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Basic Study

Soluble factors secreted by human Wharton's Jelly Mesenchymal Stromal/ Stem Cells exhibit therapeutic radioprotection - a mechanistic study with integrating network biology

Soluble factors secreted by human Wharton's Jelly Mesenchymal Stem Cells exhibit therapeutic radioprotection

Dharmendra Kumar Maurya, Mayuri Bandekar, Santosh Kumar Sandur

Abstract

BACKGROUND

Human Wharton's jelly-derived mesenchymal stromal/ stem cells (hWJ-MSCs) have gained considerable attention in their applications in cell-based therapy due to several advantages offered by them. Recently, we reported that hWJ-MSCs and their conditioned medium have significant therapeutic radioprotective potential. This finding raised an obvious question to identify unique features of hWJ-MSCs over other sources of stem cells for a better understanding of its radioprotective mechanism.

AIM

Understanding the radioprotective mechanism of soluble factors secreted by hWJ-MSCs and identification of their unique genes.

METHODS

Propidium iodide staining, endogenous spleen colony-forming assay, and survival study were carried out for radioprotection studies. Homeostasis driven proliferation assay was performed for *in vivo* lymphocytes proliferation. Analysis of RNAseq data was performed to find the unique genes of WJ-MSCs by comparing them with bone marrow mesenchymal stem cells, embryonic stem cells, and human fibroblasts. Gene enrichment analysis and protein-protein interaction network were used for pathway analysis.

RESULTS

Co-culture of irradiated murine splenic lymphocytes with WJ-MSCs offered significant radioprotection to lymphocytes. WJ-MSCs transplantation increases the homeostasis-driven proliferation of the lymphocytes. Neutralization of WJ-MSCs-conditioned medium (WJ-MSCs-CM) with G-CSF antibody abolished therapeutic radioprotection. Transcriptome analysis showed that WJ-MSCs share several common genes with BM-MSCs and ESCs and also express a high levels of unique genes such as IL1-α, IL1-β, IL-

6, CXCL3, CXCL5, CXCL8, CXCL2, CCL2, FLT-1, and IL-33. It was also observed that WJ-MSCs preferentially modulate several cellular pathways and processes which handle the repair and regeneration of damaged tissues compared to stem cells from other sources. Cytokine-based network analysis showed that most of the radiosensitive tissues have a more complex network for the elevated cytokines.

CONCLUSION

Systemic infusion of WJ-MSCs-CM will have significant potential for treating accidentally radiation exposed victims.

Key Words: Radioprotection; Mesenchymal stem cells; WJ-MSCs; Cytokines; G-CSF; Network biology

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Core Tip: This study showed the potential role of cytokine G-CSF in therapeutic radioprotection. Transcriptome analysis shows that WJ-MSCs have unique set of genes compared to BM-MSCs and ESCs. WJ-MSCs secrete several cytokines which promote cellular pathways which handle tissue repair and regeneration.

INTRODUCTION

Exposure of mice to high doses of gamma-radiation causes acute radiation syndromes (ARS), which include damage to hematopoietic, gastrointestinal, and neurovascular systems depending on the dose. Currently, extensive progress has been made to understand the molecular players leading to ARS and therapeutic options. Several agents have been reported to protect mice from ARS which include thiol-containing

compounds, phytochemicals [1-3], and several cytoprotective cytokines and growth factors (IL-1, IL-6 TNFαG-CSFGM-CSF), *etc.* [4-6].

Stem cell therapy is one of the promising strategies for the amelioration of ARS because of its ability to regenerate by sensing the damage. Mesenchymal stromal/ stem cells (MSCs) are one such type of cells that have the potential for clinical applications. They are easy to culture, possess low immunogenicity, high regenerative potential, multi-lineage differentiation abilities, and potent anti-apoptotic & immune-suppressive effects [7]. Several reports are showing the ability of MSCs to repair various tissue injuries induced by radiation and other stressors [8-13]. The mechanism of action to prevent radiation-induced tissue injury by MSCs could be paracrine secretion of several soluble factors [14]. Various paracrine factors secreted by MSCs include cytokines, chemokines, pro-survival factors, and growth factors [8, 15-19]. All these properties of the MSCs may help in recovery from hematopoietic and gastrointestinal tract injury after radiation exposure.

