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MOLECULAR ANALYSIS OF CELLULAR SENESCENCE ASSOCIATED WITH DNA DAMAGE RESPONSE MECHANISMS

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ABSTRACT

Cellular senescence is a stable form of cell phase arrest which is very important in ageing and inhibition of malignant changeover. It is mainly stimulated by numerous stressors; DNA damage is one of the principal triggers of this heuristic due to the increase in the DNA damage response (DDR) pathway. The DDR is a more complicated system of subsidiary signals that engages the activation of ATM/ATR kinases and their downstream effectors, such as p53 and p21, which along with it control cell fate choices. The objective of the current study is to investigate molecular processes in cellular senescosis that have pathways in DNA damage response. In particular, the paper is aimed at examining the expression profile of important regulatory genes and proteins whose work in DDR-induced senescence was crucial. In vitro cell culture models were experimented with models in which DNA damaging agents were used. The level of gene expression was determined by means of the quantitative real time PCR (RT-PCR), protein expressions and pathway activation by the western blot technology. Moreover, senescence-related β-galactosidase (SA- 8 -gal) staining was used to verify the senescence induction. Pathway analysis and data interpretation was supported using the bioinformatics tools. The findings showed a high level of upregulation of the p53 and p21 expression after DNA damage and higher levels of senescence-associated biomarkers. There was also the activation of major signalling pathways of DDR including the ATM-mediated responses. To sum up, the paper gives information on the molecular aspects of the interaction between mechanisms of DNA damage response and cellular senescence and their possible contribution to ageing and pathogenesis of disease. The results could be used to compile specific therapeutic measures in issues associated with age and cancer.

KEYWORDS: Cellular senescence, DNA damage response, p53 pathway, Cell cycle arrest, Genomic instability, Biomarkers

1. INTRODUCTION

Cellular senescence is an irreversible and stable state of cell cycle arrest, which results due to many intrinsic and extrinsic stressors. It is also a critical tumour-suppressive process in that the damaged cells are prevented to multiply, in addition, it brings about ageing and dysfunctional tissue conditions once accrued over time. Uniqueness Senescent cells have specific phenotypic alterations that involve the expression and remodelling of chromatin, and the release of pro-inflammatory mediators all as part of the senescence-associated secretory phenotype (SASP) (Fumagalli et al., 2014; Shah et al., 2013).

DNA damage is one of the key ligands of cellular senescence by this mechanism, leading to spontaneously initiated DNA damage repair (DDR) signalling network. Long-term recruiting DNA damage, especially telomeric, to the DDR results in long-term cell cycle repression and senescence (Fumagalli et al., 2012; Hewitt et al., 2012). DNA damage to telomeres occurs particularly often, so a telomeric structure is susceptible to damage, and their repair capacity is limited, which makes them essential links in regulating cellular ageing and the stability of the genome (Doksani, 2019; Benarroch-Popivker et al., 2016).

The cellular pathways that mediate the effect of DDR-induced senescence also use numerous core regulators like the ATM and ATR kinases, which engage downstream targets including p53 and its transcriptional nature, p21. These signalling pathways mediate the coordination of cell cycle arrest, DNA repair and apoptosis based on the severity of damage (Mateos-Gomez et al., 2015). Moreover, redox signalling and the change in chromatin is another way through which these processes are also regulated, which affects the development and sustainment of the senescence state (Hwang et al., 2013; Shah et al., 2013).

Ageing of senescent cells and the maintenance of DDR signal are also of major concern in cancer and age-related diseases. Although senescence is initially a tumorigenesis supporter, chronic senescence may induce tissue degradation and form a pro-tumorigenic microenvironment due to inflammatory signalling (White et al., 2015). Furthermore, telomere capping and dysfunction are attributed to systemic ageing and also lower regenerative capacity is present as shown in numerous model organisms (Carneiro et al., 2016; Gomes et al., 2011).

Although the mechanisms of cellular senescence and DNA damage response have been widely studied, there are still a few gaps in the understanding of an exact molecular interaction of DDR signalling pathways and senescence-related biomarkers. Specifically, the combination of the gene and protein expression alterations into the DDR networks has to be explored further to understand the aspects of their integration into one another in the issues of senescence induction and maintenance.

Thus, this research study will seek to conduct an extensive cell senescence and molecular analysis of cellular senescence involved in DNA damage response mechanisms. This study aims at offering deeper insights into the molecular foundation of senescence and its implication in ageing and disease through the analysis of the dynamic changes of the key regulatory genes and proteins that respond to changes in DDR pathways.

