p73α regulates the sensitivity of bone marrow mesenchymal stem cells to DNA damage agents

Wei Liang, Chunhua Lu, Jing Li, James Q. Yin, Robert Chunhua Zhao

**Abstract**

Human bone marrow mesenchymal stem cells (MSCs) are important cell population located in bone marrow that are thought to have multiple functions in cell transplantation and gene therapy. Although in vitro experiments have demonstrated that hMSCs are resistant to apoptosis induction by DNA damage agents such as chemotherapeutic substances used in bone marrow transplantation, the molecular mechanism underlying remains unclear. p73 is highly similar to p53 and plays crucial roles in regulating DNA damage-induced apoptosis pathways. In this study, we investigated the role of p73α in response to chemotherapeutic substances in cultured human bone marrow MSCs. Cellular chemosensitivity and DNA damage-induced apoptotic cell death were examined in the hMSCs with exogenously over-expressed p73α. Our results showed that the expression of retrovirus-driven human p73α could be successfully induced in hMSCs, the over-expression of ectopic p73α resulted in a significant increase of cellular sensitivity to cisplatin. The increase of cellular apoptosis was attributed to enhanced chemosensitivity in p73α infected cells. Moreover, immunoblot analysis indicated that the co-activation of pro-apoptotic factors Bax and p21 were observed in the p73α infected cells after cisplatin treatment. In conclusion, our findings suggested that p73α is an important determinant of cellular chemosensitivity in human bone marrow MSCs.

1. Introduction

Mesenchymal stem cells (MSCs) are multipotential cells that can be isolated from various of human tissues such as bone marrow, adipose tissue, muscle, brain and dermis of mammalian (Aust et al., 2004; Jiang et al., 2002a,b; Kawada et al., 2004; Sato et al., 2005; Zhao et al., 2005). They are able to self-renew in vitro and differentiate into osteocytes, chondrocytes, adipocytes, myocytes and even neural cells under appropriate conditions (Boland et al., 2004; Ferrari et al., 1998; Johnstone et al., 1998; Tao and Ma, 2003; Tao et al., 2005). Recently, more and more attentions have been paid to MSCs for their potential roles in treatment of various diseases including neural degenerative disorder, osteogenesis imperfecta and acute myocardial infarction (Harvey and Chopp, 2003; Horwitz et al., 2002; Stamm et al., 2004). Moreover, their unique immunologic characteristics such as low immunogenicity make MSCs excellent candidate for clinical application in dealing with autoimmunologic diseases and GVHD after the transplanta-

tion of haemopoietic stem cells (Aggarwal and Pittenger, 2005; Jorgensen et al., 2003; Le Blanc et al., 2004).

After conventional haemopoietic stem cell transplantation, the majority of hMSCs in bone marrow seems to be of recipient origin (Devine and Hoffman, 2000; Li et al., 2004), suggesting that hMSCs are not sensitive to the chemotherapy-induced damage. This assumption is further supported by the observation that in vitro cultured hMSCs are resistant to a panel of chemotherapeutic drugs, especially at clinical dose commonly used in BMT patients receiving intensive chemotherapy (Li et al., 2004). Recently, it is reported by another paper that human bone marrow MSCs contain an elevated apoptosis-induced threshold in response to DNA damage agents such as cisplatin, and the chemotherapeutic treatments result in an increased expression of p53 protein, however the increased p53 protein did not induce cell apoptosis (Mueller et al., 2006). According to previous studies, many DNA damage agents could induce the activation of endogenous p53 protein and next activate specific cell signal pathway and finally lead to cell cycle arrest and apoptosis in order to sustain the homeostasis (Mueller et al., 2006), thus the controversial phenomenon observed in hMSCs make us propose that there may be other factors involved in this process. p73, a member of p53 family, is similar to p53 in configuration and functions (Barbieri and Pietenpol, 2005). It can also
induce apoptosis response dependent or independent of p53 and probably is a determinant of chemotherapeutic response (Jost et al., 1997; Kim et al., 2006). Based on this hypothesis, in this study we attempted to link human p73 protein to the sensitivity of hMSCs to DNA damage agents. We found that exogenously expressed p73 can enhance the sensitivity of hMSCs to cisplatin, and the increased apoptosis contributed to the change of sensitivity.