The therapeutic potential of Bone marrow MSCs (BM-MSCs) against radiation injury has been well documented in several experimental studies [20-24]. Lange *et al* (2011) showed that a protective response was evoked in combating inflammatory events post-radiation exposure after systemic administration of MSCs [23]. Another study reported by Chang *et al* (2012) showed protection against whole-body irradiation (WBI) induced damage to the intestinal mucosa by enhancing angiogenesis and chemotaxis after bone marrow transplantation [11]. Existing evidence shows that MSCs "home in" to bone marrow or other injured tissues and secrete different soluble factors which help in the recovery of radiation-induced hematopoietic damage *via* induction of hematopoietic stem/progenitor cell division and differentiation [23-25]. The trophic factors (GM-CSF, TPO, and SCF) secreted by MSCs can modulate hematopoietic stem cell niche thereby rescuing endogenous hematopoiesis [23, 25].

Stem cells isolated from Wharton's Jelly of the umbilical cord (such as WJSCs, WJ-MSCs, or UCMSCs) are a unique source of MSCs. These cells did not induce any adverse effects or teratoma formation in the recipients and are hence considered safe [26].

WJ-MSCs exhibit reduced immunogenicity [27] as compared to other MSC types. WJ-MSCs have low expression of costimulatory ligands, which otherwise can stimulate immune responses [28]. Interestingly, WJ-MSCs are known to express human leukocyte antigen G (HLA-G) which helps in the expansion of immune-suppressive T_{reg} cells. Recently, our laboratory has shown the radioprotective potential of human WJ-MSCs and also conditioned media collected from culturing WJ-MSCs against ionizing radiation (IR) induced mortality in mice [8, 29].

In the present study, we have established the involvement of cytokine G-CSF in the observed radioprotection offered by human WJ-MSCs-CM. We have also constructed the cytokine network using an experimentally determined list of the cytokines secreted in mouse blood serum 4h after hWJ-MSCs transplantation in the mice. Our study showed that the PPI network constructed using cytokines is more complex in the radiosensitive tissues as compared to other tissues such as muscle. Our transcriptome analysis showed that WJ-MSCs preferentially modulated several cellular pathways and processes which are responsible for the repair and regeneration of damaged tissues.

MATERIALS AND METHODS

Chemicals and reagents

Mesenchymal stem cell expansion medium, RPMI-1640 medium, Fetal bovine serum (FBS), and Antibiotic–antimycotic mixture were purchased from Hi-Media Pvt (India). MSC qualified fetal bovine serum was purchased from Thermo Fischer Scientific. Propidium Iodide (PI), Ribonuclease A, and Triton X-100 were purchased from Sigma Chemical Co. (USA). Hoechst 33342 and carboxyfluorescein diacetate succinimidyl ester (CFSE) was procured from Molecular Probes (NY, USA). Anti-G-CSF antibody was procured from R&D Systems (Minneapolis, USA). All other chemicals used were of analytical grade and were procured from local manufacturers.

Isolation and characterization of WJ-MSCs

Ethical approval for isolation of WJ-MSCs was obtained from the Institutional ethical

review board at Bhabha Atomic Research Centre Hospital, Mumbai, India (project numbers IC-SCRBARC/2018/2 and BARCHMEC/14). Human umbilical cords were freshly collected soon after the delivery of a full-term infant by cesarean section from the donor after obtaining written consent [8]. WJ-MSCs were isolated and characterized as described earlier [8] from the umbilical cord and cultured in mesenchymal stem cell expansion medium added with 20% MSC qualified fetal bovine serum and 1% antibiotic-antimycotic mixture.

