Am J Stem Cells 2025;14(2):34-52
www.AJSC.us /ISSN:2160-4150/AJSC0164195

Original Article

Platinum chemotherapeutic-induced oxidative stress affects the transcriptional response of DNA repair genes in murine mesenchymal stem cells

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Received February 26, 2025; Accepted May 24, 2025; Epub June 15, 2025; Published June 30, 2025

Abstract: Cisplatin and oxaliplatin are among the most extensively used anti-cancer drugs in the treatment of various types of cancer. However, the cytotoxicity associated with these drugs in normal and adult stem cells is a major concern. Objectives: This study aimed to determine the oxidative stress induced by platinum drugs in murine mesenchymal stem cells (mMSCs). Methods: mMSCs were cultured and treated with cisplatin and oxaliplatin concentrations (5 μM, 15 μM, and 25 μM/L) for 1, 4, 24, 48, and 72 hours. Morphological changes and viability of cells were observed. Oxidative stress was assessed by the expression of 8-Hydroxy-2'-deoxyguanosine (8-OHdG). Necroptosis was determined by Acridine Orange/Ethidium Bromide (AO/EB) staining. Moreover, mRNA levels of DNA repair genes, particularly genes involved in mismatch repair (MMR), including MLH3, MSH2, MLH1, MSH6, and PMS2, and nucleotide excision repair (NER) pathways, such as ERCC1 were measured using Taq-Man Quantitative Real-Time Polymerase Chain Reaction (TaqMan-qRT-PCR). Results: The proliferation and morphology of mMSCs were noticeably influenced by cisplatin and oxaliplatin at 25 μM, compared to 5 μM and 15 μM by 72 hours. 8OHdG positive and necroptotic cells were significantly ($P < 0.001$) high from 24 to 72 hours among 25 μM drug-treated mMSCs. The concentration and temporal oxidative stress generated in mMSCs by cisplatin and oxaliplatin disturbed the expression of DNA repair genes at the mRNA level ($P < 0.001$). Cisplatin remarkably upregulated the expression of MLH1 and PMS2 (≥ 3.0-fold) at 24 hours, while it downregulated MSH2, MLH1, MSH6, and PMS2 (≤ 0.5-fold) at 72 hours. However, oxaliplatin noticeably caused the upregulation of MLH3 and ERCC1 expression (≥ 3.0-fold) at 24-48 hours, and downregulation of MSH2, MLH1, MSH6, PMS2, and ERCC1 (≤ 0.5-fold) at 72 hours. Conclusions: This suggests that adult stem cells in tissues and organs are highly vulnerable to platinum drugs during cancer treatment. Additional studies on localized treatments may help to prevent adverse effects on normal cells.

Keywords: Mesenchymal stem cells, oxidative stress, platinum drugs, DNA repair, excision repair cross-complementation group 1 (ERCC1), gene expression

Introduction

Cells and DNA are continuously unprotected from the oxidative environment which may originate from exogenous or endogenous sources [1]. Oxidative stress is an excessive synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. Primarily, mitochondria are the major source of ROS, which are produced constantly by oxidative metabolism as respiration byproducts. ROS encourage crucial signaling pathways and is essential for cell proliferation, survival, function, differentiation, and apoptosis. Usually, ROS level is controlled by complex antioxidant system (regulated by superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) etc.) and which prevents ROS mediated cellular damage [3]. Unregulated increased ROS levels are harmful to cells and cause the oxidation and nitration of biomolecules such as DNA, RNA, proteins, and lipids. Oxidative stress disturbs regular biological activities leading to several diseases for example hypertension, diabetes, metabolic, pulmonary, cardiovascular, and neurological diseases, etc. [4-6]. Despite this fact, studies present-

https://doi.org/10.62347/TICZ7344

ed contradictory results since ROS has a positive and negative role in tumors [7].

It has been confirmed that many chemotherapeutic agents exert their anticancer effects by the generating oxidative stress and modifying redox homeostasis in cancer cells [3]. However, long-term use of chemotherapy can reduce total cellular ROS indicating a role of ROS in drug effects [8]. The effect of cisplatin is associated with oxidative stress [9]. Mitochondria is a primary target which results in loss of sulfhydryl group of mitochondrial protein, inhibition of calcium uptake and reduced mitochondrial membrane potential.

Cell death occurs immediately after the activation of various signaling pathways; however, the specific pathway depends on the cancerous cell type. Production of ROS depends on the concentration and duration of cisplatin exposure. Cellular redox homeostasis is regulated by the thiol-containing biomolecules. In some conditions, a thiol group may form thiyl radicals that interact with molecular oxygen and generate ROS consequently. A high level of ROS can lead to apoptosis through extrinsic or intrinsic pathways [10]. Chemotherapeutic agents cause apoptosis through one of these pathways. The extrinsic pathway starts once the ligand binds with the TNF-α receptor superfamily after oligomerization and activation of procaspase-8 through adaptor molecule and forms a death-inducing signaling complex (DISC). Cellular stress can trigger intrinsic pathways, for instance, DNA impairment, which causes the release of mitochondrial cytochrome-c resulting in the procaspase-9 activation by associating with apoptosis promoting activating factor-1 (APAF-1) and the development of active apoptosome complex. Bcl-2 family proteins control this DNA damage-induced apoptosis and cytochrome c release. Cisplatin-generated stress initiates several signal transduction pathways, which contribute to apoptosis or chemoresistance [10, 11].

Platinum-based drugs are standard chemotherapeutic agents, such as cisplatin, carboplatin and oxaliplatin. These drugs are important in the management of many types of cancer, including ovarian, head, testicular, breast, bladder, and neck etc. [12]. Unfortunately, significant toxic side effects on cancer and normal cells have been observed, consequently limiting their use at high doses [13]. In addition, researchers have investigated that platinum drugs can effectively overcome tumor resistance due to the presence of metal complexes [14].

Cisplatin and oxaliplatin form covalent DNA adducts, however, oxaliplatin adducts are not required as much, while more potentially inhibits its DNA synthesis [15, 16]. Cisplatin binding to DNA subsequently arrests the replication and transcription of DNA. If damaged DNA is not recognized by the repair mechanism, it consequently leads to cell death [17]. It has been studied that only cisplatin induced DNA adducts are restored by the Mismatch repair (MMR) system. The MMR proteins, such as MutSα, detect cisplatin associated DNA adducts [18]. On the other hand, oxaliplatin DNA damage is solely repaired by the nucleotide excision repair (NER) pathway [19]. ERCC1 and ERCC2 genes are essentially involved the in NER pathway. Successful drug effect has been influenced by ERCC1 expression in colon cancer patients. Furthermore, MMR defected tumors were generally oxaliplatin sensitive but cisplatin resistant [20]. Oxidative DNA damage to 2'-deoxyguanosine by platinum drugs produces 8-Hydroxy-2'-deoxyguanosine (8OHdG) in cancer cells [21]. Loss of damage repair further promotes cytotoxic action [22]. Little information is available on the toxicity of platinum drugs in normal cells. Therefore, a comprehensive study of the normal cells' response to platinum drugs is needed to overcome the associated side effects.

Adult stem cells are extremely valuable in mammalian tissues. They enhance the regenerative response by acting as a cell reserve in generating new cells subsequent to severe injuries [23]. Mesenchymal stem cells (MSCs) have excellent competencies of self-renewal and multilineage differentiation. MSCs are being assessed for clinical trials for the management of various pathological illnesses [24], such as Osteoarthritis (OA) [25]. In the line of various discrepancies, this part of study was planned to determine the platinum drug induced oxidative DNA damage in mammalian MSCs and related consequences on DNA repair genes expression. Moreover, the mRNA levels of MMR and NER genes have been compared with mMSCs sensitivity to platinum drugs.

Materials and methods

Murine BALB/c mice

BALB/c mice were provided by the Central Animal House Facility, Dow University of Health and Sciences (DUHS), Karachi-75280, Pakistan. The study was approved by Advanced Studies and Research Board, University of Karachi (ASRB/No./04955/SC.), Karachi-75270, Pakistan.

Isolation and culturing of murine mMSCs

Mice (6-8 week) were euthanized by cervical dislocation in accordance with the protocol [26] to extract bone marrow and isolate mMSCs. The skeleton was briefly disinfected with 70% ethanol. Hind limbs were cleaned, dissected, and stored in Dulbecco's modified Eagle medium (DMEM, cat. no. 11960-044, Gibco) containing Penicillin/Streptomycin 100 mg/ml (cat. no. 15140, Gibco) on ice. Muscles were removed from femur and tibia, and bone marrow was extracted by flushing the marrow cavities with complete DMEM medium supplemented with 10% fetal bovine serum (FBS, cat. no. 16000-044, Gibco), Penicillin/Streptomycin 100 mg/ml and 2 mM L-glutamine (cat. no. 25030-081, Gibco) using a syringe and 27-gauge needle. The cell suspension was filtered through a 70-mm strainer. The cells were cultured in specific dishes (60-mm) in complete DMEM medium. Cells were incubated at 37°C in a 5% CO_2 incubator. Cellular morphology was observed under the microscope (Nikon ECLIPSE TS100, USA). After 3 hours of incubation, the medium was changed to remove non-adherent cells. Fresh medium was added after 8 hours and replaced every 8 hours for up to 72 hours. Adherent cells were then washed with Dulbecco's Phosphate Buffered Saline (DPBS without Calcium, Magnesium, cat. no. 14190-136, Gibco). When cultures reached confluence (65-70%), cells were washed with DPBS and detached by treating 0.05% trypsin/1 mM EDTA (cat. no. 25300054, Gibco) for 2 minutes at room temperature. Trypsin was neutralized with culture medium, and the cells were seeded into culture flasks (25-cm^2). The medium was replaced after 72 hours.

