study (Fig. 2), Spearman's rank correlation showed a significant positive correlation between SIRT1 and P53 ($P = 0.012$), SIRT3 and cMYC ($P = 0.010$), cMYC and P53($p = 0.000$) and SOD and HIF-1 α ($P = 0.012$) genes.

On the other side, there were no significant correlations between the expression levels of each of the following pairs: SIRT1 and SIRT2 ($p = 0.732$), SIRT1 and SIRT3 ($p = 0.331$) and SIRT2 and SIRT3 ($p = 0.691$), cMYC and SOD ($p = 0.823$), cMYC and HIF-1 α ($p = 0.491$), P53 and SOD ($p = 0.301$) and P53 and HIF-1 α ($p = 0.195$) genes.

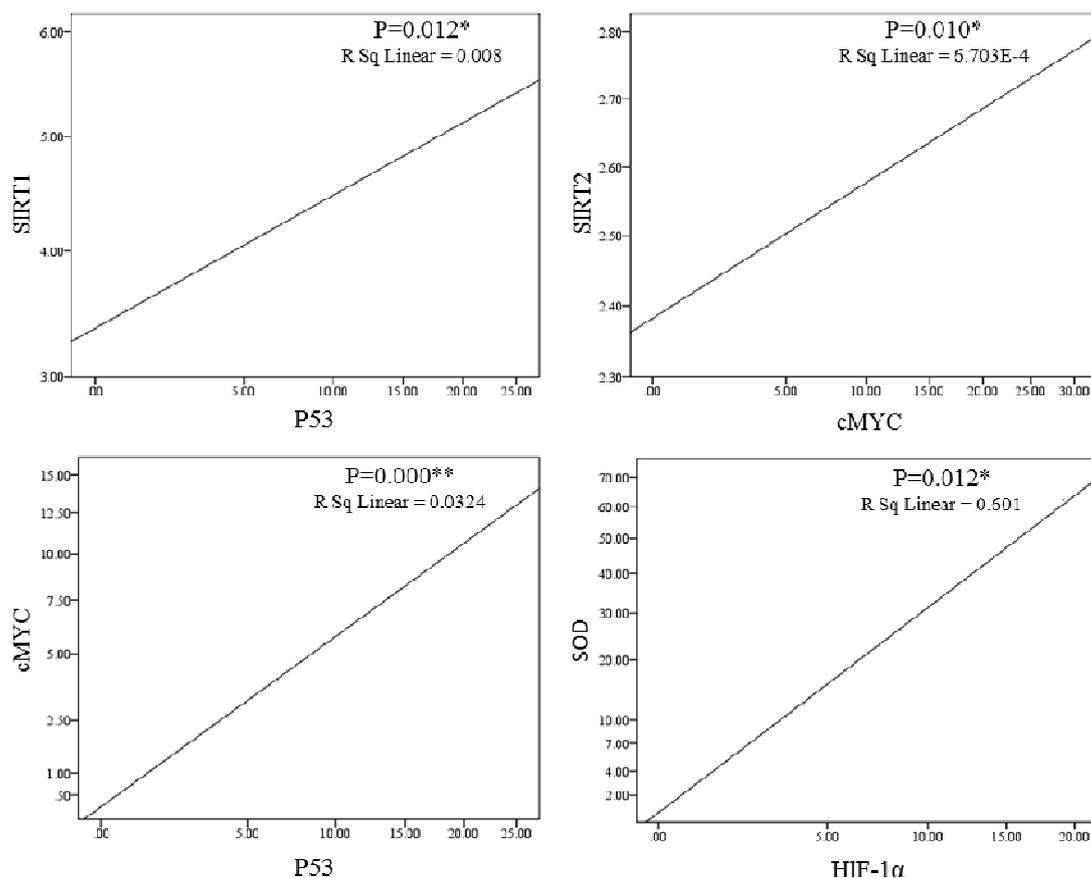


Fig. 2: Scatter plots showing the significant correlations between the relative expression levels of a pair of particular genes included in our study (* $p < 0.05$; ** $p < 0.01$)

Table 2: The correlation between the relative transcription expression levels of cMYC, P53, SOD and HIF-1 α ; SIRT1, SIRT2 and SIRT3 and the clinicopathological parameters of breast cancer patients