Preparation of WJ-MSCs conditioned media

WJ-MSCs (5x104) were cultured in 1 mL WJ-MSC complete media for 24h in 24 wells plate. After incubation, media was harvested, centrifuged at 1000 rpm for 2 minutes, and then filtered through a 0.22 µm sterile syringe filter. Conditioned media was kept in the ultra-deep freezer in aliquots and thawed as and when required.

Co-culture of WJ-MSCs / conditioned media with irradiated murine splenic lymphocytes

To study the radioprotective property of WJ-MSCs in vitro, different numbers of WJ-MSCs (6.25 x103, 12.5 x103, and 25 x103) were seeded in a 24-wells cell culture plate. The next day 2Gy gamma-irradiated murine splenic lymphocytes were co-cultured with pre-seeded WJ-MSCs. Splenocytes were incubated for 24h in complete RPMI-1640 medium (RPMI-1640 medium supplemented with 10% FBS and 100 units/mL penicillin / 100 µg/mL streptomycin). To study the radioprotective property of WJ-MSCs-CM, irradiated lymphocytes suspended in RPMI-1640 were mixed with normal medium (NM) or WJ-MSCs-conditioned medium (CM) in 1:1 ratio and cultured for 24h with 10% FBS. Cells were then washed with PBS and stained with 1 mL of propidium iodide solution (0.5 µg/mL propidium iodide, 10 µg/mL ribonuclease A, 0.1% sodium citrate, and 0.1% Triton X-100) overnight. Cells were subjected to flow cytometry and the data was analyzed using FlowJo v10 software. The pre-G1 population in the histogram represented apoptotic cells.

Neutralization of WJ-MSCs conditioned media

Conditioned media of WJ-MSCs (24h cultured) was treated with 5µg/mL of human G-

CSF neutralizing antibody. This conditioned media was incubated at 37°C for 2h. The G-CSF neutralized CM (200µl) was infused to lethally irradiated mice to investigate the role of G-CSF in therapeutic radioprotection [8].

Spleen index and endogenous spleen colony formation

Endogenous spleen colony formation is considered a gold standard assay for the demonstration of radioprotection. To study the effect of WJ-MSCs-CM or G-CSF neutralized WJ-MSC-CM on radiation-induced reduction in spleen index and endogenous spleen colony formation, mice were divided into four groups: Control, 6Gy WBI, 6Gy WBI + WJ-MSCs-CM, and 6Gy WBI + WJMSCs-CM neutralized with G-CSF. Mice were exposed to 6Gy WBI [8] and injected with 200µl WJ-MSCs-CM or WJ-MSCs-CM neutralized with G-CSF 24h after radiation exposure. Mice were sacrificed on day 15 and weights of mice and spleens were recorded. Spleens were then fixed in Bouin's solution. Spleen index was calculated by taking the ratio of spleen weight to mouse weight. The spleen CFU were counted as macroscopic colonies formed on the surface of the spleen as a result of the proliferation of surviving bone marrow stem/progenitor cells.

Survival study

For studying the effect of WJ-MSCs-CM infusion on the survival of mice, 8.5 Gy of the radiation dose was selected based on our previous study [8]. For survival study, mice were randomly divided into the following experimental groups, 1) Control: PBS alone, 2) 8.5Gy WBI, 3) 8.5Gy WBI + 24h post-irradiation WJ-MSCs-CM, and 4) 8.5Gy WBI + 24h post-irradiation WJ-MSCs-CM neutralized with anti-G-CSF. The day of whole-body exposure was considered as day 0. WJMSCs-CM or WJ-MSCs-CM neutralized with anti-G-CSF (200µl) were systemically infused through the lateral tail vein. Mice were daily monitored for 30 days.

