Correlation Between ncRNA Gene Profiles and *NEIL1* Expression in Breast Cancer

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Abstract- Endonuclease VIII-like 1, a crucial DNA repair enzyme encoded by the *Neil1* gene, is essential for shielding the genome from oxidative damage. It is an essential part of the Base Excision Repair (BER) pathway, which fixes minor but dangerous DNA defects. We describe how the Neil family contributes to cancer's harmful biological characteristics. The aim is to detect some types of ncRNA genes that can be used as molecular markers for breast cancer. The methodology involves detecting the gene expression levels of the *Neil1* gene and several ncRNA genes, including miRNA‑3912‑3p, snoRNA SNHG7, and lncRNA BC069792, using the qPCR technique. The results showed increased expression levels of the Neil1 gene (2.435), miRNA‑3912‑3p (12.34), lncRNA BC069792 (6.11), and snoRNA SNHG7 (5.08) in patients with biopsy samples, while decreased expression levels were observed for all genes in blood samples compared with healthy cases. The conclusion from this study suggests that miRNA‑3912‑3p may be considered a regulatory factor for the *Neil1* gene and a potential biomarker for breast cancer.

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Introduction

Cellular DNA is continuously vulnerable to both internal and external sources of damage. Ionizing radiation, environmental pollutants, and ultraviolet (UV) radiation are examples of exogenous causes of damage. Ultraviolet radiation from sunlight is one of the main environmental factors that causes different types of DNA damage (1). One of the primary cellular processes responsible for repairing minor, non‑helix‑distorting DNA damage caused by oxidative stress, alkylation, and spontaneous base loss is the Base Excision Repair (BER) pathway (2). Reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), the hydroxyl radical (OH), and the superoxide radical (O_2^-), are major genotoxic agents that induce endogenous damage. The accumulation of reactive oxygen species can cause DNA strand breaks and oxidative damage, posing serious threats to genome integrity (3). Each cell experiences approximately 10^4 –10^5 spontaneous DNA lesions every day. Unrepaired damage can result in base mismatches, interfere with DNA transcription and replication, and contribute to aging and cancer development (4).

Globally, cancer is a leading cause of mortality. DNA damage repair is directly linked to the development and progression of cancer. The primary mechanism for repairing oxidative DNA damage is base excision repair (5). The BER pathway is initiated by several DNA glycosylases that recognize and remove damaged bases. In humans, eleven DNA glycosylases have been identified and are classified into three categories: Nei‑like monofunctional enzymes, bifunctional glycosylases, and DNA glycosylases of the NEIL family, particularly *NEIL1* (6). We describe how the NEIL family contributes to the malignant biological

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features of cancer, including cell proliferation, resistance to chemoradiotherapy, invasion and migration, apoptosis, and stemness (7). The expression of the NEIL protein family is regulated by various mechanisms, including post‑translational modifications, mRNA editing, and transcriptional control. The structural conformations and substrate specificities of NEIL family proteins exhibit both shared and distinct characteristics (8).

The Base Excision Repair pathway's *Neil1* gene is essential for repairing oxidative DNA damage in breast epithelial cells, according to recent research (9). Genomic instability greatly contributes to the initiation of breast cancer, and non‑coding RNAs (ncRNAs) have emerged as important regulators of *Neil1* gene expression, providing deeper insights into the molecular mechanisms underlying breast carcinogenesis (10). miRNAs regulate *Neil1* genes by binding to complementary sequences in the 3′ untranslated region (3′‑UTR) of *Neil* mRNA (11). Current evidence suggests that miRNA‑3912‑3p may regulate the *Neil1* gene family post‑transcriptionally, particularly *Neil1*, which encodes a crucial DNA glycosylase involved in the base excision repair pathway. By removing oxidized DNA bases, *Neil1* helps protect the genome from instability, mutagenesis, and cancer progression (12). The aim of this study is to investigate the role of non‑coding RNAs in regulating the expression of the *Neil1* gene in breast cancer.

Materials and Methods

Case of study

Eighty individuals — 60 with breast cancer and 20 serving as a control group — between the ages of 30 and 65 were examined between November and December of 2024, according to the scientific research ethics approval (permission form number 02/2025) issued by the Ministry of Health and Environment.