Characterization of mMSCs by flow cytometry

Cells were plated in T-75 flasks containing complete medium. Cells were detached and centrifuged for 7 minutes at 1200 rpm. Cells were washed with DPBS and counted. Approximately 1×10^6 cells were incubated on ice in 1 ml FACS buffer (2% FBS, 0.1% Sodium azide, and DPBS) for half an hour. Cells were pallet down at 1200 rpm for 5 minutes. Cells were treated with 1:100 dilutions of fluorescein isothiocyanate (FITC) rat anti-mouse CD44 and CD45 antibodies (cat. no. 553133, 553080, BD Bioscience) for 30 minutes. Cells were washed twice and finally resuspended in FACS buffer (100 µl). FITC rat IgG2b, κ (cat. no. 553988, BD Biosciences) was used as an isotype control [27]. Approximately 10,000 events were acquired on BD FACS Calibur (Becton Dickinson, San Jose, CA, USA). To detect Nanog, 250 µl of fixation buffer (cat. no. 554655, BD Bioscience) was used to fix the cells (1×10^6) for 10 minutes at 4°C. Cells were washed in FACS buffer and centrifuged for five minutes at 250 xg. Cells were permeabilized using Perm Buffer I (1:10 dilution, BD Bioscience) on ice for 30 minutes. After washing with FACS buffer, cells were centrifuged, and stained with 20 µL of Alexa Fluor 488 Mouse anti-Mouse Nanog and Alexa Fluor 488 Mouse IgG1 κ Isotype Control (cat. no. 560278, 557782, BD Bioscience) individually at room temperature for 60 minutes. Finally, the cell pellet was resuspended in FACS buffer and analyzed.

Pre-treatment and IC_{50} calculation

Before the experiment, mMSCs were seeded in culture plates at a cell density of 1×10^4 to 1×10^6 cells per well. mMSCs were pretreated with varying concentrations of platinum drugs (1-100 µM) for 24 to 72 hours to assess how mMSCs respond to the effects of the drugs. The final concentration of platinum-based drugs for mMSCs was selected after calculating the IC_{50} value (half-maximal inhibitory concentration). The dose at which 50% of the mMSC population is destroyed within a specified time.

Treatment of mMSCs with platinum drugs

mMSCs (0.7×10^6) were plated in T-25 flasks for control, cisplatin (cat. no. 013965, Pfizer Perth Pty Ltd, Western Australia), and oxaliplatin (cat. no. 044878, Fresenius Kabi Oncology Ltd. Baddi, India) treatments in complete culture medium. Cells were incubated for 24 hours at 37°C (5% CO_2). After adherence, mMSCs were treated with specific concentrations of both drugs comprising 5, 15, and 25 µM for

various time intervals (0, 4, 24, 48, and 72 hours). Cellular morphology was monitored, and images were recorded.

Cell viability assay

The drug-containing medium was removed and cells were washed with DPBS. After trypsinization using 0.05% trypsin/1 mM EDTA, complete culture medium was added to the cell suspension to stop the trypsin reaction. Cells were centrifuged and washed with DPBS. 10 µl of Trypan blue exclusion dye (cat. no. T8154, Sigma) was mixed with 10 µl of cell suspension [28]. Viable cells were counted on a hemocytometer at each time point. Data were plotted after calculating mean number of viable cells.

Detection of 8OHdG by immunofluorescence staining

Cells were cultured on glass coverslips overnight and treated with 25 µM concentrations of cisplatin and oxaliplatin independently for 24, 48 and 72 hours. Drug containing media was discarded and cells were washed with DPBS (without calcium and magnesium). Cells were fixed by incubating with 4% paraformaldehyde (cat. no. 16005, Sigma) for 10 minutes at room temperature and washed twice with DPBS containing Ca^{+2} and Mg^{+2} (cat. no. 14040-091, Gibco). Blocking was carried out with 1% BSA (cat. no. 15561-020, Invitrogen) for 1 hour, followed by incubation with mouse anti-8OHdG antibody (1:200 dilution, cat. no. BS-1278R, Bioss). mMSCs were washed with DPBS two times for five minutes each. Cells were then treated with a 1:500 dilution of FITC-conjugated goat anti-rabbit IgG secondary antibody (cat. no. sc-3839, Santa Cruz Biotechnology) for 1 hour and 30 minutes. Coverslips were washed with DPBS two times for five minutes each. Images (6-8) were captured for each sample using a Nikon ECLIPSE Ti-S microscope, (Japan) equipped with a DS-L3 camera. Approximately 500 cells were counted, and the mean values of 8OHdG positive cells were calculated in all drug treated and non-treated samples.

Determination of necroptosis by dual staining with acridine orange/ethidium bromide (AO/EB)

2×10^4/ml cells were cultured in 96-well plates in the complete DMEM medium. Plates were stored at 37°C in a CO_2 incubator. Next day, mMSCs were incubated with 25 µM cisplatin and oxaliplatin for 24, 48 and 72 hours. Cells were dissociated and centrifuged at 1000 rpm for 5 minutes. The cell pellet was washed and resuspended in 25 µL of cold PBS.

Acridine Orange (AO) staining solution (cat. no. 0249.2, Carl Roth)/Ethidium Bromide (EB, cat. no. H5041, Promega) were prepared at 100 µg/mL each and mixed in equal volumes. 2 µl of the AO/EB mixture was added to the cell suspension. 10 µl of the stained mixture was placed on a slide and examined using a Nikon 90i upright fluorescence microscope (Nikon, Japan). Approximately 500 cells were calculated and morphological modifications observed in three individual samples.

Gene expression analysis by Taq-Man quantitative real-time polymerase chain reaction (TaqMan-qRT-PCR)

Total RNA was isolated from drug-treated and non-treated mMSCs by Trizol reagent (cat. no. 15596-018, Invitrogen). 500 ng of RNA was reverse transcribed into cDNA using the High Capacity cDNA PCR Master Mix (cat. no. 4368813, Applied Biosystems Foster, USA) by 2720 PCR Thermal Cycler (Applied Biosystems, Singapore). The first reaction mixture was incubated at 25°C for 10 minutes for hybridization. Further reactions were carried out at 37°C for 120 minutes, then at 85°C for 5 minutes, and finally stored by cooling to 4°C. 2.5 µl of cDNA (62.5 ng) was amplified with TaqMan Universal Real-Time PCR Master Mix (cat. no. 4324020 Applied Biosystems). RT-PCR reactions were performed on a 7300 Real-Time PCR system (Applied Biosystems, Singapore). Initial hold for 10 minutes at 95°C to activate DNA polymerase, followed by 15 seconds at 95°C, and 60 seconds at 60°C for 40 cycles. mRNA levels of target genes including MMR (e.g., MLH3, MSH2, MLH1, MSH6, and PMS2), and the NER gene (ERCC1) were quantified in mMSCs treated with 5, 15, and 25 µM of cisplatin and oxaliplatin at indicated time intervals. Gene expression was normalized to 18SrRNA expression, and fold changes were calculated relative to the untreated control group to determine upregulation or downregulation in gene expression. Probes and primers used in this study are listed in **Table 1**.

Statistical analysis

Statistical analysis was done to test the hypothesis that platinum drugs induce oxidative stress

Table 1. Sequences of probes and primers for reverse transcription quantitative PCR

Gene	Primer Sequence (5'-3') 50 nmol
18S rRNA	Forward: TAACGAACGAGACTCTGGCAT
	Reverse: CGGACATCTAAGGGCATCACAG
	Probe: [6-FAM] TGGCTGAACGCCACTTGTCCCTCTAA [TAMRA-6-FAM]
MLH3	Forward: CAGACAGCAGTGATTTAACAAGCA
	Reverse: GTAACCTGTATTTGTTCTGCAAAACC
	Probe: [6-FAM] AAAGAATCCAGTCAACCGCCCAACAAA [TAMRA-6-FAM]
MSH2	Forward: CTTGTGTGAGTTCCCCGAGAA
	Reverse: GCATTCCTTTGGTCCAATCTG
	Probe: [6-FAM] TCAGTTCTCCAATCTCGAGGCTCTTCTGA [TAMRA-6-FAM]
MLH1	Forward: TGGAAATGGTGGAAAATGCTT
	Reverse: CGCTGGTGAGGTTAATGATCCT
	Probe: [6-FAM] AAATGACAGCTGCTTGCTACCCCAGGA [TAMRA-6-FAM]
MSH6	Forward: CCCCGATCACCCTGAATTTA
	Reverse: GCTGCCACCACTTCCTCATC
	Probe: [6-FAM] CCCCACCACACTGTATGTGCCTGAAG [TAMRA-6-FAM]
PMS2	Forward: CCAAAATGGTGCAGGTCTTACA
	Reverse: CCCTGTCCGAGCTGATTAGTG
	Probe: [6-FAM] CGTACTGTATCATCTCAGCAGGCGTCCG [TAMRA-6-FAM]
ERCC1	Forward: GGCGGTACCTGGAGACCTACA
	Reverse: TGCTCCAGCTTTTCCATAAGG
	Probe: [6-FAM] CGTATGAGCAGAAGCCAGCCGACC [TAMRA-6-FAM]

18S rRNA, 18S ribosomal RNA; MLH3, MutL homolog 3; MSH2, mutS homolog 2; MLH1, MutL homolog 1; MSH6, MutS homolog 6; PMS2, Post-meiotic segregation increased 2; ERCC1, Excision repair cross-complementation group 1.

in mammalian cells, which inturn disrupts DNA repair mechanisms. Significant differences in means between drug-treated and untreated mMSCs were calculated using analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparisons test, with a significance level of $P < 0.05$. All data analysis were conducted by IBM SPSS Statistics 20.0 software. The data represent the results of three independent experiments, each performed in triplicate.

