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Computational Analysis of Interleukin 17A Activity in Breast Cancer Patients Using Bioinformatics Methods

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Abstract

In the current study, bioinformatics approach has been utilized to discover the sequences and structures analysis of IL-17A of breast cancer and compares with normal sequence from NCBI. The current study was aimed to discuss the possibility of using IL-17A as a marker for patients with breast cancer. Also, the effect of mutation on the physicochemical properties and structure of IL-17A. Sixty blood samples were examined from patients with breast cancer (aged between 20 and 75 years old). In patients with breast cancer, there were missense mutations and deletion mutations detected by BLAST. Furthermore, the current study determined the physicochemical properties of IL-17A, such as their hydrophilic nature; alpha–helical structure, and 3D structure. The results of this study show that IL-17A consider as a marker for the patient with breast cancer. Also, the mutations on IL-17A gene affected the structure and physicochemical properties of the Interleukin-17A protein complex.

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1. Introduction

This study was contributed to analyze and understand the function of Interleukin-17A protein using bioinformatics methods. Bioinformatics is the field that binds three sciences, biology, mathematics, and computer, to create the biological information for the macromolecules of organisms like DNA, RNA, and proteins focus on their compositions, genetic sequences, transcription, and the translation expression process [1]. Interleukin-17A is one of the Interleukin-17 family of pro-inflammatory cysteine knot cytokines [2]. The human IL-17A gene is 1874 base pairs long in the short arm of chromosome 6 region one band two sub-band 2 (Chr. 6 p12.2) with three exons, two introns. In rodents, IL-17A used to be referred to as CTLA8. After the similarity with a viral gene, the protein encoded by this gene is a pro inflammatory cytokine produced by activated T cells. This cytokine regulates the activities of NF-kappa B and mitogen-activated protein kinases. In addition, this cytokine can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2) and enhance the production of nitric oxide IL-17A [3]. High levels of this cytokine are associated with several chronic inflammatory diseases, including rheumatoid arthritis, psoriasis, and multiple sclerosis. Breast cancer is a group of diseases where cells in breast tissue keep changing and dividing uncontrollably, which typically forms a lump or mass [4]. This study aims to analyze sequence, compute, and predicting structures of IL-17A in patients with breast cancer using a bioinformatics tool and there are other studies that used the same science to study different genes [5-7].

2. Materials

2.1. Instruments and Equipment

Table 1: The instruments and equipment used in this study.

NO.	Equipment	Company /Origin
1	AURA TM PCR Cabinet	Italy
2	Balance	Kern PCB / Germany
3	Bio TDB-100, Dry block thermostat built	Bio San/ Germany
4	Combi-spin	Biosan/ Latvia
5	Electrophoreses	CBS, Scientific/ USA
6	Incubator	Jrad/ China
7	Microwave	Gosonic/ China
8	Microspin	Biosan/ Latvia
9	Mini-Power Supply 300V, 2200V	China
10	Multi Gene OptiMax Gradient Thermal Cycler	Labnet/ USA
11	Multiple Micropipettes Variable Volume (2-1000) ul	Slamed /Japan
12	UV.transmission	Vilberlourmat / France
13	V-1 plus, Personal Vortex for tubes	Digsystem/ Germany
14	Water distilater	China

2.2. Chemicals and Kits

Table 2: The chemicals and kits used in this study.

NO.	Material	Cat#	Company /Origin
1	Agarose	8100.11	Conda / USA
2	EasyPure® Blood Genomic DNA Kit	EE121	TransGenbiotech./china
3	2×EasyTaq PCR SuperMix	AS111-01	TransGenbiotech./china
4	6X Loading dye	21161	Intron / Korea
5	Ladder 100bp	KK6302	Kapa /USA
6	Pre mix pcr	25025	Intron / Korea
7	Primer	---	IDT/Canada
8	Red safe Nucleic acid staining souluion	21141	Intron / Korea
9	TBE buffer 10 X	IBS.BT004	Conda / USA

3. Experimental Procedure

3.1. Blood Sample Collection

This study included 60 human patients with breast cancer. Their ages were between 22 to 75 years; for each patient, drawn three milliliters of whole blood for patients was obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. In addition, whole blood was collected (about two milliliters) in a sterile EDTA tube for a genetic test. The blood samples were stored at –20°C [8].

