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hSSB1 (NABP2) as Prognostic for Worker's Exposure to Oxidative Stress

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Abstract

Background: Oxidative stress can be defined as a phenomenon brought on by an imbalance between biological system's capacity for detoxifying such reactive products and the formation and build-up of ROS in tissues and cells. X-rays have an effect on the systems of the human body, hSSB1 (NABP2) plays a crucial role in DNA damage responses by taking part in a repair regarding double-strand DNA breaks and the base excision repair of oxidized guanine residues (8-oxoguanine). Here, we show that hSSB1 stabilizes as an oligomer in response to oxidative stress, which is necessary for the hSSB1 to remove 8oxoguanine.

Methods: In this study, the detection of NABP2 (SSB-I) (Single-stranded DNA binding protein (ATM / MRN path-way)) expression through QRT-PCR is considered a key method. Depending on the Centre for Radiation Protection, employees exposed to weak doses of y -rays approximately (2-10) cGy could experience X-ray Geno toxicity. The exposure group worked in close proximity to the source (X-ray) and control group. Whole blood had been collected from all of the individuals, according to the findings.

Results: NABP2 (hSSBI) expression increased in people who were exposed for longer than lcGy and decreased in the controls. was enhanced as a result of the Geno toxicity caused by low doses of X-rays. X-ray oxidative stress stimulates. Single-stranded DNA (ssDNA)-binding proteins like OBFC2B, which bind to single-stranded DNA (NABP2; SSBI).

Conclusion: In the present study, the conclusion that has been reached is that the exposure of the individuals to the low dosages from the I-DIR had led to the occurrence of some slight symptoms that can't be noticed via outside signs as in the high dosages, therefore, it we have studied the changes in gene expression like XRCC-I, XRCC-2, FOXM-I, it was researched hSSB1 (NABP2) for the purpose of knowing effects of the I-DIR. Using radioisotopes in industry, science, and medicine, must be implemented in such a way as to keep the environment and people safe for a long time.

Introduction

Radiation is divided into two categories: ionizing and non-ionizing. Due to the ionizing ability to stimulate atom ionization, the bimolecular effects of ionizing are more affected as compared with non-ionizing. The primary sources of ionizing radiation are radioisotopes, also known as radionuclides, which are unstable. Those radioisotopes generate high-energy particles that can easily displace electrons in atoms, which makes it possible for chain reactions to occur [1]. The most common ionizing radiation sources used in research facilities or for therapeutic purposes include Cs-137, Co-60, and Europium 152.

One imaging method is radiography, which shows internal body parts using either (V or X-rays [2,3]. Body scanners, which typically use the backscatter of the X-ray, are one of the radiography procedures. The X-ray generator produces an X-ray shed at the object to create an image during standard radiography. Based on their composition and density, objects absorb a particular amount of such rays or any other radiation kinds [4].

Ionizing radiation might take place artificially or naturally in the environment, and the two types might have adverse effects on healthy individuals. Cancers induced by radiation were registered beginning in 1917 and continued until 1926. Ionizing radiation causes DNA damage either un-immediately or immediately, typically resulting in base damage that could be repaired through base expression repair (BER) [5-9]. Recombinant human SSBI was discovered by Richard et al. (2008)[10], they used database analysis to locate OBFC2B, which referred to SSBI ›they stated that the OBFC2B gene maps to chromosome 12q13.3 (**Fig.** 1). It binds exclusively to ssDNA, particularly to poly-pyrimidines, and that binding affinity has been considerably increased with the DNA substrate length. In response to DNA double-strand breaks, ATM607585 has been coupled with SSB-I and phosphorylated on the-1 17, which stabilized SSBI against proteasome degradation. SSBI accumulated in the nucleus when DNA damage was induced, in which it localized with DNA damage repair proteins at specific foci. Cellular response to the radiation-induced DNA double-strand breaks, which include the ATM activation and the ATM targets' phosphorylation, was eliminated when the SSB-I was deleted. Increased radio-sensitivity decreased DNA repair capacity, poor checkpoint activation, and increased genomic instability were all seen in cells with SSBI deficiency. According to Richard et al. (2008), SSBI impacts various cellular DNA damage response endpoints [8]. It was demonstrated that the single-stranded DNA binding (SSB) protein family member hSSB-1 is essential for preserving genomic stability [9,10]. Along with being needed for the repair of the stalled replication forks, hSSB -1 has been found crucial for starting the homologous recombination (HR) pathway's repair. hSSB1 functions by binding to ssDNA throughout the repair of the DNA double-strand breaks (DSBs) and stalled/collapsed DNA replication forks. Human ssDNA binding protein 1 (hSSB-1)/NABP2/0BFC2B was discovered recently to be a crucial part of base excision repair pathway, working with the hOGG^{-1} to repair 8-oxoG lesions [9-11].