Pathological parameters		Relative gene expression						
		SIRT1	SIRT2	SIRT3	cMYC	P53	SOD	HIF-1 α
Age	rs	0.259	0.262	0.262	0.352	0.470*	0.062	0.279
	p	0.284	0.279	0.388	0.152	0.049*	0.820	0.296
Menopausal status	rs	0.175	-0.065	0.089	0.155	0.299	0.226	0.156
	p	0.475	0.790	0.772	0.538	0.228	0.400	0.563
Histological type	rs	0.071	0.081	-0.050	-0.054	-0.018	0.120	-0.233
	p	0.711	0.675	0.826	0.787	0.928	0.558	0.253
Tumor size	rs	-0.304	-0.068	0.453*	-0.324	-0.279	0.106	0.048
	p	0.103	0.726	0.034*	0.100	0.159	0.607	0.815
Tumor grade	rs	0.148	0.079	-0.391	0.058	0.474*	0.427*	0.501*
	p	0.453	0.696	0.088	0.784	0.017*	0.037*	0.013*
Vascular invasion	rs	-0.193	0.060	0.136	-0.067	-0.268	-0.142	-0.270
	p	0.308	0.758	0.547	0.740	0.177	0.489	0.182
Lymph node metastasis	rs	-0.168	-0.097	0.117	-0.292	-0.370	-0.313	-0.344
	p	0.374	0.618	0.605	0.139	0.058	0.120	0.085
ER	rs	-0.450	0.443	0.099	0.191	-0.035	0.526	-0.366
	p	0.080	0.098	0.747	0.513	0.905	0.079	0.241
HER2	rs	0.026	0.106	0.729**	0.135	-0.115	-0.251	0.381
	p	0.926	0.719	0.007**	0.660	0.709	0.456	0.247

rs: Spearman coefficient; *: Statistically significant at $p < 0.05$; **: Statistically significant at $p < 0.01$

The correlations between the relative transcription expression levels of SIRT1, SIRT2 and SIRT3 and the selected cancer related genes: cMYC, P53, SOD and HIF-1 α , together with the clinicopathological parameters of breast cancer patients

In regard to the correlation between genes expression and the clinicopathological features of breast cancer patients (Table 2), Spearman's rank correlation showed that there were positive correlations between the relative transcription expression level of SIRT3 gene and both tumor size ($p = 0.034$) and HER2 status ($p = 0.007$), P53 gene and both patient's ages ($p = 0.049$) and tumor grade ($p = 0.017$) and both SOD and HIF-1 α genes and tumor grade ($p = 0.037$ and $p = 0.013$, respectively).

On the other hand, there was no significant correlation between the relative transcription expression of SIRT1, SIRT2 and cMYC and any of the clinicopathological parameters of breast cancer patients.

Discussion

Breast cancer, like other cancer types, has thousands of genetic aberrations that enable tumor formation and progression (Kumar *et al.*, 2009; Pleasance *et al.*, 2010). The genetic path to cancer is not related only to mutation of oncogenes or tumor suppressor genes, but also to intracellular stress adaptation and/or abnormal expression due to epigenetics alterations (Sharma *et al.*, 2010). SIRT1-3, members of Class III histone deacetylases of sirtuin family, are related to epigenetic regulatory proteins that are capable of deacetylation not only chromatin proteins, which are key elements in the regulation of gene expression, but also of non-histone proteins leading to inappropriate activation or inhibition of various cellular signaling pathways (Minucci and Pelicci, 2006; Sandoval and Esteller, 2012). However, the efficiency and physiological relevance of their activity are not known and their role in cancer still controversial (Kulić *et al.*, 2014; Teng *et al.*, 2014). In our study, we aimed to investigate the mRNA expressions of SIRT1-3 genes and a set of cancer related genes like cMYC, P53, SOD and HIF-1 α in human breast cancer. To the best of our knowledge, this is the first report that investigates the expression of this set of genes together in human breast cancer.

