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Analysis of sequence and structure for beta-2-microglobulin in patients with thyroid cancer using bioinformatics tools

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ABSTRACT

Bioinformatics tools used to employ the technology that utilizes computers to manage biological data, to analysis the sequence and structure of the protein. The current study aimed to compute the physio-chemical properties and predicting structures of Beta-2-microglobulin (B2M) in patients with thyroid cancer, 14 patients with thyroid carcinoma included in this study. The DNA was extracted and B2M gene was amplified by using specific primers for three exons of this gene. Thirty-two mutations found in the patients for first and second exons while in third exon no mutations detected. In the first exon, a point mutation at the site 44,711,557 observed in all patients except one patient. In the second exon a point mutation at the site 44,715,448 observed in all patients except two patients, these mutations recorded in the GenBank at NCBI, ENA and DDBJ databases with the number LC424501 and LC424502, respectively. The results of the ProtParam program showed that the mutations affected the physio-chemical properties of B2M protein for patients compared to B2M protein retrieved from NCBI. Also, results of secondary structure prediction of B2M protein by SOPMA tool showed that the mutations affected the percentages of alpha helix, extended strand, beta turn and a random coil of B2M protein for patients compared to B2M protein retrieved from NCBI. The results of the RaptorX server showed that the impact of mutations was clear on the tertiary structure of B2M protein for patients compared to B2M protein retrieved from NCBI.

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INTRODUCTION

Bioinformatics is an interdisciplinary research field at the mediator between biological science and computer science (Diniz and Canduri, 2017). Beta-2-microglobulin (B2M) is a protein encoded by B2M gene mapping on the long arm of chromosome 15 at position (15q21) (Maleno *et al.,* 2011). It belongs to the superfamily of immunoglobulin, the primary and secondary structure of B2M is similar to immunoglobulin G (IgG) structure (Svatonova *et al.,* 2014). Structurally, B2M protein is a component of major histocompatibility class I (MHC-1) which is also called human leukocyte antigen (HLA) in human and non covalently linked with it, the MHC-1/B2M complex found on the surface of all nucleated cells (Amighi *et al.,* 2011) to function in the presentation of foreign peptides fragments to cytotoxic CD8+ T cells (Margalit *et al.,* 2006), also acts as a chaperone during the assembly or folding of MHC-1 quaternary structure (Cresswell *et al.,* 2005). Thyroid cancer is the most common malignant diseases in the endocrine system and is rapidly increasing in incidence (Jemal *et al.,* 2011, Shahad F Obeid *et al.,* 2018). The incidence of thyroid cancer is about (three to four times) higher among women than men worldwide. It can occur at any age, but in childhood is rare (Katoh *et al.,* 2015). This study aims to analyze of

sequence, compute and predicting structures for B2M in patients with thyroid cancer using bioinformatics tools.

MATERIALS AND METHODS

The study included 14 patients with thyroid cancer (7 males and 7 females), their ages between 18 and 76 years, the patients selected from Al-Amal National Hospital for Cancer Treatment in Baghdad province/Iraq during the period from October 2017 to November 2017. Blood samples were collected from each patient that were stored at (−20 °C) until analysis (Ouda *et al.,* 2015). Written informed consent was taken from all patient, the study approved by Department of the Applied Sciences University of Technology as part of the MSc thesis.

The human DNA extracted from the whole blood samples using gSYNC™ DNA Extraction Kit (Cat. No.: GS100**/** Geneaid company**/** Taiwan**)** according to the manufacturer's protocol. Then agarose gel electrophoresis used to confirm the integrity and presence of extracted DNA (Green and Sambrook, 2012, Al-Radeef *et al.,* 2018).

The B2M gene sequences were taken from the genome database (GenBank) of the "national centre for biotechnology information" NCBI, where the NCBI reference sequence: NC_000015.10. Three primers (B2M -1, B2M -2 and B2M -3) for three exons of B2M gene designed using primer3plus program (Untergasser *et al.,* 2007) and provided by Bioneer Company/Korea. The names and sequences of forwarding and reverse primers are shown in Table (1).