hSSB-1 can also identify double-stranded DNA that contains 8oxoG. It has been shown earlier that the hSSB-1 participates in the base excision repair pathway and is essential for recruiting hOGG-1 and removing 8-oxoG residues. It has also been shown that the hSSB-1 binds to the hOGG-1 directly and it potentially helps promote glycosylase's recruitment to the damage site. Additionally, it has been demonstrated in the past that in a reconstituted assay system, hSSB1 facilitates hOGG1's removal of 8-oxoG. hSSB1 is necessary for both direct lesion repair and the beginning of oxidative stress signaling via ATM kinase and P-53 [11]. It is proposed that oxidative stress leads to the promotion of hSSB-1 oligomerization, and it is essential for the aid of 8-oxoG removal and for initiating the signaling of the ATM. Additionally, it has been demonstrated that even though the oligomerization of the hSSB-1 is required for 8-oxoG repair, it's dispensable for the hSSB-1 role in homologous recombination.

The oligomerization of the hSSB-1 potentiates its capability of binding the dsDNA that contains the oxidative lesions. The data indicated that C-41 is the key for hSSB-1 oligomerization and is needed for the hSSB1-facilitated 8-oxoG lesion repair. Whereas the earlier results have demonstrated that the hSSB-1 and hOGG-1 form a complex within the cells the location of the location of hSSB1 as shown in Fig. 1.

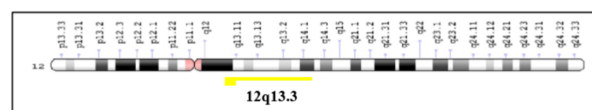

Figure 1: Human chromosome 12 ideogram, within location of hSSB1 gene maps to the chromosome 12q13.3.

Considering the abovementioned facts, the present study aims to evaluate the effects of ionizing radiation through the hSSB-1 gene expression of radiography examiners and control groups.

Methods

a. Sample collection

This study includes 25 radiography examiners with an age limit of 35-63 years. They were all male and had no history of radiotherapy, as a control sample, 20 more participants who have not been exposed to X-ray or medical treatment were chosen.

b. hSSB-1 gene expression

To study the expression of the genes, 250 µl of blood was taken and transferred directly into a tube containing the TRIzol reagent. RNA was extracted according to the kit's instructions. The kits provide a rated yield of more than 20 ug of total mRNA. Utilizing Accu Power RT Premix Kit, whole RNA has been reverse transcribed into complementary DNA (cDNA). According to manufacturers, the procedure is completed with a reaction volume of 50 UI. 20 1-11 of RNA was added to AccuPower RT Premix. Then, it was centrifuged briefly to spin down contents and remove any air bubbles as in Table 1.

Template RNA	The volume	20.0gl reaction	50.0gl reaction
	Total RNA	0.51µg-1µg	1µg-2µg
	Poly(A) RNA	0.050-0.10g g	0.10-0.20g g
Primer	OLIGO Dt18	0.50g g(100 pmole)	lg g(200 pmole)
	Sequence-Specific	1O pmole30pmole	-

Table l: Experimental protocol of (Accu Power RT Premix Kit) describes RNA reverse transcribed into complementary DNA (cDNA).

Analyses of the mRNA expression of the hSSB-1 (NABP-2) by (QRTPCR)

The primers for hSSB1 (NABP2) had the sequence as mentioned in Table 2 Forward and Reverse were produced by Alpha DNA Ltd. Then using GAPDH as (housekeeping gene). The final volume of the reaction of the PCR was 20gl, which included l ul of the forward primer, 10.0ul of the SYBR green, 2ul of the cDNA, l ul of the reverse primer, and 20 ul of free nucleus water.

Primer/probe	Sequence, hSSB-1 (NABP-2)
Forward	AGGATTCCCACTCAAATGGG
Reverse	AAAGAAATTCCGATGGAGCA
GAPDH (100 pb)	
Forward	GAAATCCCATCAATCTTCCAGG
Reverse	GAGCCCCAGCCTTCCATG

Table 2: Primers used in the study with their sequences , hSSB-1 (NABP2) and GAPDH (House-Keeping gene) primers.

As shown in Table 3, the amplified of samples by 40 cycles for 5 min at 95 °C. also annealing at 62°C for 60 sec, then the Ct value of analysis [12, 13]. for hSSB1 in order to determine the times of folding expression for each group, the levels of expression hSSB1 are normalized to housekeeping gene expression.