Primers design

The primers were designed using the Primer3 Plus (Version 4) tool, and their reference sequences were confirmed through the NCBI database, with additional verification using UCSC programs. The primers were synthesized and lyophilized by Macrogene Ltd. (Korea).

mRNA and ncRNA extraction

The Trizol kit provided by Transgeneic was used to extract mRNA and ncRNA from blood samples. Following the extraction protocol, we established a method for the simultaneous isolation of mRNA and ncRNA by applying the Trizol–phenol–chloroform principle for sample lysis and silica membrane–based purification for total RNA and ncRNAs.

Poly A polymerase (Polyadenylation)

Using DNase- and RNase-free tips at every step, the Poly A technique must be performed immediately after RNA extraction in order to enable the detection of ncRNAs during real-time PCR analysis.

Conversion to cDNA

All RNA molecules were converted into cDNA using reverse transcriptase enzyme activity (RT-PCR method) after the completion of mRNA and non-coding RNA extraction.

Determining the mRNA molecules' gene expression levels for the *Neil1* gene using the qPCR technique

qPCR was used to determine gene expression levels using specific primers for the *Neil1* gene and a housekeeping gene, as shown in Table 1:

Table 1. *Neil1* and housekeeping gene primers for mRNA molecules (13)

Primers	Sequence
***NEIL1*-F_RT**	5´CCTGATCCCCCACGACTTT´3
***NEIL1*-R_RT**	5´GCAGGTTGTCAAGCATTTC´3
H.K-F	5´GACCCAGATCATGTTTGAG ´3
H.K-R	5´CGTACAGGGATAGCACAG ´3

Determining the expression levels of ncRNA (miRNA, snoRNA, and lncRNA) genes that influence the *Neil1* gene using the qPCR technique

Specific primers designed using Primer3 software were used in this study to determine the gene expression levels of miRNA‑3912‑3p (which regulates the *Neil1* gene), snoRNA *SNHG7*, lncRNA *BC069792*, the qPCR program and reaction showed in tables 3 and 4, and the *U6* gene using qPCR, as shown in Table 2.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism software.

Table 2. miRNA, snoRNA molecules, lncRNA and housekeeping genes primers designed by primer3 software for this study

RNA	Specific gene	Primers sequence
miR-3912-3p– F	*NEIL1* gene	AACACGCTAACGCATAATATGGACA
miR-3912-3p– R	*NEIL1* gene	CAGTGCAGGGTCCGAGGT
lncRNA	BC069792 F	CCAGCCACGTTCTTCTTGGT
lncRNA	BC069792 R	AGGCCCAGTGCTGTTAAAGA
SnoRNA	SNHG7 F	GGAAGTCCATCACAGGCGAA
SnoRNA	SNHG7 R	GTCAGGATCACGCAGGACAG
U6-F	Housekeeping gene	5´ GTGCTGCTTGGGCAGCA 3´
U6-R	Housekeeping gene	5´ GAAATATGGAACGGTTC 3´

Table 3. The total volume reaction for qPCR was 20 µl

Components	Volume
SYBR Green qPCR Mix	10 µl
RT- forward primer	0,5 µl
RT- reverse primer	0,5 µl
cDNA Template	4 µl
Nuclease-Free Water	5 µl
Total volume	20 µl

Table 4. the qPCR reaction program

Stage	Temperature	Time	
Pre denaturation	95 C°	10 min	
Denaturation	95 C°	15 sec	40 cycles
Annealing -Extension	60 C°	1 min	
Melting curve analysis	95 C°	15 sec	
	60 C°	1 min	
	95 C°	15 sec	
	60 C°	15 sec	

Results

Analysis of *Neil1* gene expression in case study

The results of this study showed an increased expression level of the *Neil1* gene in biopsy samples (2.435 ± 0.3002) and a decreased expression level in blood samples (0.5315 ± 0.1280) from patients compared to healthy individuals (1.134 ± 0.3503), as shown in Table 5.

In Figure 1, the relative expression value of the Neil1 gene between biopsy samples and control samples was 0.017, while no variation was observed between blood samples and control samples.