Results

mMSCs morphology and characterization

Mouse bone marrow cultures reached approximately 65-70% confluency within two weeks, with a reduced number of hematopoietic cells. A uniform population of mBM-MSCs with complete spindle shape morphology was attained within three weeks of culture initiation. Mouse bone marrow cells were shown strong expression of typical mMSCs surface markers. Flow cytometric results revealed that these cells were positive for anti-CD44 and anti-Nanog markers, which were approximately 94.2% and 27.7% respectively. On the other hand, the hematopoietic marker CD45 was negatively expressed, as shown in **Figure 1**.

Cytotoxic impact of platinum drugs treatment on the morphology and viability of mMSCs

Cisplatin and oxaliplatin induced several distinct morphological alterations in mMSCs, including branching and constricting of cells, the prominent appearance of the nucleus, and diminished cell connections as presented in **Figure 2A-C** (**II** and **III**). Overall, minimal changes in cellular adherence were observed after 24 hours of treatment across all concentrations. However, a noticeable increase in non-adherent cells was observed in the drug-treated groups at 72 hours, indicating a time-dependent effect on mMSC attachment and survival.

It was observed that mMSCs growth was not impaired considerably at 1 hour by any concen-

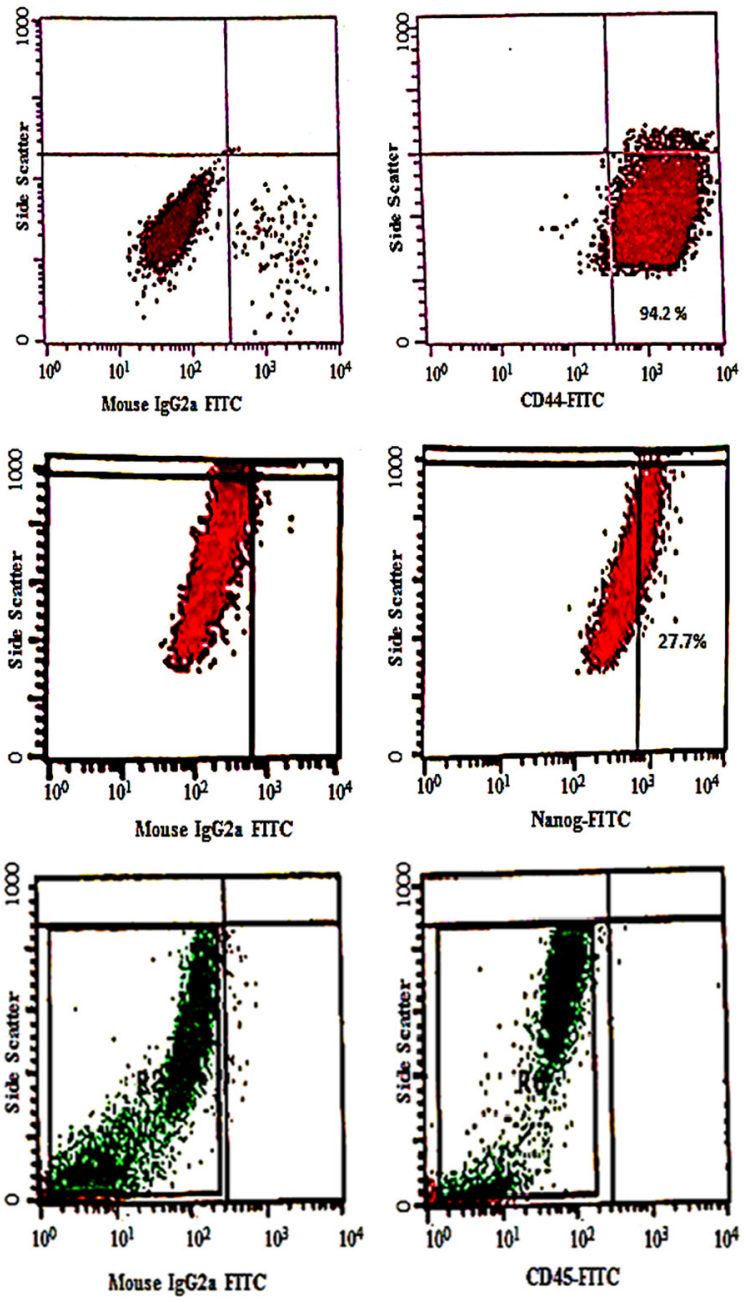

Figure 1. Detection of mMSCs markers by flow cytometry. Analysis of flow cytometry results indicated that isolated mouse bone marrow cells strongly expressed markers of MSCs CD44 (94.2%) and Nanog (27.7%). While negative expression of CD45 was detected.

tration of cisplatin. After 4 hours, mMSCs growth was insignificantly inhibited treated with 5 μM. While in 15 and 25 μM of cisplatin, considerable decrease in viable cells was observed ($P < 0.05$). mMSCs viability was significantly ($P < 0.001$) affected followed by treatment with 5, 15, and 25 μM cisplatin after 24 hours. Cisplatin treated mMSCs (5 μM, 15 μM, and 25 μM) displayed noticeably reduced viable cells at 48 hours ($P < 0.001$). An extraordinary decrease in viable mMSCs were found in each cisplatin exposed group in contrast to non-treated mMSCs by 72 hours. Additionally, the viability of mMSCs is more sensitively affected by cisplatin at 25 μM concentration compared to 5 and 15 μM over 72 hours. Overall, viable cells were decreased with increasing time and drug concentrations as shown in **Figure 3A**.

Treatment with 5 μM and 15 μM oxaliplatin has been shown insignificantly decreased in mMSCs from 1-4 hour. However, 25 μM treated mMSCs displayed an insignificant increase in cell count at 1 hour while no apparent changes were observed at 4 hours. Over 24 hours, number of 5 μM and 15 μM treated cells were found slightly higher than 25 μM treated cells which presented markedly decreased viable cells. We did not find a substantial change in 5 μM oxaliplatin treated mMSCs after 48 and 72 hours but number of cells were considerably low than control. Alternatively, viable cell count of 15 and 25 μM treated mMSCs were markedly reduced over 48 hours. Growth of 15 μM and 25 μM treated cells was greatly influenced since mMSCs were noticeably decreased by 72 hours. Overall, significant differences ($P < 0.001$) were identified among treated and non-treated groups from 24 to 72 hours by each concentration of oxaliplatin presented in **Figure 3B**. The toxic effect of 25 μM oxaliplatin on mMSCs was comparably higher than low concentrations.