Three primers (B2M -1, B2M -2 and B2M -3 primers) used to amplify first, second and third exons of B2M gene, respectively by polymerase chain reaction (PCR) using AccuPower® PCR PreMix kit (Bioneer Company/Korea) according to the manufacturer's instructions, where 5µl of template DNA, 2µl of each primer (forward and reverse) and 11µl of deionized sterile distilled water were added to AccuPower® PCR PreMix tube. The thermal cycling conditions programmed using a thermal cycler PCR to amplify the target DNA as shown in Table (2). The PCR products were electrophoresed on 2% agarose gel, the gel was visualized using UV transilluminator after staining with ethidium bromide and the size of amplified fragments was checked and photographed. The amplified fragments were estimated using DNA ladder 100 bp (Bioneer Company/Korea).

Amplified PCR products of B2M gene for 14 patients with thyroid cancer were sent to Macrogen Company/Korea for sequencing the three exons of the B2M gene for each sample. The sequences of B2M gene for patients with thyroid cancer compared with B2M gene reference sequence in the GenBank at NCBI by Basic Local Alignment Search Tool (BLAST) program (Altschul *et al.,* 1990) to detect variations in the gene sequences.

The European Molecular Biology Open Software Suite Translate nucleic acid sequences (EMBOSS Transeq) tool (https://www.ebi.ac.uk/Tools /st/emboss_transeq/) (Syromyatnikov *et al.,* 2018) and used for translation of nucleotides sequence of B2M gene for patients with thyroid cancer to amino acids sequence, while amino acids sequence of B2M protein was retrieved from NCBI. The Protein Parameters (ProtParam) program (https://web.expasy.org/protparam/) (Adekiya *et al.,* 2017) used for compute physicochemical parameters of B2M protein for patients with thyroid cancer and B2M protein retrieved from NCBI.

Using "Self Optimized Prediction Method with Alignment" SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) (Sun *et al.,* 2017) for secondary prediction structure of B2M protein, also B2M protein retrieved from NCBI and computes the percentages of alpha helix, extended strands, beta turn and random coil. The RaptorX server (http://raptorx.uchicago.edu/) was used for tertiary prediction structure of B2M protein and B2M protein retrieved from NCBI, it predicts the quality of alignment between the target (protein sequence of interest) and template (sequence of known structure) to estimate the similarity between the target and template, and then by arranging all the candidate templates according to the predicted quality (Kallberg *et al.,* 2012).

RESULTS

Detection of Three Exons of B2M Gene by PCR Technique

The results were demonstrated using the first set of primer (B2M -1F and B2M -1R) to amplify the first exon of B2M gene in patients with thyroid cancer by PCR technique showed the amplified segments had size (113 bp) and it gave a clear bands using gel electrophoresis on 2% of agarose gel as shown in Figure (1).

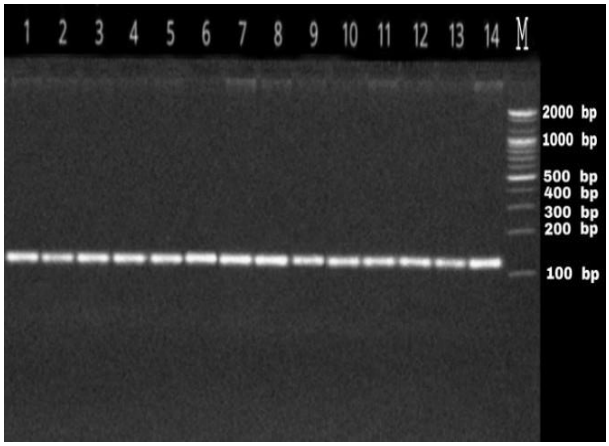

Figure 1: PCR products electrophoresed on 2% agarose gel, showing a clear band had size 113 bp for the first exon of B2M gene. Lane M: 100 bp DNA Ladder. Lane (1-14): Bands of amplified fragment for thyroid cancer patients

The results were demonstrated using the second set of primer (B2M -2F and B2M -2R) to amplify the second exon of B2M gene in patients with thyroid cancer by PCR technique showed the amplified segments had size (337 bp) and it gave a clear bands using gel electrophoresis on 2% of agarose gel as shown in Figure (2).