Enumeration of homeostasis driven proliferation of lymphocytes

To perform homeostasis-driven proliferation (HDP), lymphopenia was induced in mice by exposing them to 6Gy WBI and keeping them for 24h. Further, CFSE (20µM) labeled splenic lymphocytes (6x106 cells) were infused into lymphopenic mice with or without

WJ-MSCs through the lateral tail vein. Each group consisted of four mice. Spleens were collected 96h after injection to mice. Spleen index was calculated by recording spleen weight and mice weight. Samples (two hundred fifty-thousand lymphocytes) from each group were acquired on a flow cytometer to enumerate the frequency of donor cells and also monitor their cell proliferation.

Analysis of transcriptome data in stem cells derived from different sources

The raw data for different sources of stem cells were downloaded from the GEO database [GSE20124 & GSE48022 having GEO accession no. GSM1165510, GSM1165511 (WJ-MSCs); GSM503594, GSM503595 (ESCs); GSM1165505, GSM1165506 (BMMSCs); GSM503604, GSM503605 (Fibroblast) and platform Affymetrix Human Genome U133 plus 2.0 Array in .CEL format] and analyzed using transcriptome analysis console (TAC, AppliedBiosystems, Thermo Fisher Scientific), for normalization of signal intensity, quality control, and statistical analysis. The differentially expressed genes (DEGs) in WJ-MSCs, BMMSCs, and ESCs were compared with the fibroblasts using TAC. Further, t-test, Multiple Testing Corrections, and False Discovery Rate Prediction were employed to calculate the significance of DEGs. A threshold of 5-fold difference in gene expression between different stem cells compared to fibroblasts with a p-value <0.05 was used for statistical significance.

Gene enrichment analysis

The data obtained from the TAC was utilized to assess the biological significance. BiNGO plugin of Cytoscape was used for assigning specific biological function, molecular function, and functional pathways for genes that were enriched among the up-regulated genes [30]. The list of genes was statistically assessed by gProfiler using the following settings: Organism- Homo sapiens, statistical domain scope- Only annotated genes, significant threshold- Benjamini Hochberg FDR, and threshold- 0.05 [31].

Protein-protein interaction (PPI) network and construction of functional modules for selected cytokines/chemokines

For creating PPI, a list of the cytokines elevated in mouse serum at 4h post-WJ-MSCs transplantation, were obtained from our previous study [8]. A PPI network was

constructed for these cytokines using Cytoscape (Ver 3.7.1). This constructed network was further extended using the STRING database. The Molecular Complex Detection (MCODE) was used to select modules of the PPI network with the following parameters: degree cutoff = 2, node score cutoff = 0.2, core = 2, and max. Depth = 100. MCODE encompasses an automated algorithm that detects densely connected regions in PPI networks that may represent molecular complexes. Further, BiNGO plugin of Cytoscape was used to obtain the biological significance of the predicted modules.

Statistical analysis

All the graphs were made between means ± S.E using Microsoft Excel. Cellular data were analyzed for multiple comparison with Tukey-Kramer post-hoc test using KyPlot 5 statistical software (KyensLab Inc, Japan) with a statistical significance set at * $p<0.05$, ***$p<0.001$, and ns $p>0.05$. For survival study, Kaplan–Meier's estimate of life time analysis was done using GraphPad Prism 5 software (GraphPad software, Inc.). The difference between the estimated survival times of the two groups was evaluated by the log-rank test. The statistical significance of the log-rank test was considered if $p < 0.05$ according to chi-square distribution. ****$p<0.0001$.

RESULTS

Co-culture of irradiated splenic lymphocytes with WJ-MSCs protected against radiation-induced cell death

In vitro therapeutic potential of WJ-MSCs was evaluated using a co-culture study. Our study showed that when irradiated lymphocytes were co-cultured with a different number of pre-cultured WJ-MSCs for 24h, offered protection to lymphocytes as monitored by propidium iodide staining (Figure 1A). The extent of protection increased with an increasing number of WJ-MSCs. Subsequently, conditioned media from WJ-MSC (WJ-MSC-CM) was added to irradiated lymphocytes and cell death was measured using propidium iodide assay. It was found that WJMSC-CM offered therapeutic protection against radiation-induced apoptosis in lymphocytes (Figure 1B). The results

showed that WJ-MSCs offered protection to irradiated lymphocytes by secreting certain soluble mediators which may help in repair.