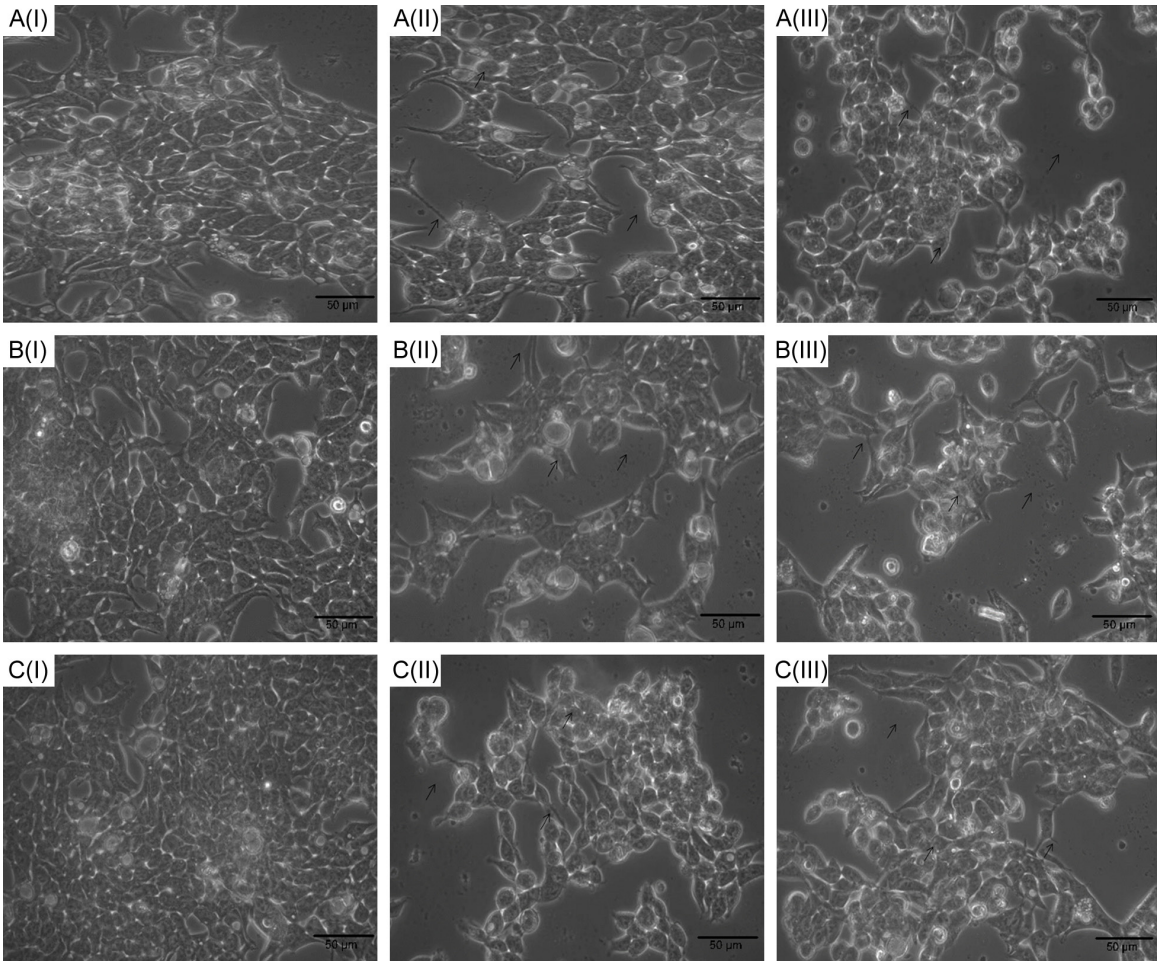

Figure 2. Cisplatin and oxaliplatin induced morphological alterations in mMSCs at different time intervals. Untreated mMSCs (A (I), B (I) and C (I)) showed spindle shape morphology at 24, 48 and 72 hours respectively. Various morphological modifications such as cell branching, constricting, decreased cell connections and prominent nucleus were observed in mMSCs treated with 25 µM cisplatin (A (II), B (II) and C (II)) and oxaliplatin (A (III), B (III) and C (III)) at different time intervals, 40X magnification, Scale bar = 50 µm.

Expressions of 8OHdG by cisplatin and oxaliplatin treatments in mMSCs

Oxidative stress was determined in 25 µM platinum drug treated mMSCs which showed the highest sensitivity. Cisplatin and oxaliplatin treated mMSCs strongly expressed fluorescence staining of nuclear DNA **Figure 4A-C** (**II** and **III**). 8OHdG positive cells were comparatively low in 24 hours cisplatin and oxaliplatin treated mMSCs. 8OHdG positive cells increased at 48 hours and showed a marked rise at 72 hours. 8OHdG expression was increased with time and significant differences have been noticed ($P < 0.001$). High fluorescent signals were showing elevated oxidative stress in cisplatin and oxaliplatin treated mMSCs especially at 72 hours. Compared with cisplatin, treatment of mMSCs with oxaliplatin resulted in a more intense increase in 8OHdG-positive cells at all time points **Figure 5**.

Evaluation of necroptosis after cisplatin and oxaliplatin treatments in mMSCs

In mMSCs, different types of cell death-associated morphological changes were observed post treatment of platinum drugs (25 µM) such as early and late apoptosis, and necrosis like changes (necroptosis) displayed in **Figure 6A-C** (**II** and **III**). Significant cell death including early and late apoptotic or necrotic cells were detected after cisplatin and oxaliplatin treatments at 24 and 72 hours ($P < 0.05$). However, cell death

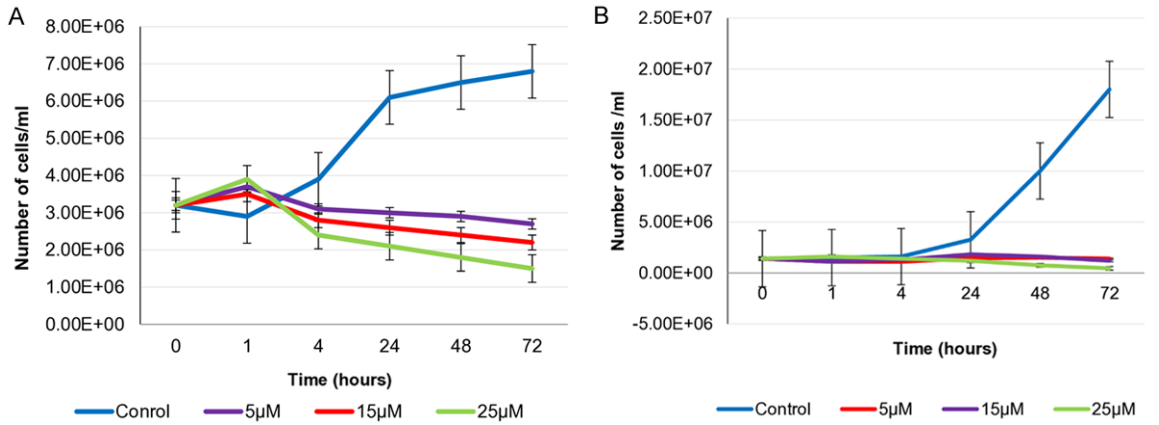

Figure 3. Time and concentration-dependent effects of platinum drugs of mMSCs. mMSCs were treated with 5, 15, and 25 μM of cisplatin (A) and oxaliplatin (B) for 1, 4, 24, 48, and 72 hours. Significantly reduced mMSCs growth was observed from 24-72 hours (ANOVA, $P < 0.05$). 25 μM concentration of cisplatin and oxaliplatin was found to be highly toxic to mMSC by the completion of 72 hours. Data was calculated as means and error bars presenting standard error of the mean.

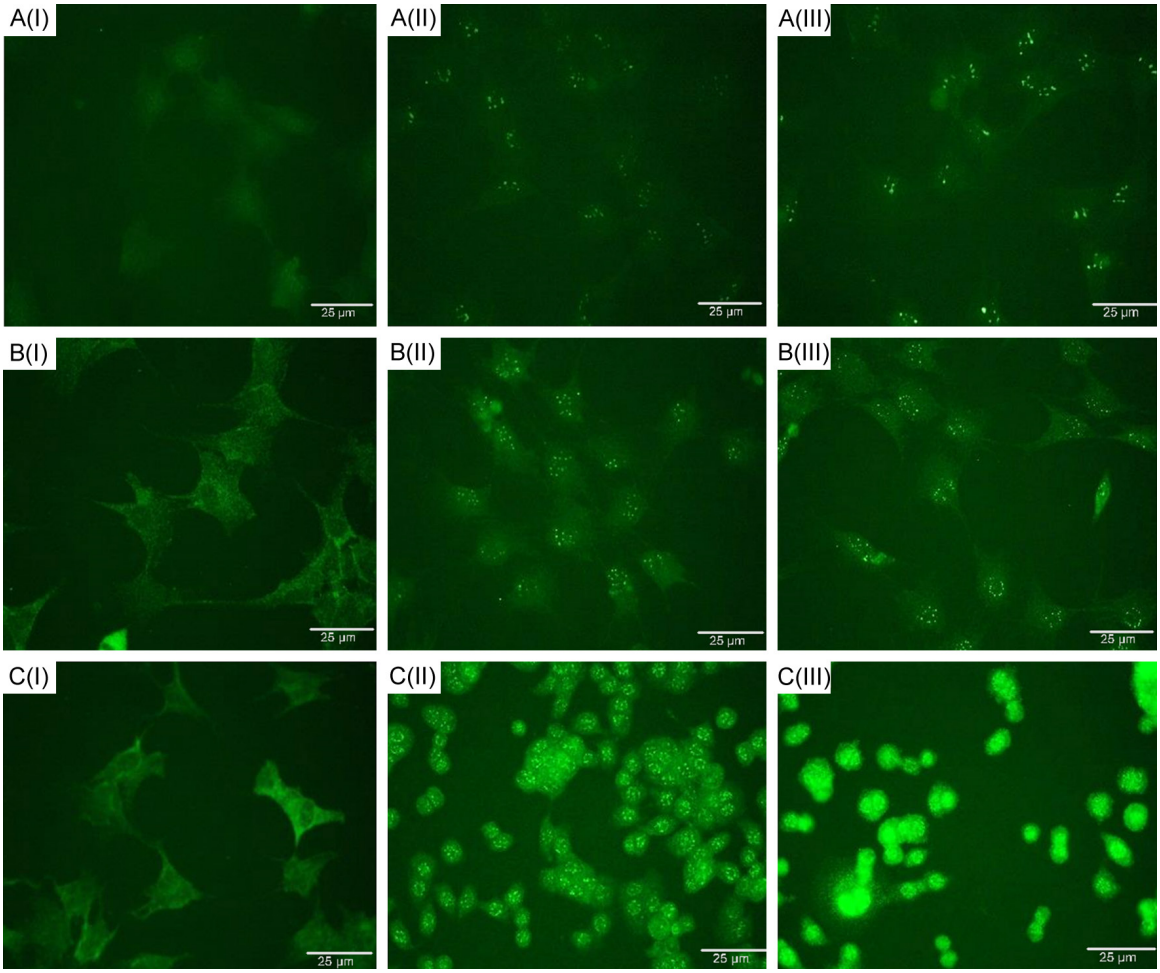

Figure 4. Detection of 8OHdG in cisplatin and oxaliplatin treated mMSCs. Strong 8OHdG immunofluorescence was observed in the nuclear region in cisplatin (A (II), B (II) and C (II)) and oxaliplatin (A (III), B (III) and C (III)) treated mMSCs at 24 (A), 48 (B), and 72 hours (C) respectively. Untreated mMSCs (A (I), B (I), and C (I)) showed negative nuclear staining of 8OHdG, (40X magnification, scale bar = 25 μm).