2. Materials and methods

2.1. Construction of retrovirus plasmids and virus production

The wt-p73x CDNA (encoding amino acids 1–636) with an influenza hemagglutinin (HA) peptide at its N-terminal was PCR amplified with Pfu DNA polymerase (TaKaRa) from pDNA3-T7-p73x (kindly provided by Dr. Kangwei Wu) using primers introducing a 5′ XhoI site and a 3′ BglII site, and ligated into the multiple cloning site of pMSCVneo (clontech) cut with these two enzymes. pMSCV contains the PMCV virus LTR which can high-efficiently drive the expression of inserted gene. The result expressing plasmids were used to transfect packaging cell line 293T with two other helper packaging plasmids pMD-MLV-OGP(gag-pol) and pVSVG(G εν)-env according to appropriate ratio. Tissue culture supernatants from 293T packaging cells transfected with those plasmids above were harvested and viral particles were concentrated by centrifuged at 50,000 × g for 3 h. Viral particles were finally collected and resuspended in culturing medium and frozen in aliquots in −80 °C for future transduction.

2.2. Human bone marrow MSCs isolation, culture and transfection, K562 cells culture

Human bone marrow were collected after obtaining informed consent from healthy volunteer donors. All the procedures were approved by the Ethics Committee at Chinese Academy of Medical Sciences and Peking Union Medical College. The methods were according to two previously published papers (Liu et al., 2006; Shi et al., 2007), Briefly, BM aspirates were collected from the posterior iliac crest containing 0.5% bovine serum albumin (BSA; Sigma), and incubated with primary antibodies against human Flk1, CD29, CD31, CD34, CD45, CD105, CD166, CD184, and α-actin (Chemicon), Bax (Santa Cruz Biotechnologies), p21 (Santa Cruz Biotechnologies), followed by an incubation with appropriate secondary antibody conjugated with HRP. Then the sections were electrophoresed by 2% agarose gel containing ethidium bromide and visualized by UV-induced fluorescence.

2.3. Cell growth curve

hMSCs were treated with or without 3 μM cisplatin or 0.005 μM camptothecin for 2 h, 24 h, and the treatment were repeated every 4 days. Then cells were collected by trypsin digestion and seeded in 24-well plates at a density of 3 × 10^5 cells/well and the cell number were counted every 24 h for up to 10 days.

2.4. Immunophenotype analysis

Cells were detached and washed with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA; Sigma), and incubated with primary antibodies for 30 min at 4 °C. To detect intracellular antigens, cells were fixed, and only adherent cells were analyzed. Total number of 5 × 10^5 cells were used in the reaction. Results were analyzed by CellQuest Pro software (BD Biosciences).

2.5. Differentiation induction in hMSCs

hMSCs were treated with or without 3 μM cisplatin or 0.005 μM camptothecin for 2 h, 24 h, and the treatment were repeated every 4 days.

Osteogenic and adipogenic differentiation of hMSCs were induced according to a published report (Hu et al., 2003). For osteogenic differentiation, cells were seeded at a density of 2 × 10^4 cm^-2 and then cultured in the following osteogenic differentiation medium for 2–3 weeks: Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FCS, 10 mmol/l β-glycerophosphate, 10^-4 mol/l dexamethasone, and 0.2 mmol/l ascorbic acid (all from Sigma). Then the cells were stained with von Kossa to show osteogenic differentiation. For adipogenic differentiation, cells at 2 × 10^5 cm^-2 were induced for 3 weeks in DMEM supplemented with 10% FCS, 0.5 mmol/l hydrocortisone, 0.5 mmol/l isobutyryl/acetanilide, and 50 μg/ml indomethacin (all from Sigma). At the end of the induction period, the cells were fixed in 10% formaldehyde for 10 min and stained with fresh Oil red-O solution (Sigma) to show lipid droplets.

2.6. RT-PCR

Total cellular RNA was extracted from induced or uninduced cells using Trizol (Invitrogen) according to the manufacturer’s protocol. cDNA was made from 1 μg total RNA using oligo dT and a SuperScript II reverse transcriptase (Qiagen) at 42 °C for 1 h. Then PCR amplification was performed for human osteopontin (OPN, 330bp), lipoprotein lipase (LPL, 238) and β-actin (204 bp) with TaqDNApolymerase (TaKara). The following primer pairs were used: OPN: 5′-CATT GAC TTC CCT GCA CCC ATC-3′; LPL: 5′-ATG AGG ACC AGA CCC CCT GTC CTC-3′/5′-TAC AGG GCG GCC ACA AAT TTT-3′; β-actin: 5′-GAG ACC TTC AAC ACC CCA GCA-3′/5′-AAT GTC ACC CAT TCC C-3′. PCR amplification was run by 35 cycles consisting of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s with 5 min of initial denaturation at 95 °C and 5 min of final synthesis, 72 °C. Amplification products were electrophoresed by 2% agarose gel containing ethidium bromide and visualized by UV-induced fluorescence.