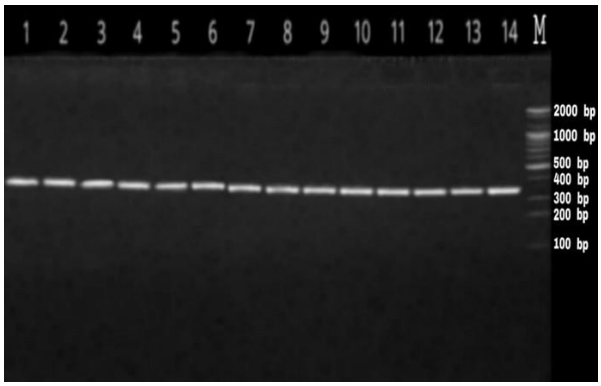

Figure 2: PCR products electrophoresed on 2% agarose gel, showing a clear band had size 337 bp for the second exon of B2M gene. Lane M: 100 bp DNA Ladder. Lane (1-14): Bands of amplified fragment for thyroid cancer patients.

The results were demonstrated using the third set of primer (B2M -3F and B2M -3R) to amplify the third exon of B2M gene in patients with thyroid cancer by PCR technique showed the amplified segments had size (101 bp) and it gave a clear bands using gel electrophoresis on 2% of agarose gel as shown in Figure (3).

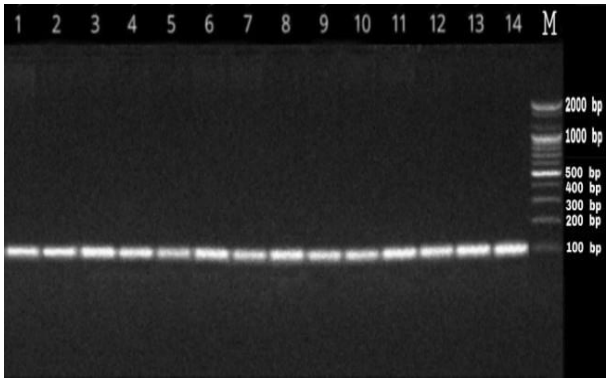

Figure 3: PCR products electrophoresed on 2% agarose gel, showing a clear band had size 101 bp for the third exon of the B2M gene. Lane M: 100 bp DNA Ladder. Lane (1-14): Bands of amplified fragment for thyroid cancer patients.

The results were demonstrated using the third set of primer (B2M -3F and B2M -3R) to amplify the third exon of B2M gene in patients with thyroid cancer by PCR technique showed the amplified segments had size (101 bp) and it gave a clear bands using gel electrophoresis on 2% of agarose gel as shown in Figure (3).

Sequencing and Alignment of Sequence of B2M Gene

The results of first, second and third exons of B2M gene sequences for 14 patients with thyroid cancer were received from Macrogen Company/Korea, then analyzed using BLAST program, where the results showed existence 32 mutations for patients in the sequence of first and second exons while in the third exon of the B2M gene, not mutations were

Table 1: The wild and mutant genetic code of DNA, type of mutations, site of mutations and the effect of mutations on the process of translation for a protein of patients with thyroid cancer in the sequence of first and second exons of B2M gene