Infusion of WJ-MSCs conditioned medium neutralized with anti-G-CSF diminished the protective effect

Our previous findings showed that infusion of WJ-MSC-CM to lethally irradiated mice protected against mortality [8]. In the present study, infusion of conditioned media 24h post-irradiation (6Gy) led to better recovery in spleen index and endogenous spleen colony formation (Figure 2A & B). However, when WJ-MSC-CM was neutralized with anti-G-CSF and infused in irradiated mice, the protective effect of WJ-MSC-CM diminished as compared to mice that were injected with non-neutralized WJ-MSC-CM. Further, when non-neutralized WJ-MSCs-CM was injected to lethally irradiated mice (8.5Gy) offered about 43% protection against mortality as compared to irradiated mice administered with WJ-MSC-CM neutralized with G-CSF (Figure 2C). It was also observed that surviving mice in the WJ-MSC-CM group recovered from WBI-induced morbidity (Figure 2D). These findings show that cytokine/growth factor G-CSF may be one factor responsible for the observed radioprotection offered by WJ-MSC-CM.

Transplantation of WJ-MSC increased homeostasis driven proliferation of lymphocytes

Transplantation of WJ-MSCs along with CFSE labeled lymphocytes into lymphopenic mice resulted in a higher frequency of CFSE positive lymphocytes in the host as compared to mice injected with CFSE labeled lymphocytes alone (Figure 3A & 3B). Further, these CFSE labeled lymphocytes divided more times in the host when injected along with WJ-MSCs as compared to mice infused with CFSE labeled lymphocytes alone (Figure 3C & 3D). Figure 3E shows that the spleen index was higher in lymphopenic mice injected with lymphocytes along with WJMSCs as compared to mice that received only lymphocytes. These results indicated that WJMSCs or some of the soluble factors secreted by them may be helping in faster recovery from radiation-

mediated injury.

Transcriptome profile of different types of stem cells

Our previous report [8] and the present findings have clearly shown that WJ-MSCs have distinct advantages over other stem cells like BM-MSCs and embryonic stem cells in ameliorating radiation injury. Hence, we want to understand the unique genes that are expressed by WJ-MSCs as compared to other stem cells and fibroblasts. For this purpose, gene expression data related to different stem cells were downloaded from the GEO database. Gene expression analysis was carried out by setting- gene-level fold change < -5 or > 5 and gene-level p-value < 0.05 (Anova method- Bayes). From Figure 4A, WJ-MSCs, BM-MSCs, and ESCs have 508, 553, and 2596 differentially expressed basal genes as compared to dermal fibroblasts respectively. Among these, the number of down-regulated genes is more. Interestingly, when WJ-MSCs were compared with BM-MSCs, 732 genes were differentially expressed, out of which 518 genes were up-regulated, and 214 genes were down-regulated in the WJ-MSCs. In contrast, when WJ-MSCs were compared with ESCs, 2701 genes were differentially expressed, out of which 1345 genes were up-regulated and 1356 genes were down-regulated. On the other hand, when BMMSCs were compared with the ESCs, 2900 genes were differentially expressed, out of which 1093 genes were up-regulated in BM-MSCs and 1807 genes were down-regulated. Figure 4B shows the percentage of differentially expressed genes in WJ-MSCs when compared with fibroblasts, BM-MSCs, or ESCs respectively. Table S1 shows the list of the differentially expressed genes which are unique to WJ-MSCs. It is clear from Table S1 that most of the differentially expressed genes of WJ-MSCs are different interleukins, growth factors (such as G-CSF), chemokines, various adhesion molecules, stemness markers, and many more which have a significant role in the tissue regeneration and repair. Some of the highly up-regulated and downregulated genes of the WJ-MSCs are plotted as shown in Figure 5A and 5B respectively.