3.2. DNA Extraction

The DNA was extracted and purified from human whole blood samples using the EasyPure® Blood Genomic DNA Kit. After the DNA was extracted, gel electrophoresis was used to confirm the presence of extracted DNA and detect the result of PCR during the presence of the standard DNA to distinguish the band size of the outcome of PCR the Agarose gel [9]. Electrical power was turned on at 70 volts for 1 hour then, the DNA moves from the cathode (-ve) to anode (+ve) poles. The Ethidium Bromide stained bands in the gel were visualized using, UV transilluminator at 365 nm and photographed [10].

3.3. Primer Design for IL-17A Gene

The whole IL-17A gene sequence was taken from the Genome Database of the national center for biotechnology information (NCBI), the NCBI reference sequence: NC_000006.12. Using primer3 software for design the rs2275913 of IL-17A [11, 12]. The forward primer sequence was 5'- GGCCAAGGAATCTGTGAGGA -3', and the reverse primer sequence was 5 GGGATGGATGAGTTTGTGCC -3'. The product size of rs2275913 was 441bp. The primers provided by Alpha DNA company/Canada as a lyophilized product for using the primers in PCR.

3.4. Polymerase Chain Reaction PCR

The PCR reaction was carried out in a volume of 25 µl. According to the manufacturer's instruction, the Master mix of EasyTaq® PCR SuperMix (Alpha DNA company, Canada) consists of 12.5µl. Where 4µl template DNA, 1 µl of each (forward and reverse), and 6.5µl of Nuclease free water (N.F.W). Electrophoresis (1 h at 70 V) was performed with the reaction solution (1% for DNA extraction, 2% for PCR amplification) with 1% agarose gel and 7µl of ethidium bromide. PCR products were visualized under UV light and photographed. The DNA ladder (100bp to 1500bp) The result of PCR (Polymerase Chain Reaction) for patients with breast cancer gave clear band after PCR analysis of the analyzed IL-17A gene, sent to macrogen company/Korea for sequencing rs2275913 of IL-17A gene for each sample.

3.5. Translation of the DNA Sequence of IL-17A Gene to Amino Acids Sequence

BLASTX server was used for translation DNA nucleotide to amino acids (https://blast.ncbi.nlm.nih.gov/ Blast. cgi) [13].

3.6. Physicochemical Properties Prediction

ProtParam online tool was used (http://us.expasy.org/ tools/protparam.html) for detection of Physicochemical properties of the patient's IL-17A with breast cancer, in addition to the IL-17A that was retrieved from NCBI. The parameters computed by the ProtParam server include the molecular weight, theoretical pI, amino acid composition, atomic composition, instability index, aliphatic index, and grand average of hydropathicity [14].

3.7. Protein Structure Prediction

PSIpred online program was used for Secondary structure predicting, where the FASTA format of the sequence was given as input [15, 16]. It provides the structural information of the protein sequence in form of coils, helices and using PHYRE2 software for 3D-structure prediction to the sequence [17]. The 3D-structure prediction involves four steps, first searching structure showing homology with the target sequence, then selecting the best template having maximum identity with the target sequence, which follows its alignment with the target and modeling the structure. On the other hand, the swiss-model is an automated system for modeling a protein's quaternary structure from its amino acid sequence using homology modeling techniques [18].

4. Results and Discussion

Genomic DNA from the blood samples was extracted following the standard protocol used in genetic studies [19]. The quantification of DNA measured by Nanodrop had revealed that the DNA concentration ranged (3-5) ng/ìl and purity ranged was (1.6-1.8). The result demonstrated that the extracted DNA's purity in all samples was sufficiently high for PCR analysis. PCR technique was used in this study for DNA isolation from the patients' blood to amplify the rs2275913 of the IL-17A gene using a specific IL-17A primer designed by primer3 software and obtained from Alpha DNA company, Canada. The PCR results were explained by the presence or absence of specific bands of an amplified gene on 1% agarose. In the current study, the primer was screened for PCR analysis using 60 DNA samples extracted from the whole blood of patients. The results of using the primer showed amplified fragment (441bp) a clear band by electrophoresis on a 1% agarose gel at 70 volts for 60 minutes, of 60 DNA samples as shown in (Figure 1). The results of the IL-17A gene amplification using PCR analysis shows that all patients gave positive result (bands formation). This result showed that the product of amplified primers of the IL-17A gene was found in all patients.