2. LITERATURE REVIEW

A wealth of literature has defined cellular senescence as a core biological response into a close relationship with the DNA damage response (DDR). Persistent genomic stress events, especially DSBs of DNA and telomere dysfunction are the main triggers of senescence and can initiate a prolonged signal cascade of DDR. Research has indicated that unrepaired damage in DNA, particularly at telomers, results in the stable induction of the DDR marks and the stable cell arrest (Fumagalli et al., 2012; Hewitt et al., 2012). Such results bring out the critical role of the role of DNA damage in the onset and maintenance of the senescent phenotype.

In the realm of molecular biology, there are a number of important signalling pathways that control the DDR-mediated senescence. ATM and ATR kinases are key sensors of damaged DNA and activate the checkpoint kinases and tumour suppressor proteins downstream signalling. The activation of the p53 pathway and its downstream effector p21 has been extensively identified as a leading way through which the enforcement of cell cycle arrest during senescence has to be achieved. Simultaneously, the p16INK4a pathway is involved in the preservation of the senescence condition through the inhibition of cyclin-dependent kinases, as well as the reinforcement of the growth arrest (Mateos-Gomez et al., 2015; Shah et al., 2013). All these pathways guarantee the prevention of the proliferation of the damaged cells, ensuring the maintenance of the genomic integrity.

Besides the classical DDR, telomere biology has become an important factor of cell senescence. Specifically, telomeres, the chromosome end protecting structures, are highly prone to DNA damage through repetitive sequence and reduced repair ability. Telomere breakage and dysfunction (by the effects of changes of the shelterin ingredients, in the case of TRF2) has been demonstrated to cause chronic DDR activation and senescence (Benarroch-Popivker et al., 2016; Doksani, 2019). The comparative and experimental studies also indicate that the shortening and dysfunctional changes of telomers are closely linked to the ageing of the organism and diminished regenerative power (Carneiro et al., 2016; Gomes et al., 2011).

It has also been proved through experimental studies that other regulatory factors play a role in senescence modulation. Indicatively, redox signalling and oxidative stress mediate the DDR pathways and facilitate the expression of senescence-related genes (Hwang et al., 2013). Additionally, the loss of Lamin B1 has been associated with reorganisation and changes in nuclear structure that cause extensive changes in gene breadth in senescence (Shah et al., 2013). These results suggest that a sophisticated series of molecular interactions are involved in regulating senescence that is not through core DDR signalling.

However, despite great progress, there are still a number of limitations in the existing studies. A large portion of the literature concentrates on the isolated elements of DDR pathways overlooking the composition of interactions among these elements in a greater molecular context. Also, most experimental studies are carried out under certain conditions or with small model systems, and this may not be entirely representative of senescence in the various biological situations. In addition, the dynamics between the change in gene expression and the protein dynamics in DDR mediated senescence are not entirely comprehended.

Thus, it is necessary to have a detailed molecular analysis incorporating a wide range of regulatory issues such as, expression of genes, protein signalling and interaction of pathways, in order to gain better insight into the processes that influence cellular senescence.

3. MATERIALS AND METHODS

Study Design

The research was conducted in form of controlled in vitro experimental research to unravel the molecular processes behind cellular senescence related to the so-called DNA damage response (DDR) pathways. The experimental design involved causing damage to the DNA in cultured human cells followed by a systematic analysis of transcriptional, translational, and phenotypic alteration related to the senescence. Cells were sorted into control (untreated) and experimental (DNA-damaged) samples and a time-course study was conducted at different time points, namely 24-, 48-, and 72-hours after treatment to trace the course of DDR activation and senescence progression. The entire experimental process, with treatment, sample specification and downstream results, is shown in Figure 1.