Steps	Temp.	Duration	Cycle
Activation of the Enzyme	95 °C	5mins	Hold
Denature	95 °C	30 sec	40
Anneal/extend	62°C	60sec	-

Table 3: Thermal profile used in expression of hSSB1 (NABP2) gene.

c. Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. T-test (Analysis of Variation-ANOVA) was used to significantly compare between means. in this study.

d. Immunofluorescence microscopy

Immunofluorescence was used to determine the localization of hOGG1 and hSSB1 as previously described, and high-content microscopy within the Cell 2200 system of imaging was used to visualize the results (GE Healthcare Life Science). At least 100 nuclei have been quantified from images after they had been analyzed with the use of the in-Cell Investigator program (GE Healthcare Life Science). As directed by the provider, 8-oxoG lesions were seen using immunofluorescence (Trevigen). Images were gathered and analysed in the manner previously mentioned [14].

Results

Normalization of cycle threshold (Ct) Values hSSB1 (NABP2)

In this work, the QPCR provides the analysis of mRNA expression of hSSB1 (NABP2) in exposed individuals and control groups. When employing relative quantification, the fold calculations for gene expression could be modified [15]. It is based on the normalization of Ct values, in which "Ct^n is defined as the difference between average Ct values of the replications of the hSSB1 (NABP2) cDNA amplifications in every one of the individual cases and those of the GAPDH. Table 4 lists the mean of the ΔCt (normalization Ct values) of every one of the study groups. The ACt mean values for the healthy control and exposed group have been, respectively, 6.34 and 4.47. A substantial difference between the study groups may be e seen (p=0.001). amongst those groups regarding mean 2^{-AACt} (p = 0.00010) of hSSB1 (NABP2) was utilized as a calibrator. Results of 2-ACt showed that the exposed group of workers Had considerably greater levels than the control group (p = 0.0001). In Table (5) the 2 - Δ Δ CT showed the results of each group, the folds of gene expression in exposed workers were 1.023 times greater than those in the healthy control group. The results show that there has been a considerable increase in the hSSB1 (NABP2) gene expression in exposed groups and demonstrate the role of hSSB1 (NABP2) in radio resistance. The 2-AACt findings were used to calculate the relative expression of the hSSB1 (NABP2) gene across all of the study groups. One of the samples from the controls had a high expression. Results of hSSB1 (NABP2) gene expression in colon cancer cells as well as CRC tissues. In analysis plots, colon cancer cell lines typically express higher amounts of the hSSB1 (NABP2) protein than the positive control MCF7. In comparison to the control group, expression of the hSSB1 (NABP2) gene was highest in exposed individuals.

Group	Mean Ct of hSSB1	Mean Ct of GAPDH	ΔCt (Mean Ct of hSSB1 - Mean Ct of GAPDH)	$2^{-\Delta Ct}$	Experimental Control groups	Fold of gene expression
Exposed group	25.73	21.26	4.47	0.045	0.045/ 0.012	3.75
Control	27.48	21.14	6.34	0.012	0.012/ 0.012	1

Table 4: Fold of hSSB1 (NABP2) expression depending on 2-ΔCt approach. showed the results of each group, the folds of gene expression in exposed workers and healthy control group.

Groups	Means Ct of hSSB1	Mean Ct of GAPDH	Mean Δ Ct Target (ct hSSB1 –ct GAPDH)	Mean ΔCt Calibrator (ct hSSB1 - ct GAPDH	$2^{-\Delta Ct}$	Δ Δ CT	$2^{-\Delta\Delta CT}$	experimental group/ Control group	Fold of gene expression
Exposed group	25.73	21.26	4.47	7.85	0.045	7.805	0.0044	0.0044 / 0.0043	1.023
Control	27.48	21.14	6.34	7.85	0.012	7.838	0.0043	0.0043/0.0043	1

Table 5: Fold of hSSB1 (NABP2) expression depending on$2^{-\Delta\Delta CT}$ approach. showed the results of each group, the folds of gene expression in exposed workers and healthy control group.

The reflection of the original mRNA that is present in samples, is crucial. Those findings clearly show that the working group has the maximum copy number of the mRNA, which corresponds to the higher expression.