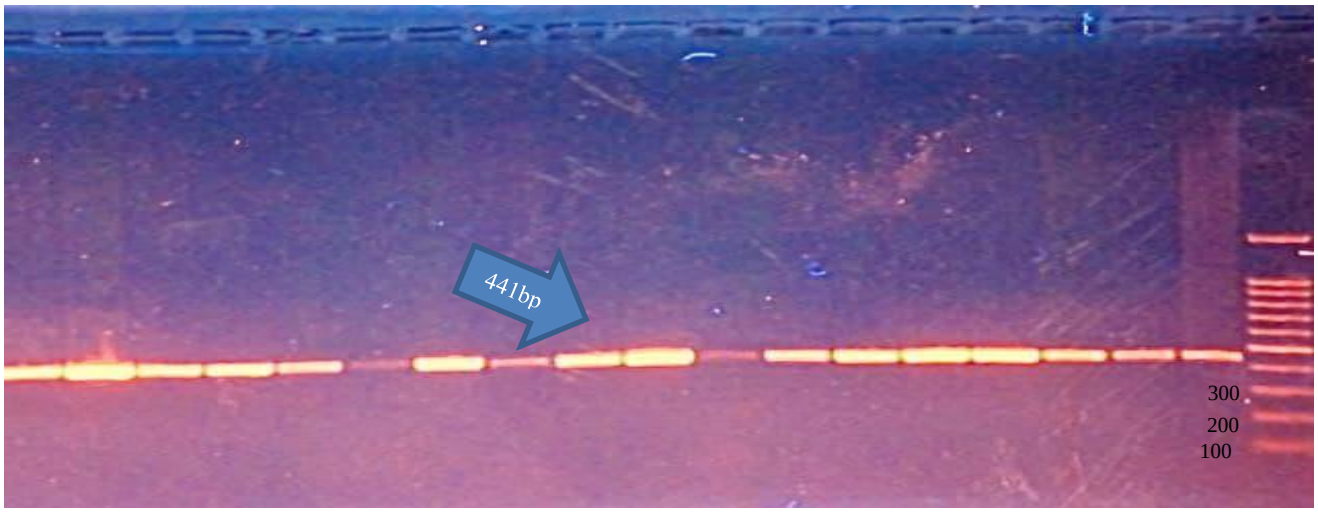

Figure 1: Detection of PCR product DNA bands of IL-17A gene using rs2275913 primers (441bp).

The amplified fragments were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide at 70 volts for 60 minutes, and photographed under UV light. Lanes (1-10) patient with breast cancer gave band for an amplified fragment. M: Marker DNA ladder size (100bp to 1500bp).

4.1. Interleukin-17A Gene Sequencing

In this study, the forward primer was used for patients with breast cancer by direct sequencing (Figure2) and the reverse primer in Fig. 3. Sequence analysis of the IL-17A gene for the PCR product of the primer indicated many genetic alterations. The mutations affect translation, and the result revealed missense mutations and deletion mutations in patients with breast cancer (Table 1).