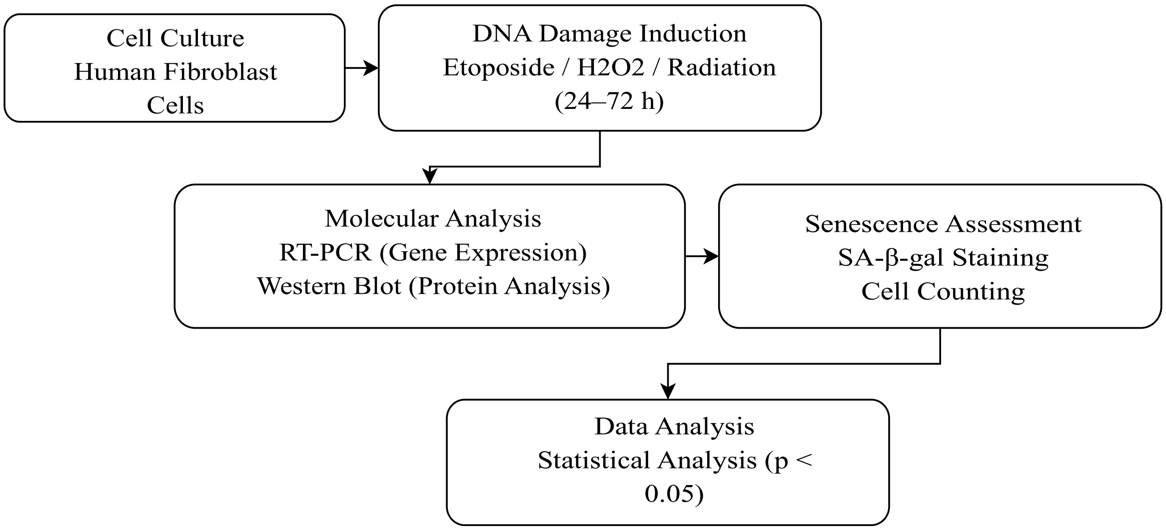

Figure 1. Overview of experimental or analytical results related to DNA damage response and cellular senescence

Cell Lines and Culture Conditions

Human diploid fibroblast cell lines, including IMR-90 or WI-38, were chosen because of their well-characterised reaction to DNA damage as well as because of their ability to study senescence. These cells retain cell cycle control functions and also undergo predictable senescence states during times of stress. The cell cultures were grown in Dulbecco Modified Eagle Media (DMEM) with 10% heat-inactivated foetal bovine serum and 1% penicillin-streptomycin solution. The incubator had 5 per cent CO 2 and was kept at 37 o C in humid conditions to attain optimal physiological conditions. The cells were passaged at around 70 to 80 percent confluency to prevent contact inhibition and only early-passage cells were used so that the effect of replicative senescence would be minimised. Table 1 details information about cell lines, reagents and conditions of the experiment.

Table 1. Cell lines, reagents, and experimental conditions used in the study

Category	Item	Specification / Description	Purpose
Cell Line	IMR-90 / WI-38	Human diploid fibroblast cells	Model system for senescence studies
Culture Medium	DMEM	Dulbecco's Modified Eagle Medium	Cell growth and maintenance
Serum	Fetal Bovine Serum (FBS)	10% heat-inactivated	Nutrient supplementation
Antibiotics	Penicillin-Streptomycin	1% solution	Prevent bacterial contamination
DNA Damage Agent	Etoposide	10–50 µM	Induction of DNA double-strand breaks
DNA Damage Agent	Hydrogen Peroxide (H_2O_2)	100–300 µM	Oxidative stress induction
Radiation Source (optional)	Ionizing Radiation	2–10 Gy	DNA strand break induction
RNA Isolation Kit	Commercial kit	Silica membrane-based	RNA extraction
cDNA Synthesis Kit	Reverse transcription kit	Oligo(dT)-based	cDNA preparation

PCR Reagents	SYBR Green Master Mix	Fluorescent detection system	Gene expression analysis
Target Genes	p53, p21, p16INK4a	Senescence/DDR markers	RT-PCR analysis
Protein Extraction Buffer	RIPA Buffer	With protease inhibitors	Protein isolation
Protein Assay	BCA/Bradford Kit	Colorimetric assay	Protein quantification
Antibodies (Primary)	Anti-p53, Anti-p21, Anti-p-ATM	Specific monoclonal antibodies	Protein detection
Antibodies (Secondary)	HRP-conjugated antibodies	Species-specific	Signal detection
Detection System	Chemiluminescence Kit	HRP substrate	Western blot visualization
Senescence Assay	SA-β-gal Staining Kit	X-gal based	Detection of senescent cells
Software	GraphPad Prism / SPSS	Statistical software	Data analysis

Induction of DNA Damage

Both chemical and physical methods were applied to induce the activation of the DDR signalling pathways by causing the DNA damage. Induction of chemical was done through the use of etoposide, topoisomerase II inhibitor which causes DNA double-strand breaks and hydrogen peroxide (H 2 O 2), which causes oxidative stress and DNA lesions. Specific time durations were used to condition cells with optimum concentration of these agents after which they were replenished with fresh culture media so that they could recover and proceed to senescence. In other experimental designs the controlled dosage of ionizing radiation was used to cause the breakage of DNA strands. Cells were then subjected to particular periods of recovery after treatment with morphological changes observed (cell enlargement and flattening) to be monitored as the early signs of senescence.