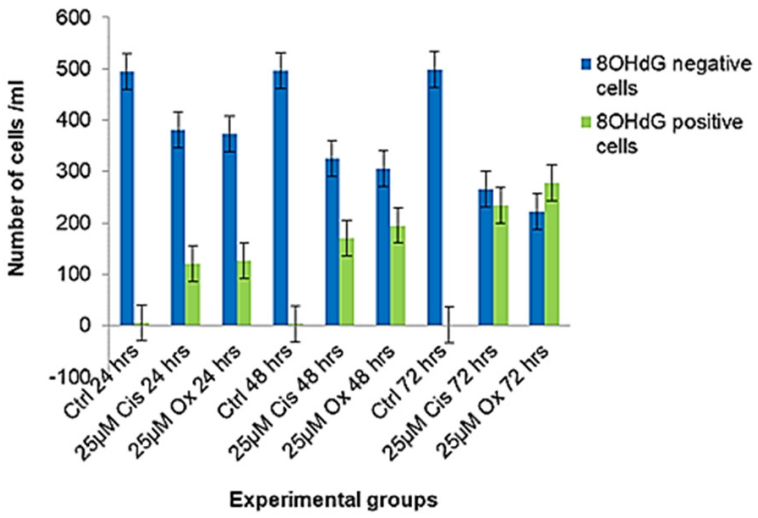

Figure 5. Assessment of 8OHdG nuclear staining in cisplatin and oxaliplatin treated mMSCs. Approximately a total of 500 cells were counted in control and 25 μM cisplatin and oxaliplatin treated mMSCs at 24, 48, and 72 hours. Data has been presented as means, with error bars indicating standard error of the mean. Means of 8OHdG positive cells is statistically significant compared to the mean of 8OHdG negative cells (ANOVA, $P < 0.001$).

was also considerably high in cisplatin and oxaliplatin treated mMSCs at 48 hours ($P < 0.05$). A maximum early apoptotic cells were observed in oxaliplatin-exposed mMSCs, while late apoptotic cell in cisplatin treated mMSCs at 72 hours. Cells with early apoptosis were detected as granular crescent-shaped or yellow-green in color and presented chromatin condensation or nuclear fragmentation, which were stained with AO. Whereas, cells showing late apoptosis were in orange fluorescence stained with EB. A higher number of necrotic cells with increased volume and orange fluorescence were observed in both treatment groups at 72 hours. Overall, necroptosis was prominently increased with the duration of drug exposure **Figure 7**.

Transcriptional influence of cisplatin and oxaliplatin treatments in mMSCs on DNA repair genes

Quantitative analysis of MLH3 mRNA level has been found comparatively high at 24 hours in mMSCs treated with 5 μM, 15 μM and 25 μM of cisplatin than non-treated. Highest fold change was calculated in 25 μM treated sample (1.9-fold). In the same way, MLH3 expression was noticeably increased in all experimental groups including 5 μM, 15 μM and 25 μM (1.9, 1.9, and 2.2-fold) by 48 hours. On the contrary, a significantly decreased fold change (< 1.0) was determined among treated groups of mMSCs at 72 hours $P < 0.001$. Another MMR gene, MSH2 expression was risen (> 2-fold) in all cisplatin treated mMSCs at 24 hours. Similarly, MSH2 mRNA level was greater in 25 μM drug treated mMSCs which showed 1.7-fold increased expression in comparison to control at 48 hours. Oppositely, MSH2 expression were found significantly reduced (< 1.0-fold, $P < 0.001$) in 5 μM, 15 μM, and 25 μM treated cells by 72 hours. Moreover, mMSCs exposed with 5, 15, and 25 μM cisplatin for 24 hours indicated higher mRNA levels of MLH1 which were mentioned as 3.2, 3.7, and 4-fold respectively. mRNA levels were found persistently high over 48 hours. On the other hand, a substantial fold reduction were found in the mRNA levels among 5, 15, and 25 μM of cisplatin compared to non-treated mMSCs, and showed significant differences (< 1.0-fold, $P < 0.001$).

In the current study, 15 and 25 μM cisplatin treated mMSCs showed comparatively high MSH6 mRNA levels while to some extent low in 5 μM at 24 hours. Related mRNA level elevated specifically in 25 μM drug treated mMSCs and showed 1.8-fold greater expression by 48 hours. Then it declined in all cisplatin treated mMSCs on 72 hours than non-treated group (< 1.0-fold, $P < 0.001$). Recently, exposure of mMSCs by indicated cisplatin concentrations displayed 1.9, 2.6, and 2.9-fold high PMS2 mRNA levels in 5, 15, and 25 μM treated mMSCs individually at 24 hours. Then PMS2 level was increased significantly by 48 hours. Conversely, it were found expressively low (< 1.0-fold, $P < 0.001$) among all treated groups over 72 hours.

Furthermore, ERCC1 expression was significantly elevated at 24 hours post cisplatin treatment, but no considerable change was observed in 5 μM and 15 μM cisplatin treated

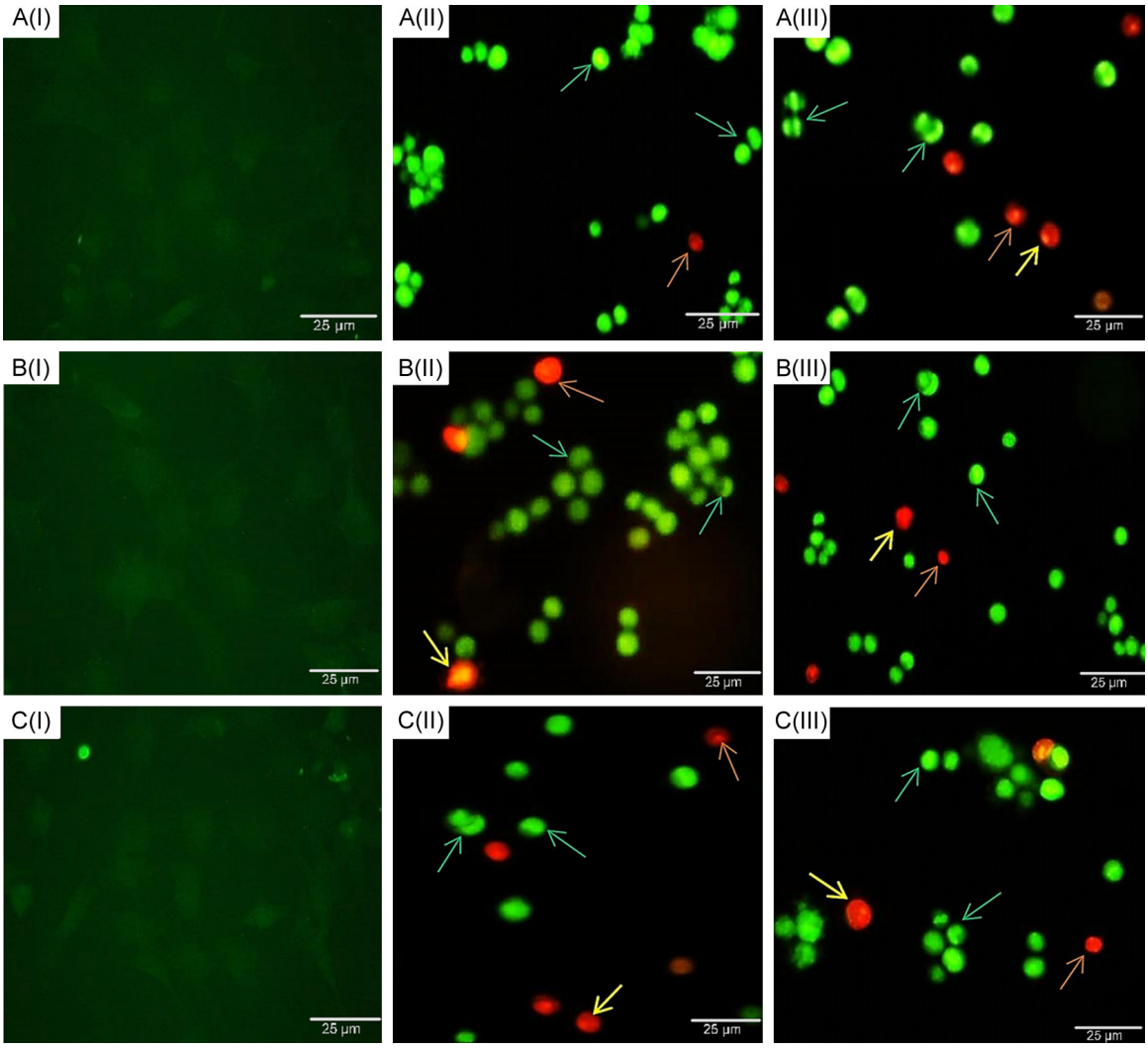

Figure 6. Expressions of necroptosis in cisplatin and oxaliplatin treated mMSCs. Necroptosis was observed at 24 (A), 48 (B), and 72 hours (C) respectively. (A (I), B (I) and C (I)) Normal cells (A (II), B (II) and C (II)) mMSCs treated with 25 µM cisplatin and (A (III), B (III) and C (III)) oxaliplatin. It was observed that these drugs induced various cellular changes associated with apoptosis e.g. granular and crescent yellow-green shapes (AO nuclear staining) showing early-stage apoptosis (Green arrows). However, orange fluorescence is presenting late apoptotic cells (Orange arrows) stained by EB. Necrosis was seen in cells with increased volume (Yellow arrows). (40X magnification, scale bar = 25 µm).

mMSCs. While 25 µM treated cells presented 1.9-fold high expression than non-treated. In the same way, 15 and 25 µM treated cells displayed 1.4 and 2.2-fold elevated expression after 48 hours ($P < 0.001$). Oppositely, ERCC1 expressively high in 5 µM while significantly low in 15 and 25 µM treated mMSCs at 72 hours (< 1.0-fold, $P < 0.001$). The mRNA expression profile of MMR and NER genes in response to cisplatin drug is shown in **Figure 8A-F**.