Table 5. Relative Neil1 gene expression levels between study group

Neil1	Blood	Biopsy	Control
Mean	0.5315	2.435	1.134
Std. Error of Mean	0.1280	0.3002	0.3503

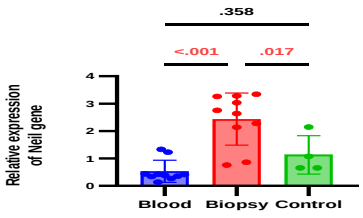

Figure 1. Gene expression levels of Neil1 gene in study groups

The study's observed increase in *Neil1* gene expression may indicate that the gene is activated in breast cancer tissue but inactivated in blood samples. This differential activity, which may be influenced by various biological factors, could reflect elevated DNA damage in breast cells, thereby driving the overexpression of the *Neil1* gene. According to recent research, the primary function of *Neil1*, which encodes the NEIL1 protein, is to repair DNA lesions within the BER pathway in cancer-related conditions. The activity of this gene is often evaluated in response to increased DNA damage.

Functional suppression of the *Neil1* gene in cancer cells can impair their ability to repair oxidative DNA damage, leading to the accumulation of mutations and contributing to genomic instability. Although this deficiency negatively affects normal cells, it can provide cancer cells with an evolutionary advantage by increasing mutational diversity, which promotes tumor progression and resistance to treatment (15).

An essential measure of the precision of the statistical estimate and the reliability of the diagnostic performance of the *Neil1* gene is the 95% confidence interval (CI), which represents the range within which the true value of the area under the curve (AUC) is expected to fall.

In this study, the confidence interval (95% CI) represents the range within which the true AUC value lies with 95% statistical confidence. The lower limit of the confidence intervals for both groups (blood and biopsy) is higher than the neutral value of 0.5, corresponding to P values less than 0.05, indicating that the results in both groups are statistically significant. This suggests that the ability of *Neil1* gene expression to distinguish between patients and the control group is genuine and not due to chance. It is also noteworthy that the confidence interval for the biopsy test begins at a higher value compared with the blood test, indicating more consistent diagnostic accuracy.

Analysis of miRNA3912-3p gene expression

The results of this study showed an increased level of miRNA-3912-3p expression in patients with biopsy samples (12.43 ± 1.001) and a decreased level in patients with blood samples (0.573 ± 0.1353) compared with healthy women (2.018 ± 0.6058), as shown in Table 6.

Table 6. Relative miRNA3912-3p gene expression levels between study group

MIR 3912-3p	Blood	Biopsy	Control
Mean	0.5732	12.43	2.018
Std. Error of Mean	0.1353	1.001	0.6058

In Figure 2, the relative expression value of miRNA‑3912‑3p between biopsy samples and healthy women was <0.001, while no variation was observed between blood samples and control samples.

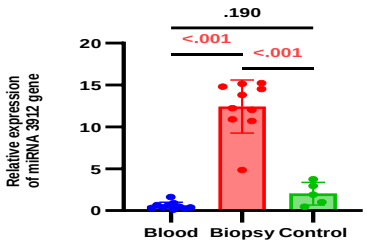

Figure 2. Gene expression levels of miRNA 3912-3p in study groups

On the other hand, miRNA‑3912 exhibits significant upregulation in tumor tissue, indicating a role in promoting malignant behavior. Because it provides cancer cells with an additional survival advantage, these cells tend to retain this miRNA intracellularly rather than releasing it into the bloodstream. It is believed that miRNA‑3912 contributes to the inhibition of tumor suppressor genes and interferes with cell‑cycle regulation and apoptotic pathways, thereby enabling cancer cells to proliferate and adapt to the harsh conditions of the tumor microenvironment (16).

The most reliable indicator of the dependability of the area under the curve (AUC) is the confidence interval. With a *P* of 0.0022, we observe a very narrow and ideal interval of (1.000-1.000) in biopsy comparisons, demonstrating the gene's complete discriminative ability and strong statistical significance as a biomarker.

In contrast, the finding loses its scientific reliability when the confidence interval for blood samples expands to (0.4329-1.000), crossing the neutral value of 0.5, along with a *P* of 0.0864 (greater than 0.05).

Statistically, this indicates that the discriminatory ability of the gene in blood samples may not be better than random chance, rendering these results clinically insignificant.

Analysis of lncRNA BC069792 gene expression

The results of this study showed that lncRNA BC069792 expression levels were markedly elevated in tumor biopsy samples (6.110 ± 0.6875) compared with healthy control tissues (1.146 ± 0.4375). In contrast, its expression was significantly reduced in the blood samples of breast cancer patients (0.6432 ± 0.1278). These findings are summarized in Table 7.

According to Figure 3, the relative expression difference of lncRNA BC069792 between biopsy and control samples was statistically significant ($P = 0.001$), whereas there was no significant variation between blood and control samples.