Genes Expression in Human Breast Cancer Samples

SIRT1 is detected in many types of cancers, but its possible role in cancer has posed a dilemma (Kulić *et al.*, 2014). Yuan *et al.* (2013) suggested that SIRT1 has a dual role in the development of tumors as a tumor suppressor or promoter depending on the type of tumor and the spatial distribution of SIRT1 upstream and downstream factors. In breast cancer, Wang *et al.* (2008) showed that SIRT1 gene expression is reduced and it acts

as a tumor suppressor gene, while Ashraf *et al.* (2006) showed that there was no association between SIRT1 gene expression and breast cancer. In our study, we found a significant increase in SIRT1 gene expression ($p = 0.035$) in malignant samples and this finding supports other reports that SIRT1 may act as an oncogene and contribute to tumor development in breast cancer (Cao *et al.*, 2014; Santolla *et al.*, 2015).

SIRT2 upregulation or downregulation is detected in many cancer types (Hiratsuka *et al.*, 2003; Dan *et al.*, 2012; Chen *et al.*, 2013; Liu *et al.*, 2013; Ming *et al.*, 2014). Ashraf *et al.* (2006) showed that there was no significant difference in SIRT2 gene expression between breast cancer biopsies and normal breast tissue. In our study, there was a significantly low SIRT2 gene expression in malignant samples of breast cancer patients ($p = 0.032$) and this support the claim that SIRT2 may function as a tumor suppressor by maintaining cellular mitotic integrity and its dysfunction leads to genetic instability and tumorigenesis (Hiratsuka *et al.*, 2003; Kim *et al.*, 2011).

Additionally, the deregulation of SIRT3 expression has been observed in different cancers (Huang *et al.*, 2014; Liu *et al.*, 2014; Yan *et al.*, 2014). Chen *et al.* (2014) mentioned that SIRT3 can function either as a tumor promoter or suppressor depending on tumor type, cellular stresses or cell death stimuli. In breast cancer, Ashraf *et al.* (2006) found no significant differences in SIRT3 gene expression between malignant and normal breast biopsies. However, our finding support other results reporting (He *et al.*, 2014) that there was a significant high level of SIRT3 gene expression in breast cancer patients ($p = 0.033$). Thus, SIRT3 could function as a tumor promoter and plays a prosurvival role in cancer (Alhazzazi *et al.*, 2011).

Our data reported that 33.3% of the tumor samples had overexpression of cMYC gene, however, there was no significant difference in cMYC gene expression between the malignant and non-malignant samples in breast cancer ($p = 0.235$). This is in agreement with studies reporting that cMYC overexpression at the mRNA level is in 22 to 35% of breast tumors (Bieche *et al.*, 1999; Scorilas *et al.*, 1999).

Moreover, an increasing number of studies indicate that a subset of mutated P53 are oncogenic and actively participate in neoplastic transformation (Weisz *et al.*, 2007). Our results have shown that 55.6% of the tumor samples had P53 overexpression even though there was no significant differences in P53 expression between malignant and non-malignant samples in breast cancer ($p = 0.690$).

In the meantime, a defect in SOD is experimentally proved to be associated with several types of cancer such as hepatocellular carcinoma and brain tumor (Elchuri *et al.*, 2005; Aggarwal *et al.*, 2006). Previous investigators

have measured the enzymatic activities of the SODs in breast cancers. Huang *et al.* (2007) suggested that SOD can act as a tumor suppressor by decreasing growth and survival of breast cancer cells. On the other hand, some studies have reported a correlation between high SOD level and invasiveness of breast cancer (Tsanou *et al.*, 2004; Kattan *et al.*, 2008). Er *et al.* (2004) speculated that upregulation of SOD expression induced by oxidative stress or local inflammation may contribute a selective growth advantage to tumor cells compared to their normal counterparts (Khan *et al.*, 2010). In agreement to other studies (Tas *et al.*, 2005; Rajneesh *et al.*, 2008), our findings have shown that 66.6% of the tumor samples had overexpression of SOD gene and that there was a significant difference in the expression levels of this gene in malignant versus non-malignant samples ($p = 0.046$).

Interestingly, all experimental data indicate that HIF-1 is over-expressed in many human cancers, mainly in the earliest detected neoplastic lesions (Talks *et al.*, 2000; Mandriota *et al.*, 2002). Our data described 42.3% of the tumor samples with HIF-1 α overexpression but no significant difference between malignant and non-malignant breast cancer samples ($p = 0.428$) have been reported.