Oxaliplatin induced remarkably high expression of MLH3 in 5 µM, 15 µM and 25 µM treated mMSCs at 24 hours, which is about 3.7, 4.0 and 4.2-fold respectively. Over 48 hours, MLH3 expression was further increased among treated cells. MLH3 expression in 25 µM treated mMSCs was relatively greater than other treated and non-treated mMSCs. The expression was significantly diminished in 15 and 25 µM treated cells by 72 hours (< 1.0-fold, $P < 0.001$). However, insignificant fold change was observed in 5 µM treated cells. In comparison with non-treated mMSCs, MSH2 expression was detected markedly high in 15 µM and 25 µM treated mMSCs as 1.9 and 2.4-fold at 24 hou-

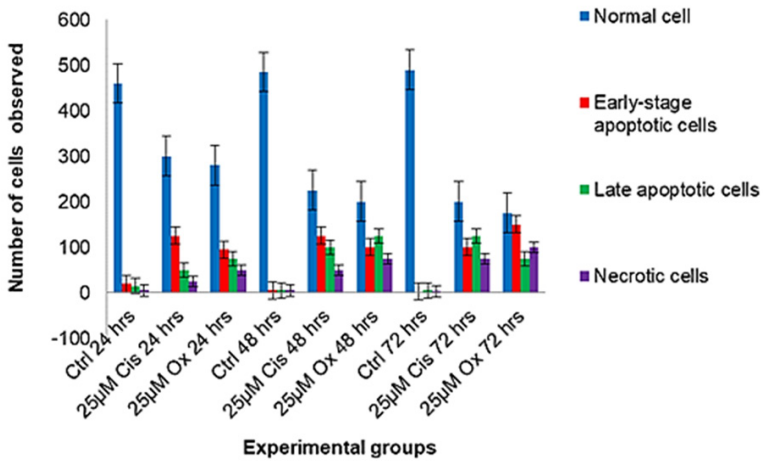

Figure 7. Necroptosis analysis in cisplatin and oxaliplatin treated mMSCs. Cisplatin and oxaliplatin treated mMSCs exhibited strong necroptosis signals at 24 to 72 hours. 25 μM platinum drug treated mMSCs were showed extreme numbers of early-stage and late apoptotic cells, and necrotic cells than untreated mMSCs $P < 0.05$, calculated by ANOVA. Data was plotted as means, by error bars showing standard error of the mean.

rs. Similarly, MSH2 gene expression persistently elevated among all oxaliplatin treatment groups over 48 hours. On the other hand, 5, 15 and 25 μM oxaliplatin treated cells displayed noticeably reduced (< 1.0-fold, $P < 0.001$) MSH2 expression by 72 hours. Besides other MMR genes, MLH1 expression was prominently high in mMSCs after oxaliplatin treatments (5 μM, 15 μM, and 25 μM) on 24 hours. Moreover, it was raised continuously about 2.0, 1.9 and 2.3-fold correspondingly, at 48 hours ($P < 0.001$). On the contrary, MLH1 gene level was considerably decreased among all treated groups (< 1.0-fold, $P < 0.001$) by 72 hours.

Presently, treatment with 5 μM of oxaliplatin indicated considerably low MSH6 mRNA level (< 1.0-fold, $P < 0.001$). However, MSH6 expression was significantly high in 15 μM and 25 μM at 24 hours. MSH6 transcript level was increased in all treatment groups, but only 25 μM treated mMSCs showed 2-fold high expression by 48 hours ($P < 0.001$). Quite the opposite, MSH6 expression was observed remarkably low (< 1.0-fold, $P < 0.001$) in drug treated mMSCs over 72 hours. Another target gene, the PMS2 expression level was significantly low in 5 μM and 15 μM, while high in 25 μM treated mMSCs at 24 hours. In addition, drug treated mMSCs groups showed a noticeable increase in PMS2 mRNA specifically in 25 μM treated mMSCs which showed 2-fold high expression at 48 hours ($P < 0.001$). While significant fold drop was detected among all oxaliplatin treated mMSCs by 72 hours (< 1.0-fold, $P < 0.001$).

Furthermore, ERCC1 mRNA level was moderately high upon 5 μM oxaliplatin treatment. Vice versa, it was significantly high in 15 μM and 25 μM treated mMSCs at 24 hours. ERCC1 expression remained consistently high and 5 μM, 15 μM, and 25 μM treated cells displayed 2.0, 2.9, and 3.4-fold increased ERCC1 expression respectively by 48 hours. Conversely, mMSCs exhibited significantly low (< 1.0-fold, $P < 0.001$) ERCC1 expression after exposing to each concentration on 72 hours. The mRNA levels of target genes in response to oxaliplatin treatment are exhibited in **Figure 9A-F**.

Discussion

Various platinum-based drugs have been tested on different types of cancers like non-small cell lung carcinoma, ovarian carcinoma, head, and neck squamous cell carcinoma, colorectal cancer, pancreatic and gastric tumors. The drug response of normal cells is completely unknown. Presently, the initial culture was contained heterogeneous types of cells. However, round-shaped cells were gradually decreased, and the growth rate increased over time upon subculture. It has confirmed that repeat medium change is an effective protocol for achieving the net population of mMSCs [27]. Whereas, frequent changing of culture media prevents adherence of non-specific cells on culture dishes [26]. The present results of immunophenotype identification markers are consistent with past studies have shown MSCs positive markers CD44 and Nanog, while negative CD45 [4]. Moreover, a study determined the expression of CD44 and CD45 surface markers in bone marrow MSCs of Balb/c mice [26].

Studies reported that chemotherapy affects rapidly growing normal, as well as cancer cells.

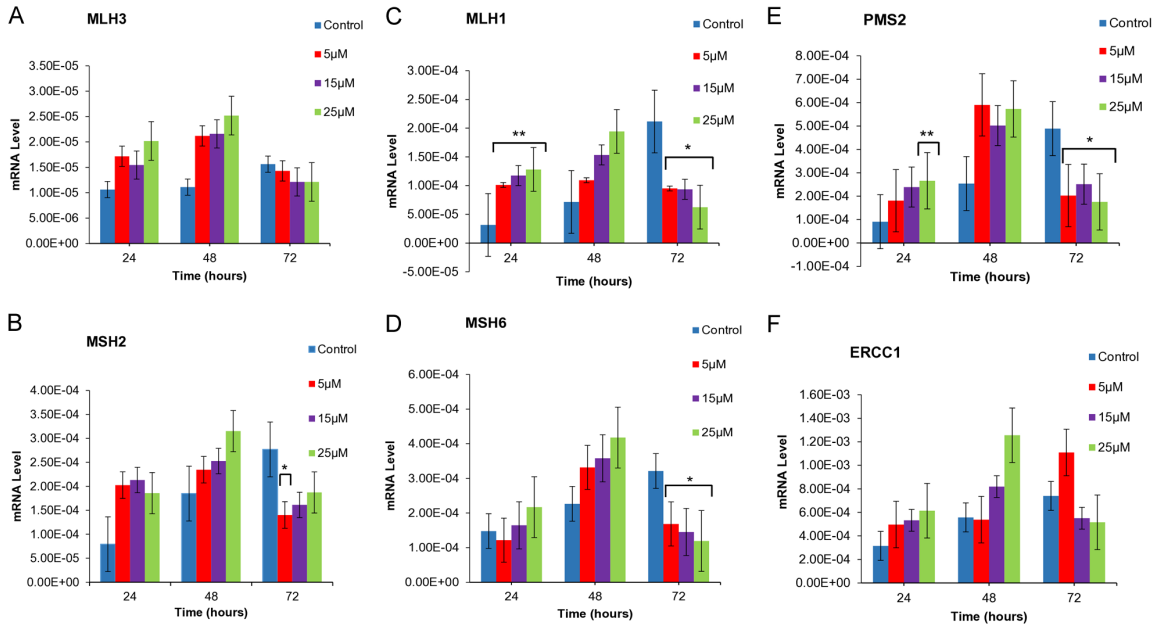

Figure 8. Time and concentration-dependent effects of cisplatin on DNA repair genes expressions in mMSCs. TaqMan-qRT-PCR analyses of DNA repair genes including (A) MLH3, (B) MSH2, (C) MLH1, (D) MSH6, (E) PMS2, and (F) ERCC1. mRNA levels were quantified at indicated concentrations (5, 15, and 25 μM) of cisplatin (at 24, 48 and 72 hours). Average mean differences were determined by ANOVA, $P < 0.001$ was considered statistically significant. Data presented as means, by error bars displaying standard error of the mean, ** (≥ 3.0-fold high), * (≤ 0.5-fold low).