Gene enrichment analysis for unique genes of WJ-MSCs

To explore the biological function of these identified unique genes of WJ-MSCs, gene enrichment analysis is performed using gProfiler for their gene ontology and functions. Table S2 shows the gene ontology of unique genes from WJ-MSCs analyzed using gProfiler. A total of 32 biological processes were found to be influenced by these genes. Some of the functions of these genes are involved in the multicellular organismal process, positive regulation of macromolecule metabolic process, positive regulation of nitrogen compound metabolic process, positive regulation of the biological process, positive regulation of the metabolic process, cytokine response. Some key molecular functions identified are cytokine activity, signaling receptor activator activity, receptor-ligand activity, cytokine receptor binding, molecular function regulator, and growth factor receptor binding. These genes belong to different cellular compartments like the extracellular matrix, extracellular space, and cell surface. To further understand how these differentially expressed genes influence the function of WJ-MSCs, we used prior knowledge of biological pathways reported in the literature. Figure 6A & 6B show two of the major signaling pathways, cytokines, and inflammatory response pathway, and NF-kB survival pathway, which regulate cell survival and death.

PPI network and their cluster analysis

Our previous findings showed that conditioned media from cultured WJ-MSCs or serum collected from WJ-MSCs infused mice contained several cytokines, chemokines, and growth factors [8]. The information obtained from serum samples was used to construct an expanded PPI network (Figure 7A). When this PPI is analyzed for their tissue specificity, most of the radiosensitive tissues such as blood, bone marrow, intestine, and spleen show a more complex network as compared to the radio-resistant tissues such as skin, liver, and muscles (Figure 7B) indicating that the WJ-MSCs secreted cytokines may play a major role in the radiosensitive tissues compared to radio-resistant tissues. When the extended cytokine PPI was subjected to cluster analysis using MCODE, six distinct clusters were obtained. These clusters were analyzed for

their functions and found that they are responsible for the modulation of the immune system and related functions (Figure 8).

DISCUSSION

Stem cell-based therapy is receiving significant attention in the field of regenerative medicine for treating various disorders and injuries [32]. During unplanned radiation exposure (such as radiation accidents), significant tissue damage is observed, and if not repaired/regenerated it can develop into ARS. To avoid ARS, regenerative therapy will be a good therapeutic option. In the past, the therapeutic potential of BM-MSCs for radioprotection has been demonstrated in several experimental studies [20-24]. The first case of human cord blood transfusion (locus-mismatched, unrelated HUCB transfusion) to lethally irradiated 39-year-old male exposed with neutrons to 8-10Gy in the uranium-processing plant was documented in a radiation accident at Tokai, Japan in 1999 who lived for 210 days [33]. Azzam *et al* (2010), showed that combinatorial administration of hUCB cells with an antibiotic (Levaquin) showed recovery from hematopoietic and gastrointestinal tract syndromes in lethally irradiated mice [34].

Wharton's Jelly is another alternate source of MSCs and are safe compared to other sources due to no adverse effects or teratoma formation in the recipients [26]. WJ-MSCs lack costimulatory ligands, which otherwise can activate robust immune responses in the host [28]. In addition, preclinical studies have shown anti-tumor activity of WJ-MSCs [35-38]. Recently, we have shown that infusion of WJ-MSCs either 4 or 24h post-irradiation significantly protected against lethal irradiation [8]. We also demonstrated that infusion of WJ-MSCs conditioned medium also offered therapeutic radioprotection. The possible mechanism by which WJ-MSCs offered radioprotection could be *via* secreting cytoprotective cytokines such G-CSF, IL-6 and Nrf-2 [8]. G-CSF has received FDA approval for the treatment of myelosuppression induced by radiation during radiological incidents/accidents [39, 40]. Shim *et al* (2013) have shown that hUCB-MSCs treatment significantly elevated the number of peripheral leukocyte counts in irradiated

mice. They also reported that treatment with hUCB-MSCs was more effective than G-CSF treatment in supporting the proliferation of various cells in the bone marrow [41].