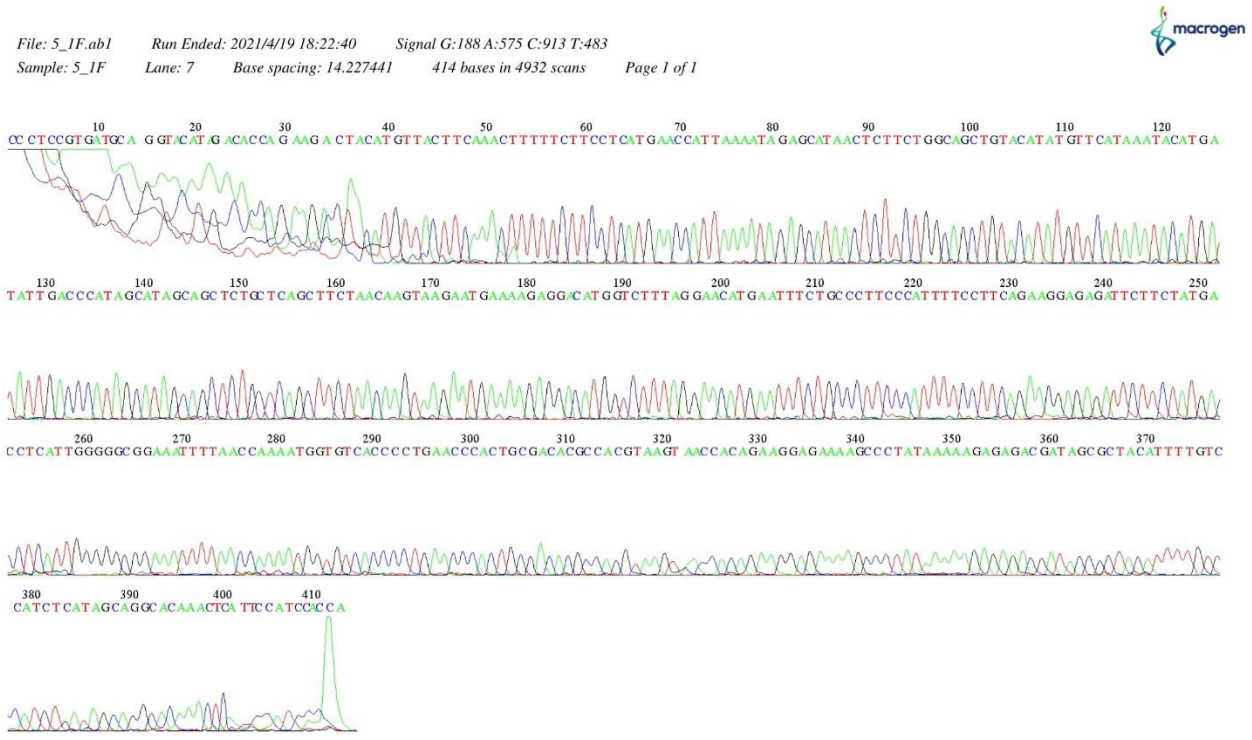

Figure 2: The chromatogram for the forward sequence of amplified the IL-17AF primer for a patient with breast cancer by DNA sequencer.

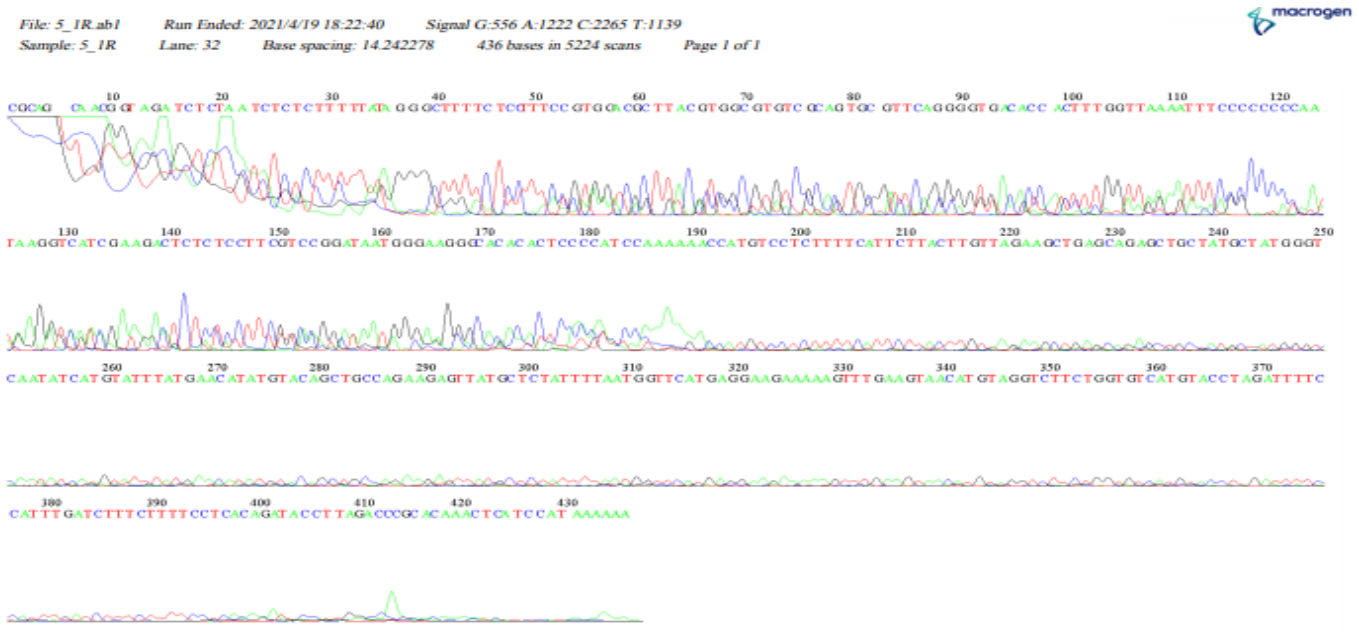

Figure 3: Show the chromatogram for the forward sequence of amplified the IL-17AR primer for a patient with breast cancer by DNA sequencer

Table 3: The wild and mutant genetic code of DNA, type of mutation, and the effect of mutation on the translation process in some patients' proteins with breast cancer in rs2275913 of IL-17Agene.