No. of patient	No. of exon	Wild-type of DNA	Mutant type of DNA	Site of mutation	Type of mutation	Change in amino acids	Effect on translation
1	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
2	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
3	First		Not	detected	any	mutation	--
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
4	First	ATG	AAG	44711548	substitution	Methionine (start codon)-Lysine	MM
	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
5	First	TCT	TAT	44711551	substitution	Serine-Tyrosine	MM
	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
6	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
7	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
8	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
9	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
10	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	GTT	GAT	44715441	substitution	Valine-Aspartate	MM
	Second	TCA	TCG	44715448	substitution	Serine-Serine	SM
11	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
12	First	TCT	TAT	44711551	substitution	Serine-Tyrosine	MM
	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	First	GCC	G-C	44711563	deletion	Alanine-Del.	FM
	Second		Not	detected	any	mutation	
13	First	TCT	TAT	44711551	substitution	Serine-Tyrosine	MM
	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM
14	First	TCC	T-C	44711557	deletion	Serine-Del.	FM
	Second	TCA	TC-	44715448	deletion	Serine-Del.	FM

FM- Frameshift mutation; MM-Missense mutation; SM- Silent mutation

detected as shown in Table (3), also the results showed five missense mutations, twenty-six frameshift mutations and one silent mutation in patients with thyroid cancer, where the mutations affected the process of translation for protein as shown in Table (3).

In all patients with thyroid cancer except (patient No. 3), a point mutation has been observed in the same position and it had the same effect in the sequence of the first exon for B2M gene when the cytosine was deleted from the codon for serine at the site 44,711,557 and affected the process of translation for protein causing frameshift mutation as shown in Table (3), and Figure (4) shows the mutation at the site 44711557 in the first exon sequence of B2M gene for a patient out of all patients with thyroid cancer compared to the reference sequence of B2M gene in the GenBank at NCBI

as part of the comparison results by the BLAST program.

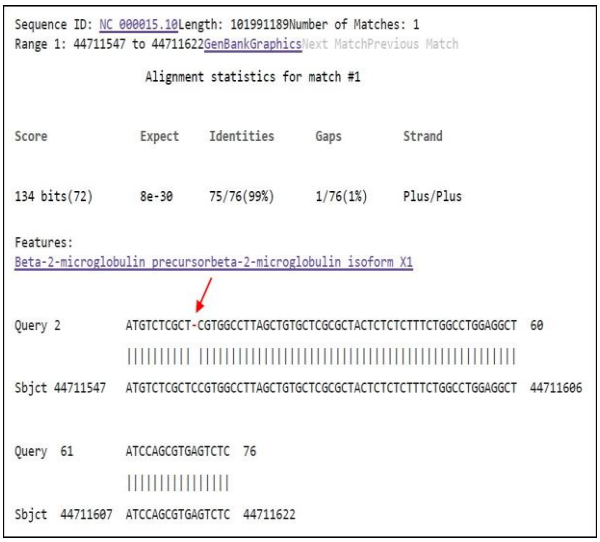

Figure 4: The results of comparison between the sequence of first exon of B2M gene for patient No.1 (query) and reference sequence of B2M gene in the GenBank at NCBI (sbjct) by BLAST program, where the mutation appeared at the site 44,711,557 (indicated by the red arrow) by deletion of cytosine (C) from the sequence of the first exon for B2M gene of the patient.

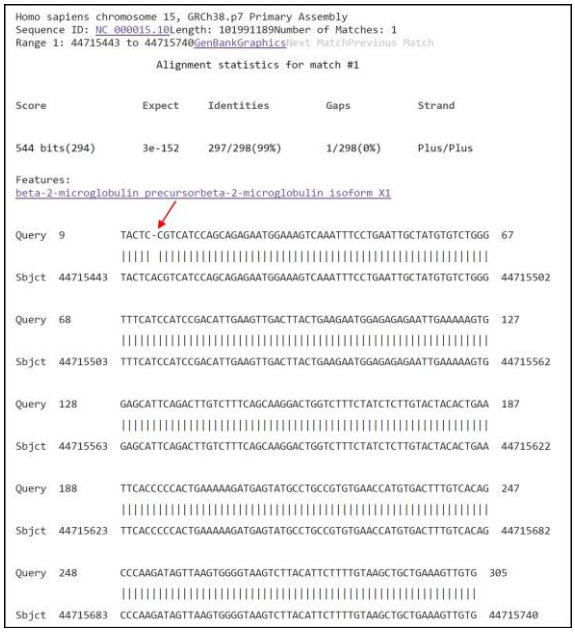

Figure 5: The results of comparison between the sequence of the second exon of B2M gene for patient No.1 (query) and reference sequence in the GenBank at NCBI (sbjct) by BLAST program, where the mutation appeared at the site 44,715,448 (indicated by the red arrow) by deletion of adenine (A) from the sequence of second exon for B2M gene of the patient.