But for the normal groups, exposure to I-DIR results in differing Ct values. The findings indicate a difference in the values of the Ct between control groups and the exposed workers as well as significant proof that the expression regarding the hSSB1 (NABP2) gene rises in exposure groups, making it possible to employ the hSSB1 (NABP2) gene as biomarker for the early identification of DNA damage. Those scenarios could account for the expression of hSSB1 (NABP2) and the lack of hSSB1 (NABP2) mRNA in the peripheral blood of healthy individuals. As a result, the expression of hSSB1 (NABP2) mRNA in whole blood could have significant clinical value in the prognosis and diagnosis of low dose ionizing radiation expression. Every quantitative PCR reaction is carried out twice for every sample. Samples from healthy and working people have been run in every one of the runs, along with non-primer and non-template controls. This was necessary to specify the calibrator and to perform the statistical analysis for each group. Fig. (2) and Fig. (3) exhibit plots of the amplification and dissociation curves for the hSSB1 (NABP2). Plots of every one of the runs have been recorded, which includes amplification plots and the dissociation curves.

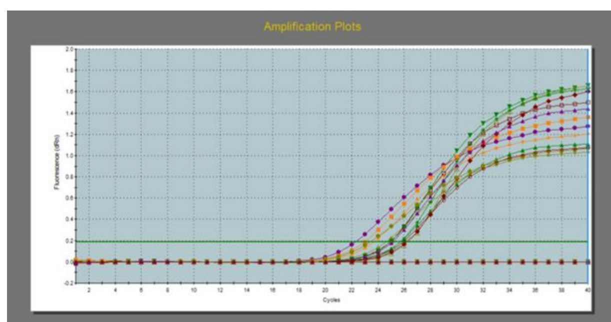

Figure 2: Plots of hSSB1 (NABP2) amplifications through the QPCR. Samples involved group exposure of the individuals. Ct values range from 22.27 to 28.10, where a photograph has been taken directly of the QPCR machine.

Figure 3: Plots of hSSB-1 (NABP2) amplification curves through the QPCR. Samples involved group exposure of the individuals.

Discussion

A previous study discovered a new role for the hSSB-1 in response to oxidative stresses, along with its critical role in repairing dsDNA breaks through homologous recombination. By facilitating the recruitment of hOGG1 to the damaged chromatin, hSSB1 facilitates the excision of 8oxoguanine, which is necessary for ATM activation [16-18]. This data supports my previous research on the effects of irradiation suppression on XRCCI, and it also supports the notion that irradiation-induced hSSB1 (NABP2) could act as a mediator for DNA repair. The findings provide evidence for the anti-tumor effects of radiation in recent years.

Large-scale gene expression studies have been used to identify new genes associated with cancer that may serve as therapeutic targets or prognostic indicators. These researchers found elevated expression of hSSB1 (NABP2) in nearly all of the most common types of human cancer, including those of the breast, lung, prostate, brain, bladder, colorectal, and pancreas. The results showed that hSSB1 oligomerizes in response to oxidative stress, increasing its affinity for DNA containing 8-oxoG. Cellular signaling response to oxidative stresses depends on hSSB1 oligomerization. It's interesting to note that whereas hSSB-1 oligomerization is necessary for its participation in the elimination of 8-oxoG, it is not necessary for its impact on the repair of double-strand breaks by homologous recombination [19]. As in figure2 the control group was included in the samples. The Ct values were between 26.280 and 35.020, where the QPCR machine was immediately photographed. Additionally, the results show that the aforementioned mRNA can act as precise markers or be sensitive to oxidative stress in whole blood in those who have been exposed to it. These

results are comparable with those of [20]. Results from [21-22] have revealed that mRNA expression of the hSSB-1 (NABP-2) in the whole blood has a clinical value be important for diagnosing genetic instability and cancer coinciding with the ones of whose results show which efficiently knockdown genes of the hSSB1 (NABP2) sensitize colon tumor cells for radiation in vitro or in vivo as molecule overexpressed in the primary colon tumor cells. As an alternative, some large base damage (8-hydroxylamine, thymine glycol) could be removed through the NER pathway. This is especially true for long-lived cells like neurons, which naturally supply a large portion of oxidative damage [23].

In the present study, the conclusion that has been reached is that the exposure of the individuals to the low dosages from the I-DIR had led to the occurrence of some slight symptoms that can't be noticed via outside signs as in the high dosages, therefore, it we have studied the changes in gene expression like XRCC-I, XRCC-2, FOXM-I, it was researched hSSB1 (NABP2) for the purpose of knowing effects of the I-DIR. Using radioisotopes in industry, science, and medicine, must be implemented in such a way as to keep the environment and people safe for a long time.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

RA and NT conceived the idea:

JT and FR designed the experiments: All authors contributed to the article and approved the submitted version.

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