Patient sample number	Wild genetic code of DNA	Mutant genetic code of DNA	Type of mutation at DNA level	Wild genetic code of RNA	Mutations in genetic code of RNA	Effect of the translation
1	AGA	AAA	Transition	AGAArg	AAALys	Missense
2	CCT	CTT	Transition	CCUPro	CUULeu	Missense
3	CAG	C-G	Deletion	CAGGlu	C-G	Frameshift
4	ACC	AAC	Transversion	ACCThr	AACThr	Silent
5	CAG	CTG	Transversion	CAGGln	CUGLeu	Missense
6	CCA	TT-	Deletion	CCAPro	UU-	Frameshift
7	CTG	CGG	Transversion	CUGLeu	CGGArg	Missense
8	CAG	CTG	Transversion	CAGGln	CUGLeu	Missense
9	CTG	CGG	Transversion	CUGLeu	CGGArg	Missense
10	AGA	AAA	Transition	AGAArg	AAALys	Missense
11	TGT	TCT	Transversion	UGUCys	UCUSer	Missense
12	CTG	CGG	Transversion	CUGLeu	CGGArg	Missense
13	CAG	C-C	Deletion	CAGGln	C-C	Frameshift
14	CAG	C-G	Deletion	CAGGlu	C-G	Frameshift
15	CTG	CGG	Transversion	CUGLeu	CGGArg	Missense

4.2. Sequence Analysis

Primary structure analysis provided the physicochemical properties of IL-17A. The molecular weight of IL-17A for patients with breast cancer was (15185.56 MW). In contrast, the molecular weight for IL-17A retrieved from NCBI FASTA format protein was (14687.63MW). The isoelectric point of a protein is an essential feature because it is at this point that the protein is least soluble [20, 21]. The isoelectric point was computed for IL-17A patients with breast cancer was 10.01, while the pI for IL-17A that retrieved from NCBI was 9.91 below 10, so

they are likely to precipitate in acidic buffers. This result agrees with studies of [18, 22] that stated that the mutation causes significant changes in the sequences. This affected on physiochemical properties of the protein, especially on protein stability

4.3. Structure Analysis

The secondary structure of this study gave the following alpha helix predicted values 27.41% IL-17A of patients with breast cancer, while the alpha helix prediction of IL-17A that retrieved from NCBI was 29.83%. The secondary structure of this study gave the following β- turn predicted values 8.87% for IL-17A of patients with breast cancer while β- turn prediction of IL-17A was retrieved from NCBI was 7.25%. The secondary structure of this study gave the following random coil predicted values 63.72% for patients with breast cancer, while the random coil prediction of IL-17A that retrieved from NCBI was 62.92%. For IL-17A of a patient with breast cancer, out of all the templates given by the PHYRE2 server, the one with the highest % i.d. (100 %) was used to predict the 3D structure. For IL-17A of a patient with breast cancer, For IL-17A retrieved from NCBI, out of all the templates given by the PHYRE2 server, the one with the highest % i.d. (100%) was used to predict the 3D structure. The current study recorded the mutations on IL-17A gene for patients with breast cancer caused different effects on the structure of protein compared with IL-17A retrieved from NCBI as shown in the result of structure analysis such as the numbers and positions of alpha helix, β- turn and coil. This result agrees with studies of [18, 22] stated that mutation cause large changes in the sequences that affect the structurally of protein. Although some studies show the effect of certain molecules or substances on some diseases [23-25], the present study has shown that pathways of protein folding are largely unaffected by changes in the sequence.

5. Conclusions

In the current study, IL-17A is considered a marker for patients with breast cancer. The genotype at site 52186234 was found to be associated with cancer disease. The mutations on IL-17A gene for patients with breast cancer affect the physicochemical properties of the Interleukin-17A protein complex such as molecular weight, pI, percentage of amino acid, and protein stability compared with IL-17A retrieved from NCBI. In addition, the mutation affected on structures of IL-17A for the current study by changing the number and position of alpha helix, β-turn, and coil. This affected IL-17A, which led to the loss of host defense IL-17A for patients with breast cancer.

Conflict of Interest

The authors declare that they have no conflict of interest.

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