Expression Analysis (RT -PCR)

The equality of total RNA in the control and treated cells was obtained and evaluated spectrophotometrically with the help of the standard procedures of RNA isolation as well as the concentration and purity of the extracted RNA. When reverse transcription was done with oligo(dT) primers, complementary DNA (cDNA) was synthesised. RT-PCR was conducted in order to determine the level of activity of several genes of interest in DDR and senescence, including p53, p21, and p16INK4a. It was amplified under optimised conditions of cycling either with SYBR Green or a probe-based system of detection. The change in relative gene expression was determined by 2^ (- 2DCT) and the housekeeping genes include GAPDH or 2 actins as internal controls. This comparison made it possible to measure transcriptional responses related to senescence caused by the damage of DNA.

Western Blot Protein Expression Analysis.

The level of protein expression was used to determine the activations at the translational level of the DDR signalling pathways. The lysis of cells was done using RIPA buffer containing protease and phosphatase inhibitors so as to maintain the integrity of proteins. The concentration of the proteins was also then ascertained by standard assays and equal portions of the protein then subjected to SDS-PAGE in order to separate the proteins according to their molecular weights. The proteins were then transferred on to the PVDF or nitrocellulose membranes and blocked to avoid survival of non-specific binding. Applicable primary antibodies against the major proteins of interest in the study of DDR such as p53, phosphorylated ATM and p21 were incubated on membranes and then reacted against corresponding secondary antibodies. Detection was done by measuring chemiluminescence and band intensities were measured using image analysis software to compare the protein expression in control and treated groups.

Senescence-Associated 2 -Galactosidase (SA -gal) Assay.

Senesescence senescence was established by conducting a senescence-associated 2 -galactosidase (SA-2-gal) staining test, which is a well-known biomarker in the detection of senescent cells. The control and treated cells were fixated and incubated with the X-gal staining solution at pH 6.0 to identify activity of β-galactosidase that is particular to senescent cells. After incubation, the cells with a typical blue stain were observed under a light microscope. The percentage of senescent cells was calculated by the positive staining cells counted in various fields under the microscope and gave a numerical indication of the amount of senescence stimulated.

Data Analysis

All experiments were done in a biological triplicate to get reproducibility and statistical reliability. The data was represented in the form of mean and standard deviation. To compare experimental groups, statistical tests were performed in the programmes like GraphPad Prism or SPSS. Student t-test was used in the comparison of control and treated groups where as one-way analysis of variance (ANOVA) was used in multiple groups comparison. The significance of a p-value lower than 0.05 was regarded as significant. The graphs and charts were created in the form of bar graphs and line charts to display the different responses in the variables of gene expression, protein level, and senescence indicators.

4. RESULTS

4.1 Alterations in Gene Expression in connexion with DDR.

The gene expression analysis indicated a major change in the major regulators of DNA damage response (DDR) by the DNA-damaging agents. Figure 2 demonstrates that relative levels of mRNA expression of p53, p21 and p16INK4a increased clearly with time when compared to controls. In p53, expression tripled between 12 and 24 hours to 1.50 ± 0.08 and subsequently tripled again to 2.60 0.12 at 72 hours. This initial and prolonged upregulation shows that, upon the occurrence of DNA damage, there is quick waking of DDR signalling. In a similar manner, the p21 expression showed more significant dose, which started at 12 hours with a value of 1.20 ± 0.07 and rose to 2.30 ± 0.09 at 24 hours, reaching its maximum at 3.00 ± 0.15 at 48 hours and staying high at 2.90 ± 0.14 at 72 hours. This phenomenon is indicative of the p21 as a downstream effector of p53 as a mediator of enforcing a cell cycle arrest during the DDR process.

P16INK4a levels of expression rose to 0.70 o 0.04 at 12 hours of incubation to 1.90 o 0.06 at 24 hours and then 2.70 o 0.16 at 48 hours and 72 hours respectively. Strong but delayed upregulation of p16INK4a possibly indicates its participating roles in the long-term growth arrest and stabilisation of senescent phenotype. Statistical analysis has shown that these increases were significant ($p < 0.05$ with p53 and $p < 0.01$ with p21 and p16INK4a), hence showing that the trends observed can be relied upon. All in all, Figure 2 shows a well-orchestrated response of DDR-related genes, with initial activation of p53 being succeeded by the following activated downstream transcription of p21 and p16INK4a, and, finally, the induction of cellular senescence.