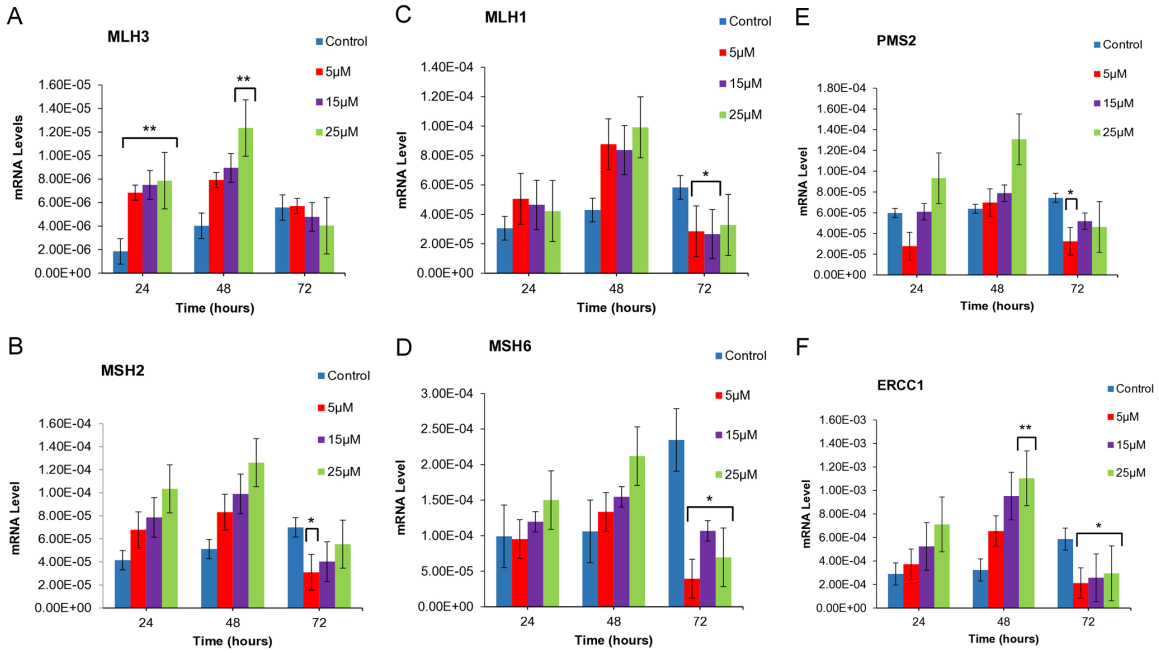

Figure 9. Time and concentration-dependent effects of oxaliplatin on DNA repair genes expressions in mMSCs. TaqMan-qRT-PCR analyses of DNA repair genes including (A) MLH3, (B) MSH2, (C) MLH1, (D) MSH6, (E) PMS2, and (F) ERCC1. mRNA levels were quantified at indicated concentrations (5, 15, and 25 μM) of oxaliplatin (at 24, 48 and 72 hours). Average mean differences were calculated by ANOVA, $P < 0.001$ was considered statistically significant. Data presented as means, by error bars displaying standard error of the mean, ** (≥ 3.0-fold high), * (≤ 0.5-fold low).

However, drug effects are conditionally dependent on concentration and types of cells [29]. Present results are comparable with these outcomes since mMSCs growth was inhibited with increasing time and concentration of cisplatin [30]. Low-level significant effects were observed at 5 μM and 15 μM concentrations of cisplatin after 24 hours. However, viable mMSCs were gradually decreased with the time of drug exposure. The study presented the morphology of hMSCs, and differentiated fibroblasts were not altered after incubating with 200 and 1000 ng/mL cisplatin at 24 hours [31]. In parallel, the current study displayed dose dependent modifications in cisplatin treated mMSC adherence, survival together with morphology e.g. prominent nucleus, branching of cells, reduced cell-to-cell attachment, and colony formation capacity. In addition, viable mMSCs were more promptly decreased when treated with 25 μM concentration by 72 hours. A past study indicated that pseudopodia highly increased in hADSCs undergoing cisplatin treatment with 10 mg/ml concentration. Drug treated hADSCs started branching and constricting from cells, clumping of endosomes and prominent nucleus. Furthermore, it has been investigated that low cisplatin concentrations (2.5 and 5 μg/ml) have no noticeable effect on viability, morphology, and ultrastructure of hADSCs. Moreover, study showed that MSC2 group exhibited significant decrease ($P < 0.05$) in adherence on 1000 ng/mL dose of cisplatin [32].

Similar to cisplatin, oxaliplatin also produced time and concentration dependent cytotoxic effects on mMSCs. Viable mMSCs treated with 25 μM oxaliplatin were remarkably lower than 5 μM and 15 μM from 24-72 hours. Consistently, greater oxaliplatin toxicity has been shown in culture and differentiated Schwann cells at 3 μM concentration separately at 48 hours. Although considerable alterations in shape were observed. While a significant decline in viable Schwann cells was monitored upon 24 and 48 hours of treatment with different concentrations of oxaliplatin, paclitaxel, and cisplatin individually [33]. Previous studies revealed significantly reduced clonogenic potential of HCT116 after exposure to clinically relevant concentrations of oxaliplatin [34]. Similar outcomes presented, such as proliferation arrested in oxaliplatin treated colorectal cancer cells than the control group [35]. In the current study, oxaliplatin treated mMSCs underwent various morphological changes such as branching and constricting of cells, and prominent appearance of the cell nucleus, etc. Moreover, several non-adherent cells were examined especially at higher drug levels by increasing time. A study showed L-OHP (oxaliplatin) sensitive cells displayed round and polygonal morphology. On the other hand, Colo320R and HT-29R cells (L-OHP resistant) were exhibited epithelial-to-mesenchymal conversion including pseudopodia formation, loss of cell polarity, fusiform and increased spaces [36].

Previous studies have confirmed oxidative stress-induced cell death in cancer cells exposed to chemotherapeutic drugs. Presently, a significant level of oxidative stress has been caused by 25 μM cisplatin and oxaliplatin in normal mMSCs. The expression of 8OHdG was comparatively low at 24 hours in cisplatin and oxaliplatin treated mMSCs. However, fluorescent signals were relatively high at 48 hours. Significant strong 8OHdG expressions were detected by 72 hours, showing elevated oxidative stress. Likewise, cisplatin causes renal oxidative stress in mice has been verified by 8OHdG expression, ROS dependent kidney injury and cell death [37].

In addition, cisplatin cytotoxicity was increased at ≥ 15 μM concentration with excessive ROS production and cells death of mouse Sertoli cells [38]. In comparison to control kidneys tubular cells, an elevated number of 8OHdG positive nuclei was seen at days 5 to 14 and then progressively reduced in CDDP treated rats [39]. Oxidative stress has been documented to play active roles in CDDP associated acute renal failure (ARF) which suggests severe toxic effects of cisplatin on normal kidney cells [40]. Another study supported oxaliplatin related cellular damage by generating ROS [41]. Increased level of mitochondrial superoxide and enteric neuronal death has been caused by oxidative stress in oxaliplatin treated Balb/c mice [42]. Moreover, oxaliplatin induced mitochondrial oxidative stress have been reported in Wistar rat's liver [43].

In the context of cisplatin and oxaliplatin related oxidative stress particularly in mMSCs, can overwhelm the antioxidant defense system,

leading to cellular damage, including DNA strand breaks, and disruption of normal cellular functions. The expression of antioxidant enzymes like superoxide dismutase (SOD), catalase, or glutathione peroxidase (GPx) may be upregulated in an attempt to mitigate cellular damage. However, prolonged stress condition might exhaust these defenses, further compromising repair mechanisms and cellular integrity. Drug associated oxidative damage impaired the expression of DNA repair genes and diminished ability to repair damage. This could lead to the accumulation of mutations or chromosomal instability, which can compromise the ability of mMSCs to differentiate and proliferate.

It has been described that cisplatin activates apoptosis in sensory epithelial cells [44]. Currently, detection of apoptotic or necrotic mMCs has confirmed high levels of cisplatin and oxaliplatin cytotoxicity. The number of dead cells was found relatively low at 24 hours and then rose at 72 hours. Equally, significant DNA damage and apoptosis were detected in the cisplatin injected mouse renal cells [45]. 200 and 1000 ng/mL of cisplatin did not cause considerable apoptosis at 24 hours in hMSCs and differentiated fibroblasts [31]. Furthermore, cisplatin induced cell death and temporal stimulation of caspase-3/7 has been investigated in the HK-2 culture [37]. However, cisplatin induced cell death in a dose dependent mode in UBOC1, HK2, and SH-SY5Y cells at 5, 10, and 20 μM on 24 hours of treatment [46]. Oppositely, hMSCs were not showed apoptosis after cisplatin treatment. Instead of high concentration, hMSCs and fibroblasts showed prolonged G2 phase accumulation (> 96 hours) at 4 hours [31]. In current study, 25 μM oxaliplatin meaningfully induced apoptosis in mMSCs at 72 hours. Alternatively, a study postulated oxaliplatin associated apoptosis only in cancer cells but not in normal cells [47]. Surprisingly, low concentration of oxaliplatin stimulated apoptosis on 24 hours [35]. On the other hand, tumors and normal prostate cells (PrEC), and fibroblasts WI-38 displayed oxaliplatin resistance [47]. Presently, it was observed that oxaliplatin treated mMSCs indicated somewhat greater cell death than cisplatin. Accordingly, oxaliplatin is more effective than cisplatin in the induction of cell death. Extensive apoptotic changes with DNA fragmentation were seen in A2780 and CEM cells treated with 2 μM and 10 μM oxaliplatin at 24 hours, respectively. Whereas, an equal dose of cisplatin exhibited parallel apoptotic effects with greater DNA-Pt adducts [47].

It is revealed that cisplatin-associated cytotoxicity interfere with transcription or DNA replication. Repetitive futile cycles of synthesis pass through damaged DNA by the functional MMR system, and eliminate new strands and finally cause cell death [48]. Recent study found that MSH2 expression was > 2-fold high among cisplatin treated mMSCs at 24 hours while significantly reduced by 72 hours, at that time cells death also increased. It has been shown that cisplatin related apoptosis depends on the MSH2/MSH6 expression and the breakdown of MLH1 [49]. Alternatively, repairing of cisplatin intrastrand adducts is not affected by the down-regulation of MSH2 expression. In contrast, mouse embryonic fibroblasts and cancer cells displayed greater repair of cisplatin intrastrand adducts and interstrand cross-links (ICLs) with MSH2 reduction at 48 and 72 hours. Moreover, cisplatin resistance in MMR deficient cells is due to the rapid repair of ICLs [50].

