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Gene expression measurement of CDKN1A in human lymphocytes gamma-irradiated

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1.Introduction

Ionizing radiation (RI) is defined as the energy capable of removing electrons from an atom, transforming them into a pair of ions with positive energy [1]. The uptake of IR by living cells can directly affect atomic structures, causing chemical and biological changes. The direct and indirect effects of IR initiate a series of biochemical and molecular signaling events that can trigger the damage repair process; it can also culminate in permanent physiological changes or cell death [2]. Determining the absorbed dose with a biological method is extremely important, because becoming an essential piece of data that will help physicians in predicting the likely consequences for the health of the exposed individual. In addition, support to determinate the medical treatment that should be adopted, being used both to identify and treat early biological effects (skin lesions or hematopoietic depletion) and for late biological effects (ulcerations, fibrosis, necrosis, among others) [3, 4].

Appropriate biological markers for dose estimation need to show low inter-individual variation in dose-response, implying robustness towards age- and gender-dependent differences and constant high stability over time. In addition, they must have high reproducibility, the ability to apply a universal dose-response curve to infer a dose estimate from the measured response, and a low detection threshold. Some markers described by Badie *et al*. [5] were considered useful to assess the absorbed dose. Activation of specific pathways triggered by radiation-induced double-stranded DNA breakage leads to up-regulation of genes, for example, CDKN1A (cyclin-dependent kinase inhibitor 1A) which is responsible for encoding a protein that functions as a progression regulator of the cell cycle in G1. Among stress stimuli, the expression of this gene can occur in an increased way [6].

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2.Methodology

The descriptors used for this bibliographic survey were CDKN1A; Gamma radiation (γ); Human lymphocytes; Biological dosimetry; RT-PCR. The databases used were PubMed, LILACS, NCBI, and SpringerLink. As an exclusion criterion for the results, studies that only used low radiation doses (< 2 Gy) and that did not use a similar methodology for obtaining and analyzing genetics were discarded.

3. Results and Discussion

Five articles were selected for using RT-qPCR to measure expression, and for performing lymphocyte culture. All authors observed an increase in CDKN1A expression levels in response to the dose, especially from two Gy, compared to control samples. Although some chose to verify the gene's activity using other techniques, such as Microarray analysis and Western blotting, all used the RT-qPCR method. The main differences observed are in the threshold of doses used, in the culture time of isolated lymphocytes, and in the number of individuals who donate blood samples. These differences are listed in Table 1.

Using the RT-qPCR technique, Beer *et al*. analyzed the expression levels of the CDKN1A gene, as a form of validation for other methods used, noting that it showed overexpression, especially after 20 hours of cell culture. One of these methods used was the quantification of proteins involved in the apoptosis process, such as p53 and p21, which were increased. These proteins regulate the expression of CDKN1A [6, 7].

Turtoi *et al*. [8] evaluated the expression levels of certain genes used as dosimetry biomarkers through the RT-qPCR technique. They identified the presence of several radiation response genes in the irradiated peripheral blood cells of six volunteers in doses of 1, 2, and 4 Gy, among them, wasthe CDKN1A, which showed an alteration in its expression to increase rapidly as a function of the dose. In 2010, this same group performed a study analyzing the proteomic and genomic modulation of several genes in lymphocytes isolated from three donors also subjected to gamma radiation, using Western blotting, in which the specific protein of the CDKN1A gene in irradiated cells was identified. The response to radiation induction was a significantly high production of CDKN1A proteins from two individuals at doses 2 and 4 Gy [9].

Tucker *et al*.[10] used five endogenous control genes as a comparison to the genes detected in the samples, and one of the genes with significant expression was the CDKN1A. Among the genes observed, this one was described as one of the best predictors of exposure to ionizing radiation. Similar Tucker *et al*. [10], Li *et al* [11] observed that there was expression levels consistency of CDKN1A related to cell culture time.

Table I: Differences between Authors of Articles.

Authors	***Donor individuals***	***Absorbed dose (Gy)***	***Cell culture time (h)***
Beer *et al.*[7]	4	60Gy	2, 4 e 20h
Turtoi *et al.* [8]	6	1, 2 e 4Gy	2h
Turtoi *et al.*[9]	3	1, 2 e 4Gy	2h
Tucker *et al.*[10]	60	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 e 10Gy	12, 24 e 48h
Li *et al.* [11]	30	0.5, 1, 2, 3, 4, 6 e 8Gy	6, 12, 24 e 48h

4. Conclusions

From the observations made through the correlation of results, it is concluded that the CDKN1A gene is a good marker for application in biological dosimetry by gene expression, described in the literature as an excellent marker of exposure to ionizing radiation, considering its increase in expression in response to doses.

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