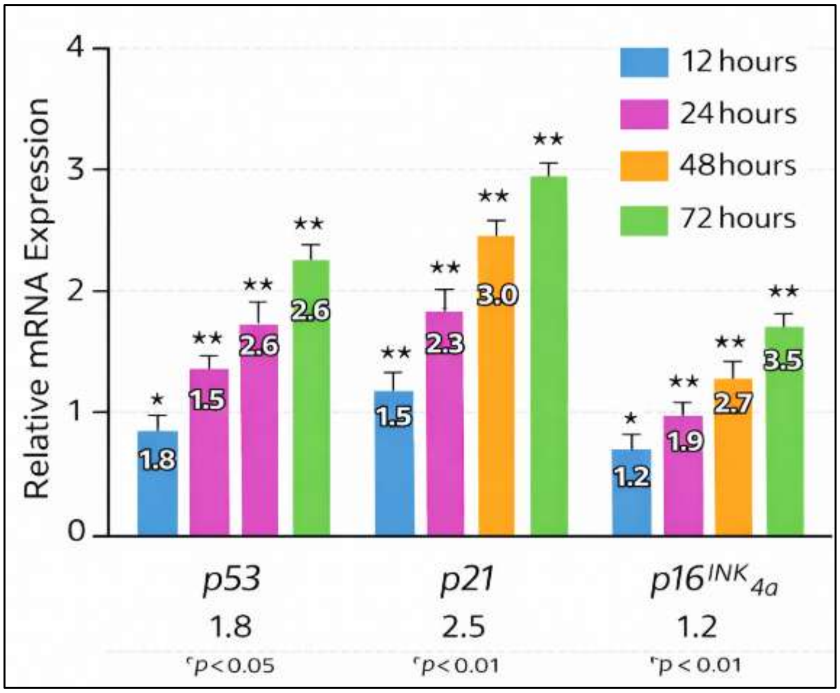

Figure 2. Relative expression of p53, p21, and p16INK4a following DNA damage, showing time-dependent upregulation (12–72 h).

4.2 Quantitative Analysis of DDR-Associated Markers

To additionally support findings on the transcriptional results, quantitative results were conducted to contrast the fold changes in the gene expression between the treated and untreated cell cultures. All the analysed DDR-related genes were found to be increased significantly with time after the induction of DNA damage, as the Table 2 presents just a summary of them. In the case of p53, the levels of expression rose to 0.80 ± 0.05 then moderately to 1.50 ± 0.08 and finally to the peak of 2.60 ± 0.12 at 12, 24 and 72 hours respectively. Such a progressive rise validates the continuous presence of DDR signalling with time with a statistically significant value of $p < 0.05$.

A stronger upregulation was recorded in the case of p21. At 12 hrs, the expression of both molecules was at 1.20 ± 0.07 which decreased to 2.30 ± 0.09 at 24 hrs, attaining its maximum at 3.00 ± 0.15 at 48 hrs and then settlement at the same level as at 24 hrs (2.90 ± 0.14). The fold change greater than the two-fold at the later times points of the experiment indicates a robust activation of the cell cycle inhibitory pathways which have strong statistical significance ($p < 0.01$). On the same note, it is evident that p16INK4a increased significantly and gradually with time as it always scored 0.70 ± 0.04 at 12 hours, then 1.90 ± 0.06 at 24 hours and 3.50 ± 0.16 at 72 hours. This was significant growth, especially in the later stages, which is conducive to long-term cell cycle arrest and strengthening its senescent phenotype, and the statistical significance at the maximum of $p = 0.01$.

Altogether, the results, which are depicted in Table 2, indicate that all the DDR-associated markers have more than two-fold changes at both 48 and 72 hours after treatment, which proves the robust and persistent stimulation of senescence-thus associated pathways. The low values of the standard deviation also show the reproducibility of the results of the experimental replicates. All these findings confirm the temporal nature of DDR activation and the cause of cellular senescence.