The current study explores the possible mechanism by which WJ-MSCs are offering therapeutic radioprotection using network biology. Our findings showed that the co-culture of WJ-MSCs with irradiated lymphocytes leads to protection against radiation-induced cell death in lymphocytes (Figure 1). The observed radioprotection could be due to the release of certain soluble mediators such as radioprotective cytokines, which other investigators had earlier reported [42]. A homeostasis-driven proliferation study showed that infusion of WJ-MSCs along with lymphocytes into lymphopenic mice led to increased lymphocyte division as compared to the group where lymphocytes alone were injected (Figure 3). We also observed an improved spleen index in these mice. This study indicates that WJ-MSCs alone or the soluble factors secreted by them may be helping the infused lymphocytes to survive and proliferate in a lymphopenic environment. These factors may be helping the host lymphocytes to survive radiation-induced death, which in turn can protect against opportunistic infections in irradiated mice. Our earlier results have shown that WJ-MSCs homed to the radiosensitive tissues in irradiated mice [8]. Thus, the infused WJ-MSCs after reaching the target site, favor the repair of damaged tissues and alter the microenvironment similar to other MSCs by secreting different cytokines and growth factors [42]. The experiments carried out using a conditioned medium of WJ-MSCs also show a survival benefit to lethally irradiated mice. Interestingly, when this conditioned media was neutralized with an anti-G-CSF antibody, WJ-MSC-CM failed to protect mice against radiation-induced mortality. This finding corroborates well with the hypothesis that WJ-MSCs might be secreting radioprotective cytokines in the observed radioprotection. However, administration of WJ-MSC-CM to lethally irradiated mice offered only about 40% protection indicating that other mechanisms are also playing a role in WJ-MSCs mediated radioprotection. Alternatively, the concentration of G-CSF present in the WJ-MSC-CM may not be equivalent to G-CSF secreted by infused WJ-MSCs in vivo. It is well known that tissues/organs like bone marrow, spleen, and GI tract where there is active cell division

taking place are highly sensitive to radiation-induced cell death. Our cytokines / chemokines-based network analysis show that they are more complex in the radiosensitive tissues as compared to radio-resistant tissues indicating their role in tissue radioprotection.

Inflammatory response during infection that offers a protective mechanism requires coordination among various types of immune cells. After exposure to lethal doses of radiation, proinflammatory mediators recruit inflammatory cells to the damaged sites and inflamed tissues. These events are regulated by a proinflammatory transcription factor NF-κB which translocates to the nucleus to initiate the transcriptional activation of pro-survival factors and inflammatory cytokines. Inflammatory cytokines, namely interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which are produced upon whole-body radiation exposure, evoke systemic responses leading to tissue injury/repair [43]. IL-6 is a pleiotropic cytokine that enhances hematopoietic cell recovery following WBI when combined with G-CSF [39]. The acute inflammation following exposure to IR must be terminated soon after resolving the injury by elevating anti-inflammatory mechanisms at the damaged area and prevent the onset of chronic inflammation and subsequent damage to tissues. The pathway analysis carried out by us using the RNA-seq data taken from the GEO database reveals that many of the differentially expressed genes are IL-1α, IL1-β, IL-6, CXCL3, CXCL5, CXCL8, CXCL2, CCL2, FLT-1 and IL-33 in WJ-MSCs as compared to fibroblasts (Figure 5). When these sets of genes were further analyzed to get more insights into biological functions, cytokines and inflammatory response pathways and photodynamic induced NF-kB survival pathway seemed to be highly modulated (Figure 6 A & B), which was not seen in BM-MSCs and ESCs (Fig S1 & S2). There are several reports on the beneficial role of cytokines in radioprotection. Infusion of recombinant interleukin-1 (IL-1) to mice protects against the lethal effects of ionizing radiation [44-46]. Interestingly, intravenous infusion of IL-1 and stem cell factor (SCF) to mice before exposure to a lethal dose of whole-body irradiation showed synergistic radioprotection [45, 47, 48]. CXCL5 has also been reported to play a role in the acute phase of radiation-induced salivary gland

damage [49]. Bergonie and Tribondeau have shown that IL-1 and TNF-α can induce IL-2 receptor, IL-6, colony-stimulating factor, and acute-phase proteins which may be responsible for the observed radioprotective effects [50].