In the current results, MSH6 mRNA levels were moderately high in cisplatin-treated mMSCs at 24 and 48 hours, while it reduced at 72 hours, with a significant drop in viable cell count. Similarly, Polβ deficient cells did not show a prominent increase in cisplatin resistance after downregulating MSH6 [50]. Whereas, MSH6 expression is required to facilitate cisplatin sensitivity, confirmed by comparing the MSH6 deficient and proficient DLD-1 cells [51]. In addition, cisplatin-resistant cancer cell lines exhibited a 90% reduction in MMR proteins, specifically MLH1 and MSH2 [52]. Recent outcomes showed > 3-fold higher overexpression of MLH1 post 24 hours of exposure to cisplatin (5 μM, 15 μM, and 25 μM). Expression was persistently high at 48 hours, especially in 15 μM and 25 μM treated cells. Later, MLH1 mRNA levels dropped significantly within 72 hours. In comparison to this, a past study investigated that MLH1-proficient cells were more sensitive to cisplatin. MLH1 binds to PMS1, PMS2, or MLH3 and forms a heterodimer [53]. PMS2 and MLH3 are possibly weak with MLH1 expression. Cells with active MLH1 displayed more sensitivity to cisplatin [54]. A study demonstrated that cisplatin causes apoptosis through augmenting the interlinkage among MMR protein MLH1/PMS2 and p73 in MMR-proficient cells

[55]. This study determined similar time and concentration dependent elevated MLH3 and PMS2 expression after cisplatin treatment from 24-48 hours. While a substantial decrease in fold-change was detected at 72 hours. In a previous study, MLH1 was downregulated in human and mouse cells under hypoxia, but mRNA levels of MSH2, MSH6, and PMS2 remained unchanged. Furthermore, the PMS2 expression level was monitored low, for the reason MLH1 deficiency destabilizes the PMS2 [56].

Moreover, cisplatin sensitivity was enhanced after the double knockdown of the XPF/ERCC1 complex in non-small cell lung cancer cells [57]. At present, ERCC1 expression was persistently high in 5 μM treated cells over 72 hours, while cells showed low sensitivity. However, ERCC1 expression was significantly decreased by 25 μM treatment, indicating greater drug sensitivity. Previous research has shown a decrease in XPA and ERCCI levels, with satisfactory outcomes of cisplatin treatment of testicular cancer patients. While, overexpression of ERCC1 is associated with cisplatin resistance [58].

A clinical study has been revealed that MMR actively responds to cisplatin. However, oxaliplatin related DNA adducts are not essentially recognized by the MMR pathway. MMR defected cells did not show oxaliplatin resistance [59]. In addition to cisplatin, the status of selected DNA repair genes was determined after oxaliplatin exposure in mMSCs. Comparisons of mRNA expression with cellular sensitivity to oxaliplatin explored that mMSCs exhibited remarkable sensitivity to oxaliplatin. Noticeably high MLH3 and MSH6 expression has been determined after 24 and 48 hours of treatment. The level of expression was dependent on the dose and time. Over 72 hours, MLH3 and MSH6 expressions have been significantly reduced than non-treated mMSCs. It is hypothesized that survival failure, coupled with a reduction in the repair process leads to a rapid increase in cell death. It has been tested that no substantial effect of hMSH3 or hMSH6 expression on oxaliplatin cytotoxicity was observed. Tumors with MMR defects may respond better to oxaliplatin than cisplatin or carboplatin [60]. The current study found that the expressions of MSH2 and PMS2 were consistently high at 24-48 hours, and markedly decreased in 72 hours.

A study reported that when MSH2-/- mice treated with ROS inducing agent (potassium bromate), tumor progression has been increased in the small intestine [61]. In contrast, it has been hypothesized that the proportion of oxaliplatin DACH ligands inhibit DNA damage recognition by MSH2 proteins [62]. Besides, MLH1 expression was moderately raised in mMSCs upon oxaliplatin treatment from 24 to 48 hours then it declined. Studies exhibited that cells with reduced hMLHI expression showed a low level of cisplatin resistance but insignificantly resistant to oxaliplatin. Additionally, MLH1 deficient cells showed a weakened antioxidant response and more sensitivity to the ROS-generating drug [63].

Furthermore, ERCC1 is an essential part of the NER pathway, which is thought to aid in the emergence of drug resistance and also affects platinum drug tolerance in normal tissues [64]. The level of ERCC1 expression was increased in 15 μM and 25 μM oxaliplatin treated cells at 24 and 48 hours. Vice versa, mMSCs showed an excessive reduction in ERCC1 by 72 hours. However, reduction of ERCC1 mRNA levels has been given satisfactory clinical results followed by platinum drug therapy [65]. Quantitative measurement of ERCC1 mRNA has been monitored higher after 20 hours of exposure to oxaliplatin in the A2780 ovarian cell line [66]. Existing results illustrated that mMSCs fail to proliferate when drug damage is not repaired.

Hence, a successful detection of 8OHdG expression showed cisplatin and oxaliplatin generated oxidative stress in mMSCs. These outcomes support the hypothesis that platinum drugs induce oxidative stress that disturbs DNA repair gene expression in mMSCs. In particular, cisplatin significantly affected the expression of MLH1, MSH6, and PMS2, while oxaliplatin MLH3, MSH6, and ERCC1 genes. Initially, most genes were seen to be upregulated and then downregulated with increasing drug exposure time and cell death.

mMSCs are known for their DNA repair capabilities, which are essential for maintaining their stemness and regenerative potential. The alteration in DNA repair gene expression due to chemotherapeutic agents may affect the balance between DNA repair, cell cycle progression, and apoptosis. The functional decline in DNA repair systems could push mMSCs towards senescence or even trigger programmed cell death.

Disturbance in the DNA repair machinery can affect the transcription of mRNA coding for DNA repair proteins. Under oxidative stress, the transcriptional activity of DNA repair genes may be dysregulated. In some cases, this can lead to a decrease in the mRNA levels of essential repair enzymes, making the cell more prone to accumulate mutations or chromosomal aberrations. This might compromise the mMSCs' ability to recover from damage. Persistent oxidative stress and faulty DNA repair mechanisms could potentially lead to genetic instability in mMSCs. This might affect their differentiation capabilities and potentially contribute to tumorigenesis.

It may be possible when oxidative DNA damage occurs by platinum drug, due to the sensitive nature of normal cells damage is not repaired. So that cells are unable to survive further, it ultimately leads to cell death. Moreover, a recent study confirmed that mMSCs sensitivity to the platinum drug does not only depend on its interaction with target DNA but also the response of DNA repair. Further investigations are required to adopt effective ways to overcome the side effects of chemotherapy treatment. Developments of oxidative stress inducing anticancer therapies are challenging for cancer cell death without altering normal cells physiology. The present findings prompted us to explore the effect of platinum drugs that need to be tested on normal stem cells from human sources at the genome and protein levels.

The oxidative stress generated by cisplatin and oxaliplatin treatment can indeed disturb the normal expression of DNA repair genes at the mRNA level in normal mMSCs. This disturbance may impair the ability of mMSCs to repair DNA damage, thereby affecting their survival and proliferation. Understanding how these drugs influence the DNA repair machinery in mMSCs is crucial for improving therapeutic strategies and minimizing side effects, particularly in the context of cancer treatment.

Conclusion

The concentration and temporal oxidative stress was generated in mMSCs by cisplatin and oxaliplatin, leading to altered expression of DNA repair genes at the mRNA level. Cisplatin upregulated MLH1 and PMS2 at 24 hours, while it downregulated MSH2, MLH1, MSH6, and PMS2 at 72 hours. In contrast, oxaliplatin caused upregulation of MLH3 and ERCC1 at 24-48 hours, and downregulation of MSH2, MLH1, MSH6, PMS2, and ERCC1 expression at 72 hours. These findings suggest that adult stem cells are highly susceptible to damage from anticancer drugs present in normal tissues and organs during chemotherapy. Further research into localized treatment strategies may help to minimize undesirable side effects on adult stem cells.

Disclosure of conflict of interest

None.

Abbreviations

18S rRNA, 18S ribosomal RNA; 8OHdG, 8-Hydroxy-2'-deoxyguanosine; ANOVA, Analysis of variance; AO, Acridine Orange; APAF-1, Apoptosis promoting activating factor-1; CAT, Catalase; DISC, Death-inducing signaling complex; DMEM, Dulbecco's Modified Eagle Medium; EB, Ethidium bromide; ERCC1, Excision repair cross-complementation group 1; FACS, Fluorescent activated cell sorting; FBS, Fetal Bovine Serum; GPx, Glutathione peroxidase; GSH, Glutathione; hADSCs, Human adipose-derived stem cells; ICLs, Interstrand cross-links; MLH1, MutL homolog 1; MLH3, MutL homolog 3; MMR, Mismatch repair; mMSCs, Murine mesenchymal stem cells; MSH2, mutS homolog 2; MSH6, MutS homolog 6; NER, Nucleotide excision repair; PCR, Polymerase chain n reaction; PMS2, Post-meiotic segregation increased 2; RFS, Relapse free survival; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; SOD, Superoxide dismutase; XPA, Xeroderma Pigmentosum Group A.

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