Also in all patients with thyroid cancer except (patient No.10 and patient No.12), a point mutation was observed at the same position and it had the same effect in the sequence of the second exon for B2M gene when the adenine was deleted from the codon for serine at the site 44715448 and affected the process of translation for protein causing frameshift mutation as shown in Table (3), and Figure (5) shows the mutation at the site 44715448 in the second exon sequence of B2M gene for a patient out of all patients with thyroid cancer compared to the reference sequence of B2M gene in the GenBank at NCBI as part of the comparison results by the BLAST program. These mutations in the sequence of first and second exons of B2M gene were recorded in the GenBank at NCBI, European Nucleotide Archive (ENA) and DNA Data Bank of Japan (DDBJ) databases with the number LC424501 and LC424502, respectively.

Sequence Analysis

The primary structure of B2M protein for thyroid cancer patients and B2M protein retrieved from NCBI were analyzed and computed the physiochemical properties by ProtParam program, where the results are shown in Table (4). The results of ProtParam program showed that the molecular weight (M.W.) of B2M protein decreased in samples of patients with thyroid cancer as compared with M.W. of B2M protein retrieved from NCBI as shown in Table (4). ProtParam program predicted that B2M protein for patients with thyroid cancer was unstable, while B2M protein retrieved from NCBI was stable as shown in Table (4). Also, the results of this paper revealed that the isoelectric points (pI) of B2M protein for patients with thyroid cancer were (pI > 7), while pI of B2Mprotein retrieved from NCBI was (pI < 7) as shown in Table (4), this means that the mutations had a significant effect on the physiochemical properties.

Structures Analysis

The secondary structure of B2M protein was predicted by SOPMA tool and the results revealed that the mutations affected and changed the percentages of the extended strand, alpha helix, random coil and beta-turn of B2M protein for thyroid cancer patients compared to B2M protein retrieved from NCBI are shown in Table (5). The tertiary structure (3-D structure) of B2M protein was predicted by RaptorX server and the results showed that the mutations affected the tertiary structure of B2M protein for thyroid cancer patients as compared with the structure of B2M protein retrieved from NCBI. Moreover, this study had shown that changes in the B2M protein sequence caused by mutations did not largely affected the protein folding process.

Table 2: The results of physiochemical properties of B2M protein for patients with thyroid cancer and B2M protein retrieved from NCBI by Prot Param program

	Patients With Thyroid		Cancer
No. of patient	The molecular weight (M.W.)	Instability index (II) *	Isoelectric point (pI)
1	13049.19	61.80	9.73
2	13049.19	61.80	9.73
3	12626.13	53.25	9.72
4	13046.17	57.84	9.86
5	13125.28	56.71	9.65
6	13049.19	61.80	9.73
7	13049.19	61.80	9.73
8	13049.19	61.80	9.73
9	13049.19	61.80	9.73
10	13027.58	61.35	10.39
11	13049.19	61.80	9.73
12	12842.80	57.80	8.95
13	13125.28	56.71	9.65
14	13049.19	61.80	9.73
	Retrieved	From	NCBI
	13714.57	33.82	6.06

* The value of instability index less than 40, the protein is stable. The value more than 40, the protein is unstable.

Table 3: The percentages of the extended strand, alpha helix, random coil and beta turn in a secondary structure of B2M protein for thyroid cancer patients and B2M protein retrieved from NCBI by SOPMA tool.