Table 7. Relative lncRNA BC069792 gene expression levels between study group

LncRNA BC	Blood	Biopsy	Control
Mean	0.6432	6.110	1.146
Std.Error of Mean	0.1278	0.6875	0.4375

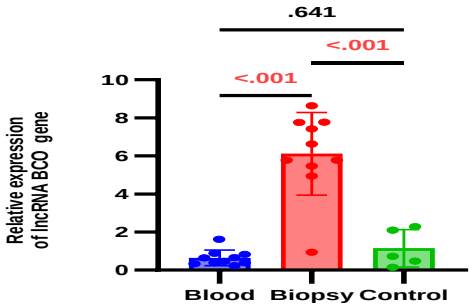

Figure 3. Gene expression levels of lncRNA BC gene in study groups

The Neil1 gene is a regulatory and DNA repair gene that works to rewire the molecular environment inside cancer cells, which is why there is a significant rise in lncRNA expression in biopsy samples. This integration promotes tumor growth and metastasis by weakening DNA repair systems, increasing genomic instability, and activating the transcription of oncogenes.

The Neil1 gene is affected by the overexpression of lncRNA levels, which disrupts the function of Neil1 gene production by either suppressing it or creating a protein that is improperly balanced. By changing the epigenetic state of its regulatory region, the lncRNA may also directly disrupt the transcription of Neil1.

According to the results, proper Neil1 function is important for human health and lifespan, and Neil1 gene abnormalities may cause a number of malignancies and associated disorders, as well as Alzheimer's disease, diabetes, obesity, and other conditions (17).

Analysis of snoRNA SNHG7 gene expression

The results in this study showed elevated expression of the snoRNA gene in biopsy samples (5.083±0.5735), and a reduction of its level in blood samples of patients (0.5329±0.1123) compared to healthy women (1.432±0.5666), as shown in Table 8.

Table 8. Relative snoRNA SNHG7 gene expression levels between study group

snoRNA	Blood	Biopsy	Control
Mean	0.5329	5.083	1.432
Std. Error of Mean	0.1123	0.5735	0.5666

In figure 4, the relative value for the expression of the snoRNA gene between biopsy and control samples was 0.004, while there was no variation between the blood samples and the control samples.

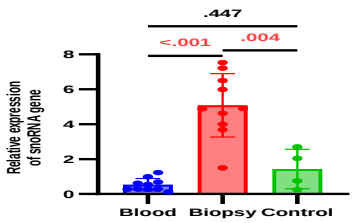

Figure 4. Gene expression levels of snoRNA SNHG7 gene in study groups

The dual pattern of snoRNA expression shown in the figure illustrates a functional shift associated with carcinogenesis, in which these molecules are activated within tumor tissue to support metabolic adaptation and rapid cell growth, while remaining suppressed in the bloodstream. By directly affecting ribosome biogenesis and indirectly influencing the regulation of key genes involved in DNA repair pathways such as the BER mechanism, including the *NEIL1* gene, snoRNAs help create a molecular environment that promotes genomic instability and the progression of breast cancer.

Correlation between miRNA-3912-3p and *Neil1* gene expression

The results obtained using Pearson's correlation showed a negative correlation between miRNA-3912-3p and Neil1 gene expression in both biopsy and blood samples (r= −0.382 and r= −0.217, respectively), with a *P* of 0.545, showed in table 9.

Table 9. Correlation between miRNA and mRNA levels between patients groups

Correlations	R	95% confidence interval	*P*
miRNA3912-3p (blood) vs. *Neil1*	-0.2177	-0.7452 to 0.4774	0.5457
miRNA3912-3p (biopsy) vs. *Neil1*	-0.3825	-0.8157 to 0.3256	0.2754

The results of the study showed an inverse relationship between miRNA molecules and DNA repair genes. The correlation value between miRNA-3912-3p and the Neil1 gene was −0.2177 in blood samples and −0.3825 in biopsy samples. Therefore, miRNA-3912-3p may act as an inhibitory regulator; that is, higher miRNA expression levels lead to a decrease in DNA repair gene expression, and vice versa.

In the present study, we report for the first time that miRNA-3912-3p may be considered a regulatory factor of the Neil1 gene and a potential biomarker for breast cancer. In addition, lncRNA and snoRNA may also have direct roles in breast cancer cases.

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