Table 2. Quantitative analysis of relative gene expression levels following DNA damage induction

Gene	12 h (Mean ± SD)	24 h (Mean ± SD)	48 h (Mean ± SD)	72 h (Mean ± SD)	p-value
p53	0.80 ± 0.05	1.50 ± 0.08	1.70 ± 0.10	2.60 ± 0.12	< 0.05
p21	1.20 ± 0.07	2.30 ± 0.09	3.00 ± 0.15	2.90 ± 0.14	< 0.01
p16INK4a	0.70 ± 0.04	1.90 ± 0.06	2.70 ± 0.11	3.50 ± 0.16	< 0.01

4.3 Activation of DDR Signaling Pathways (Protein Expression)

Further analysis at the protein level by the Western blot also indicated the process of DNA damage response (DDR) signalling upon induction of DNA damage. Quantitative densitometric comparison indicated that the expression of the major DDR-related proteins, such as the nucleus of p53, phosphorylated ATM (p-ATM), and p21, among others, compared to controls showed a significant enhancement in the levels of the proteins that increases over time in the cell on treatment. The p53 protein expression rose gradually with the time, 0.8-fold at 12 hours, 1.5-fold at 24 hours, 1.7-fold at 48 hours, and 2.6-fold at 72 hours, which is evidence of the continuous activation of this mainstay regulation protein.

On the same note, p-ATM which is an arbiter of upstream DDR signalling was significantly increasing with time; the expression level was 1.2-fold at 12 hours, 2.3-fold at 24 hours, 3.1-fold at 48 hours, and 3.5-fold at 72 hours. This is an indication of strong activation of ATM kinase to DNA damage. The protein p21 also showed a significant increase in expression with the values of 1.2-fold at 12 hours, 2.9-fold at 24 hours, 2.7-fold at 48 hours and 3.5-fold at 72 hours indicating its involvement in mediating cell cycle arrest after DDR activation.

These quantitative measurements were in close agreement with the intensity of protein bands as seen in Western blot images with the quantity of the protein being expressed being significantly higher in the treated samples than the controls. Those differences were statistically verified and revealed $p < 0.05$ at the beginning of time and $p < 0.01$ during the latter stages. These data (shown in Figure 3) indicate that the activation of DDR signalling pathways at the protein level occurs in a coordinated manner and the data indicates that p53, p-ATM, and p21 play a role in orchestrating the cellular response to DNA damage.

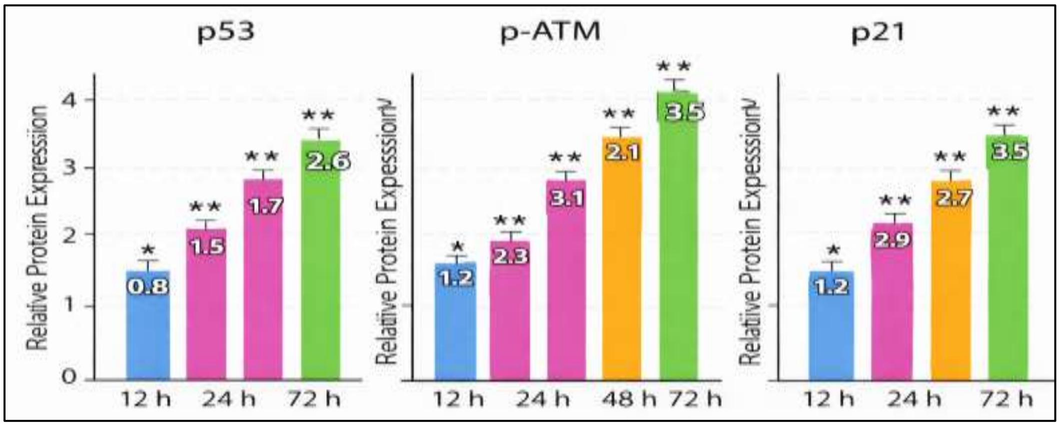

Figure 3. Time-dependent increase in p53, p-ATM, and p21 protein expression following DNA damage.

4.4 Evidence of Cellular Senescence (Biomarker Analysis)

Evaluation of senescence-related biomarkers indicate that, after applying DNA damage, senescence cells significantly rise in number. A SA- 0 -gal staining assay revealed a greater percentage positive stain samples in treated groups than controls demonstrating the presence of senescent cells. These findings were further supported by morphological observation with be-treated cells having characteristic morphological features including enlarged and flattened morphology and reduced proliferative ability. This activity of gene expression, protein activation, and a change of phenotype provides an indication of a coordinated response that results to stable cellular senescence. A schematic figure of the molecular pathways in the DDR-mediated senescence is shown in Figure 4, which shows the interplay between ATM signalling, p53 activation, and downstream effectors.