	Patients	With	Thyroid	Cancer
No. of patient	Alpha helix	Extended strand	Beta-turn	Random coil
1	34.23%	24.32%	7.21%	34.23%
2	34.23%	24.32%	7.21%	34.23%
3	43.86%	16.67%	6.14%	33.33%
4	31.53%	24.32%	7.21%	36.94%
5	33.33%	25.23%	7.21%	34.23%
6	34.23%	24.32%	7.21%	34.23%
7	34.23%	24.32%	7.21%	34.23%
8	34.23%	24.32%	7.21%	34.23%
9	34.23%	24.32%	7.21%	34.23%
10	49.56%	12.39%	3.54%	34.51%
11	34.23%	24.32%	7.21%	34.23%
12	27.68%	28.57%	12.50%	31.25%
13	33.33%	25.23%	7.21%	34.23%
14	34.23%	24.32%	7.21%	34.23%
	Retrieved	From	NCBI	
	20.17%	30.25%	8.40%	41.18%

DISCUSSION

The presence of point mutations at the site 44,711,557 in the sequence of the first exon or the site 44,715,448 in the sequence of the second exon for B2M gene in a large number of patients with thyroid cancer indicates a relationship between these mutations in the sequence of gene for patients and this type of cancer, so it is possible to consider these mutations as one of the causes of thyroid cancer. These results were compatible with the studies (Serra *et al.,* 2001, Wiest *et al.,* 2003) which revealed that neurofibromatosis was caused by "point mutations" in the neurofibromin 1 gene. Also, these results were compatible with another study which revealed that cystic fibrosis is caused by a "mutation" in the CFTR gene (Bobadilla *et al.,* 2002).

The B2M protein for thyroid cancer patients was unstable while B2M protein retrieved from NCBI was stable, where the instability index was used to estimate protein stability. The instability index less than 40, was predicted as stable. The value more than 40, predict the protein may be unstable (Kumar *et al.,* 2018).

The pI of B2M protein for thyroid cancer patients were more than 7 while pI of B2M protein

retrieved from NCBI was lower than 7, where the pI is the pH at which the molecule carries no net electrical charge, and it is useful for understanding stability of the protein charge, where depending on the pH of the buffer, which determines the solubility and migration of the protein. If the pH of the buffer is more alkaline (>) than pI, the charge of protein becomes negative. If the pH of the buffer is more acidic (<) than pI, the charge of the protein is positive (Kumar *et al.,* 2018, Kucukkal *et al.,* 2015), this means that mutations had a significant effect on the physical and chemical properties of the protein. Also, the results of this paper are in agreement with studies of (Kucukkal *et al.,* 2015, Kamel *et al.,* 2018) demonstrating that the mutations causing large changes in the protein sequences and this affected the physio-chemical properties of the protein, particularly on protein stability.

The mutations in the sequence of B2M gene were caused various effects on the secondary structure for B2M protein by changing the percentages of an extended strand, alpha helix, random coil and beta turn, and tertiary structure of B2M protein for patients with thyroid cancer compared to B2M protein retrieved from NCBI. These results are in agreement with the study (Won *et al.,* 2011) which revealed that mutations caused large changes in the protein sequences that affected the secondary structure of the protein. Also, the results are in agreement with studies (Kamel *et al.,* 2018, Ahmed *et al.,* 2014) which demonstrated that mutations caused large changes in the sequence of a protein that affected the tertiary structure of the protein.

CONCLUSIONS

The mutations at the site 44,711,557 and site 44,715,448 were detected to be associated with thyroid cancer. The mutations in the sequence of B2M gene for patients with thyroid cancer effect on the physio-chemical properties of B2M protein like M.W., pI and stability of protein as compared with B2M protein retrieved from NCBI. Also, the mutations affected the structures of B2M protein by changing the percentages of alpha helix, extended strand, beta turn and random coil, and tertiary structure of B2M protein which may result to change in protein function by not bind with the HLA complex.

Conflicts of Interests

All authors have none to declare

Author contributions

All author contributed equally

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