CONCLUSION

Our study shows that the co-culture of irradiated lymphocytes with WJ-MSCs protects the lymphocytes against radiation-induced cell death. Infusion of WJ-MSCs along with lymphocytes increases homeostasis-driven proliferation. Neutralization of WJ-MSCs-CM with anti-G-CSF antibody reduced the radioprotective ability. Network biology and transcriptome profile analysis of WJ-MSCs indicate the radioprotective role of WJ-MSCs secreted cytokines. Thus, WJ-MSCs-CM may be used as a therapeutic option during the recovery of radiation-exposed victims.

ARTICLE HIGHLIGHTS

Research background

Exposure to high doses of ionizing radiation is known to cause acute radiation syndromes (ARS), such as damage to hematopoietic, gastrointestinal, and neurovascular systems depending on the dose. To avoid ARS, regenerative therapy will be a good therapeutic option. So, stem cell therapy may be one of the promising candidates to ameliorate ARS because of its regenerative and damage sensing potential.

Research motivation

Stem cells isolated from Wharton's Jelly of the umbilical cord are a unique source of MSCs, which has been reported to be safe when administered to recipients without inducing any adverse effects or teratoma formation. Recently, we reported that hWJ-MSCs and their conditioned medium have significant therapeutic radioprotective potential in lethally irradiated mice. These findings motivated us to identify a unique feature of hWJ-MSCs over other sources of stem cells for the understanding of its

radioprotective mechanism and deciphering the role of the G-CSF present in hWJ-MSCs-CM.

Research objectives

The main objective was to understanding the radioprotective mechanism of soluble factors secreted by hWJ-MSCs and identification of their unique genes.

Research methods

Propidium iodide staining, endogenous spleen colony-forming assay, and survival study were carried out for radioprotection studies. Homeostasis driven proliferation assay was performed for *in vivo* lymphocytes proliferation. Neutralization of G-CSF with anti-G-CSF was done to investigate the role of G-CSF in therapeutic radioprotection. Analysis of RNAseq data was performed to find the unique genes of WJ-MSCs by comparing them with bone marrow mesenchymal stem cells, embryonic stem cells, and human fibroblasts. Gene enrichment analysis and protein-protein interaction network were used for pathway analysis.

Research results

Co-culture of irradiated murine splenic lymphocytes with WJ-MSCs offered significant radioprotection to lymphocytes. WJ-MSCs transplantation increases the homeostasis-driven proliferation of the lymphocytes. Neutralization of WJ-MSCs-conditioned medium (WJ-MSCsCM) with G-CSF antibody abolished therapeutic radioprotection. Transcriptome analysis showed that WJ-MSCs share several common genes with BM-MSCs and ESCs and also express a high level of unique genes such as IL1-α, IL1-β, IL-6, CXCL3, CXCL5, CXCL8, CXCL2, CCL2, FLT-1, and IL-33. It was also observed that WJ-MSCs preferentially modulated several cellular pathways and processes which are responsible for the repair and regeneration of damaged tissues compared to other sources of stem cells. Cytokines-based network analysis showed that most of the

radiosensitive tissues have a more complex network for the elevated cytokines.

Research conclusions

This study showed the role of cytokine G-CSF present in WJ-MSCs-CM in eliciting therapeutic radioprotection. Systemic infusion of WJ-MSCs-CM may have significant potential for treating accidentally radiation exposed victims.

Research perspectives

WJ-MSCs-CM holds significant therapeutic radioprotective ability and has translational potential for its use during radiation accidents.

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