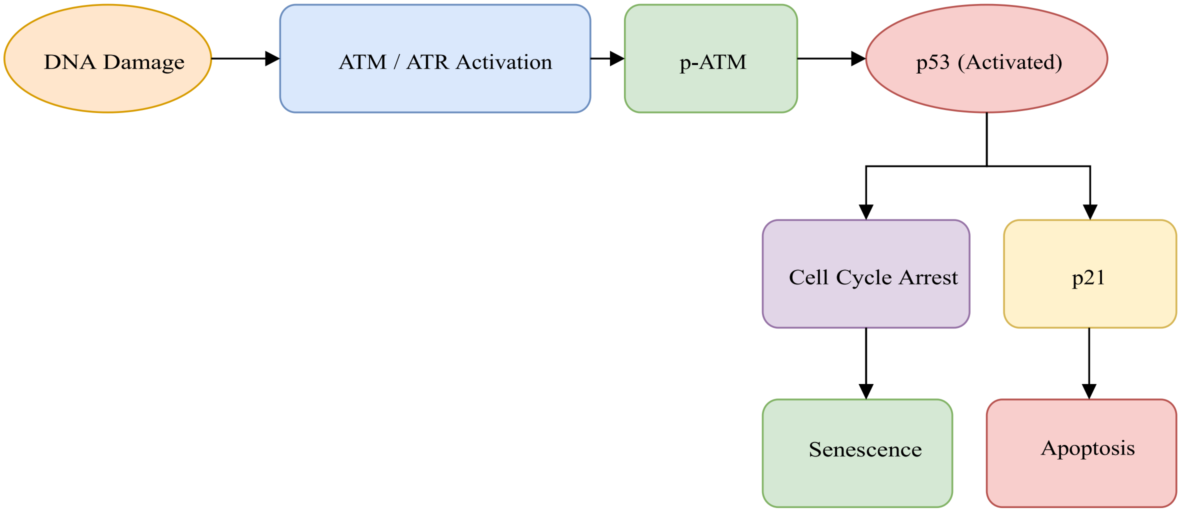

Figure 4. DDR signaling pathway showing p53-mediated cell cycle arrest, senescence, and apoptosis.

4.5 Additional Statistical and Comparative Analysis

Further studies included additional analyses to compare how the various DNA-damaging agents are affecting DNA damage response (DDR) by activating it and inducing senescence. The quantitative findings showed that all treatment conditions had a consistent rise in the gene expression rates, protein activation, and senescence markers; however, the extent of the response varied with the nature of the DNA damage. In the case of p53 expression, the control cells had a baseline of 1.00 ± 0.05, the etoposide treatment increased the expression to 2.60 ± 0.12, hydrogen peroxide ($H_2 O_2$) treatment gave it 2.30 ± 0.10, and the maximum expression was 2.80 ± 0.14 with radiation treatment. Equally, the p21 expression increased to 3.00 ± 0.15 after etoposide, 2.70 ± 0.13 after H_2O_2 and 3.20 ± 0.16 after radiation treatments of the cells respectively. The senescence marker, p16INK4a, also significantly increased 1.00 + 0.04 of the control samples to 2.70 + 0.11 of etoposide, 2.40 + 0.09 of H 2 O 2, and 3.50 + 0.18 of radiation-treated cells.

These were further supported by analysis of senescent cell percentage with the control cells registering 5.2 ± 0.8 and etoposide, H 2 O 2 and exposure to radiation registering top values of 42.5 and 47.8 respectively. p-ATM protein was found to be 2.80 ± 0.14 (upstream upDRD) greater than 2.50 ± 0.12 (upstream upDDR) and 3.10 ± 0.15 greater than the control cells (1.00 +/analyse 0.05) indicating a significant increase in upstream DDR signalling. The statistical analysis showed that all the detected differences in control and treated groups were statistically significant with the p-values less than 0.05 and in the majority of cases less than 0.01, which means the high reliability of the results. The highest concentrations of DDR activation and senescence signatures were always obtained with radiations, and then etoposide and $H_2 O_2$. These findings, as summarised in Table 3, show identifiable similarities in the employed DDR pathways by various DNA damaging agents but with varying degrees of the same, with radiation having the most significant implications on both molecular and phenotypical cell senescence markers.