Correlations between Different Genes in Human Breast Cancer Samples

The correlation between the expression levels of SIRT1-3 and cancer related genes have been shown to be significantly positive between SIRT1 and P53 genes ($p = 0.012$). This is in accordance with Wilking and Ahmad (2015) who reported that P53 positively regulates the transcription of SIRT1 and promotes its activity. In a feedback loop, overexpression of SIRT1 would lead to its own repression via P53 acetylation and inactivation. Kozako *et al.* (2014) showed that in response to DNA damage and oxidative stress, SIRT1 deacetylate and inactivate P53 and blocks its nuclear translocation, leading to the accumulation of P53 in both the cytosol and mitochondria. Thus, we suggest that inhibition of the function of P53 protein by SIRT1 may induce the cell to produce more P53 to compensate its function and this supports the oncogenic consequences of SIRT1 overexpression. Interestingly, SIRT3 gene expression was significantly correlated with the expression of cMYC oncogene ($P = 0.010$) in this study. This finding supports the idea that SIRT3 can act as a tumor promoter and can have oncogenic consequences in breast cancer. Moreover, there was in this study a significant positive correlation between the expression levels of cMYC and P53 genes ($p = 0.000$) as previously observed in lymphoma, leukemia, non-small cell lung carcinoma and hepatocellular carcinoma (Gaidano *et al.*, 1991; Morkve *et al.*, 1992; Kawate *et al.*, 1999). c-Myc can transactivate the P53 promoter and thus may induce

expression of P53 (Reisman *et al.*, 1993), mutant P53 protein is capable of transactivating c-myc promoter, or loss of wild-type P53 alleles is coupled closely with induction of gene amplification in some cells (Kawate *et al.*, 1999). Thus, inactivation of P53 in tumors with deregulated Myc expression may be one mechanism by which cells have evaded control of tumorigenesis by cell death. We also found a significant positive correlation between the expression of SOD and HIF-1 α genes ($P = 0.012$). There are multiple sources of reactive oxygen in tumors and SOD can act as antioxidant scavenger thus these can clearly influence HIF1 activity in a hypoxia-independent way (López-Lázaro, 2007; Dewhirst *et al.*, 2008).

Correlations between Genes Expression and Clinicopathological Parameters of Breast Cancer Patients

Concerning the correlation between SIRT1-3, cancer related genes and the clinicopathological data of the patients, we observed significant positive correlations between the relative transcription levels of SIRT3 and both tumor size ($p = 0.034$) and HER2 status ($p = 0.007$); between the relative transcription levels of P53 gene and both patient's ages ($p = 0.049$) and tumor grade ($p = 0.017$); and between tumor grade and the relative transcription levels of both SOD ($p = 0.037$) and HIF-1 α ($p = 0.013$). Together, these results might mean that SIRT3 abnormalities are early events in breast tumorigenesis (Desouki *et al.*, 2014) and have a role in tumor progression, that P53 deregulation by mutation or inhibition is frequently associated with tumor progression (Fearon and Vogelstein 1990; Sidransky *et al.*, 1992; Kemp *et al.*, 1993) and finally that overexpression of SOD and HIF-1 α genes can support cancer cell progression against oxidative stress and hypoxic conditions.

Conclusion

Briefly, SIRT1 gene overexpression can have an oncogenic function in human breast cancer and can promote P53 gene expression through inactivation of P53 protein. Thus, P53 overexpression is stimulating to compensate its function and this may be one mechanism by which tumor cells evade control of cell death. Additionally, SIRT3 gene overexpression can act as an oncogene and its role is not only limited to tumor promotion but extends to play a role in tumor progression as well. On the other hand, SIRT2 gene expression is downregulated and can act as a tumor suppressor gene in human breast cancer. Our data reported that cMyc gene expression stimulates both SIRT3 and P53 overexpression and that SOD overexpression supports tumor formation and has a role in human breast cancer. In the meantime, SOD overexpression can stimulate HIF-1 α expression through

H₂O₂ production and this can influence HIF-1 activity in a hypoxia independent way. To summarize, SIRT1-3 can act not only as epigenetic markers but can have a direct important role in human breast cancer through their interaction with other genes.

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Author's Contributions

All authors contributed equally in this work.

Ethics

The authors declare that this article is original and corresponds to the ethical norms specified by the Online Journal of Biological Sciences.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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