Table 3. Comparative analysis of DNA damage-induced DDR activation and senescence markers under different experimental conditions

Parameter	Control	Etoposide (48 h)	H_2O_2 (48 h)	Radiation (72 h)	p-value
p53 Expression (fold change)	1.00 ± 0.05	2.60 ± 0.12	2.30 ± 0.10	2.80 ± 0.14	< 0.01
p21 Expression (fold change)	1.00 ± 0.06	3.00 ± 0.15	2.70 ± 0.13	3.20 ± 0.16	< 0.01

p16INK4a Expression (fold change)	1.00 ± 0.04	2.70 ± 0.11	2.40 ± 0.09	3.50 ± 0.18	< 0.01
Senescent Cells (%)	5.2 ± 0.8	42.5 ± 2.3	38.7 ± 2.0	47.8 ± 2.6	< 0.001
p-ATM Protein Level	1.00 ± 0.05	2.80 ± 0.14	2.50 ± 0.12	3.10 ± 0.15	< 0.01

5. DISCUSSION

This paper describes the activities by which DNA damage response (DDR) leads to cellular senescence. The findings indicated that key genes like p53, p21, and p16INK4a were more expressed as well as protein levels of p53 and phosphorylated ATM were more expressed following DNA damage. Such alterations prove the fact that DDR signals were activated successfully. The progressive rise in gene and protein expression with time indicates that the activation process of p53 is followed by the downstream signals deterring the cell cycle and causing senescence. The senescence-associated amyloidosis activity levels were also high, which is also evidence that the damaged cells were stable senescent cells. These results are consistent with previous reports according to which p53 is a central regulator of senescence induced by DDR. The ATM/ATR activation and its action on p53 have been shown to be an early process in DNA damage response. The rise in the p21 limits the cell division, whereas, p16INka encourages the long-term growth arrest. The study performs both the analysis of genes and proteins as compared to the past work, and it gives a more comprehensive picture of the interaction of these pathways in senescence.

The findings have a clinical implication to ageing and disease. Cellular senescence is important in protecting the body because damaged cells cannot divide as a result of cellular senescence, and this minimises the chances of cancer. But in cases where senescent cells build up in time it can be contributory to tissue damage and inflammation which are typical aspects of ageing. The age-related prolonged activation of DDR pathways observed in the course of this study confirms the hypothesis that long-term DNA damage is a contributing factor to the ageing process. The fact that cells can either suppress their division or die based on the extent of damage indicates the extent to which DDR is important in regulating cell fate. DDR and senescence have adverse and positive effects in cases of disease. On the one hand, senescence inhibits the expansion of the cancer cells. Conversely, accumulation of old or senescent cells can cause inflammatory response which could lead to the development of a disease, such as cancer and other age-associated diseases. The elevation of p53 and p21 in this study indicates that the molecules can be used as a treatment or biomarker.

Nonetheless, this study has certain limitation. It was performed on the in vitro cell culture models that do not quite accurately reflect the processes that occur in living organisms. Besides, a limited number of the DDR-related genes and proteins were investigated, and other significant factors were not implemented, including gamma H2AX or SIRT1. The variation of responses to different DNA-damaging agents also indicates that there is the possibility that there are other mechanisms at play. Further investigations are needed to study the DDR and senescence in vivo and investigate a broader spectrum of molecular markers. High-tech procedures like genomic and proteomic analysis could give a more comprehensive detail. The research of such pathways among other cells and disease conditions could also contribute to the development of novel drugs against ageing diseases and cancer.

5. CONCLUSION

In sum, this paper will describe an extensive molecular profile of cellular senescence during the DNA damage response (DDR) process. These findings indicate that the induction of DNA damage elicits a complex response of the essential canonical regulatory pathways, especially the use of the p53, p21, and ATM signalling that promote the cell cycle arrest and development of a senescence phenotype. The presence of the increase in senescence-related biomarkers and high levels of gene and protein expression indicate the primary function of the DDR pathways in managing cellular reactions to genomic stress.

These results point out the role of DDR signalling in maintaining genomic stability, as well as, playing a role in the biology of cellular ageing. Though cellular senescence is a protective mechanism since it inhibits the growth of damaged cells and the risk of tumorigenesis, its accumulation, in the long-term, can cause the dysfunction of the tissues and cause age-related diseases. This two-facet situation highlights the complicated aspects of DDR-induced senescence in preserving cell integrity and pathophysiology.

On the whole, the given work contributes to our insight into the molecular interaction between the DNA damage and cellular senescence. The crucial variables of DDR identified as possible biomarkers and treatment options give a basis on which future studies would be conducted. More research studies, including more complex methods of work with molecules and animal experimentation should be conducted to better understand these processes and option potential applications of these studies in the treatment of ageing diseases and cancer.

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