2.7. SDS-PAGE and Western blot

MSCs were rinsed with PBS and lysed in RIPA buffer (25 mM Tris–HCl (pH 8.0), 150 mM NaCl, 2.7 mM KCl, 1% Triton X-100, 1 mM PMSF) supplemented with protease inhibitor mixture (all from sigma), suspended K562 cells were collected by centrifugation at 1500 × g for 5 min and rinsed with PBS for two times and then lysed in RIPA buffer, insoluble components were removed by centrifugation at 12,000 × g. Protein concentration were determined by the Brodfard method. After boiling in SDS loading buffer (62.5 mM Tris–HCl (pH 6.8), 2% SDS, 0.1% mercaptoethanol, 0.01% bromophenol blue) for 5 min, 40 μg protein per lane was separated by SDS-PAGE and then transferred to Immobilon-P membranes (Millipore) with actin using as loading control. Non-specific antigen–antibody binding on the membranes was blocked with 5% nonfat dry milk in TBST/0.1% Tween 20 for 1 h and probed with the following primary antibodies for 1 h in TBST/0.1% Tween 5% nonfat milk at the respective concentrations: anti-HA tag (12C5A, Roche), p73 (Chemicon), p53 (BD), actin (Chemicon), Bax (Santa Cruz Biotechnologies), p21 (Santa Cruz Biotechnologies), followed by an incubation with appropriate secondary antibody conjugated with HRP and visualized by enhanced chemiluminescence (Millipore) according to the manufacturer's instructions.

2.8. Cell survival assay

Cell proliferation was measured using the method of XTT according to the manufacturer’s instruction. Briefly MSCs or K562 cells were seeded into flat-bottom 96-well plates at a density of 3 × 10^3 or 105 cells per well in 100 μl of culture medium, respectively. After 24 h, cells were treated with or without cisplatin or camptothecin at a final concentration range of 10^-2–10^-4 M or 0.0005–5 μM, respectively. Then cells were cultured to continue for another 2–3 days and cell survival was measured by (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-5-((phenylamino)carbonyl) 2H-tetrazolium hydroxide; XTT) (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer’s instruction. The colorimetric readout in this assay reflects the number of metabolically active mitochondria, and hence viable cells in a given well. The resulting spectrophotometrical absorbance was measured at 490 nm. Values were normalized relative to untreated controls. Data shown were representative of three independent experiments.

2.9. Apoptosis analysis

Relative caspase 3 activity was analyzed by the method of substrate cleavage assay. WT-p73x or vector transfected human marrow mesenchymal stem cells were treated with or without 10 μM cisplatin for 48 h. Detached death cells were discarded, and only adherent cells were analyzed. Total number of 5 × 10^5 cells were harvested and the caspase 3 activity was analyzed using Caspase-3 Colorimetric Activity Assay Kit (Chemicon International, Inc.) according to the manufacturer’s instructions. 100 μg of protein in each cell extract was used in the reaction. Results
were read in a microplate reader at 405 nm. Fold increase in caspase-3 activity was determined by comparing the OD reading from the treated cells with the level of the untreated control. For TUNEL analysis, adherent cells were rinsed three times with PBS, and fixed in 4% paraformaldehyde and then permeabilized with 0.1% Triton-X-100. The next step proceeded as described in the manufacturer’s instruction (In Situ Cell Death Detection Kit, Fluorescein, Roche Molecular Biochemicals). TUNEL-positive cells were detected by flow cytometry. Data shown were representative of two independent experiments. For Hoechst staining, cells were fixed in 4% paraformaldehyde for 10 min and then Hoechst 33342 was added at a final concentration of 10 μg/ml. Finally the results were showed under the fluorescent microscope.

3. Results

3.1. Morphology, phenotypes and differentiation potential of cultured hMSCs cells

The cultured hMSCs displayed a characteristic of spindle-shape or fibroblast-like morphology and replicated exponentially for up to 10 population doublings (Fig. 1B). The morphology was maintained through repeated passages under non-stimulating conditions. Flow cytometry detection of a set of cell surface antigen showed that hMSCs were positive for CD29, CD44, CD105, CD166, Flk1, but failed to express the CD31, CD34, CD45, and HLA-DR, and CD184 (Fig. 1A). When subjected to respective differentiation-inducing medium, hMSCs showed a typical morphological change of osteogenic and adipogenic differentiation (Fig. 1D and E). Extracellular calcium deposition was showed by von Kossa stain and intracellular lipid droplets stained by Oil red. Analysis of gene expression confirmed the differentiation fact and revealed the expression of specific osteogenic and adipogenic gene in hMSCs (Fig. 1C).

3.2. Chemosensitivity of hMSCs versus K562 cells

The in vitro chemosensitivity of MSCs and K562 was determined by XTT assay. Consistent with previous reports (Mueller et al., 2006), hMSCs were more resistant to cisplatin than K562 cells when treated with a gradually elevated dose of cisplatin (Fig. 2A). The same result was also seen in camptothecin treated group (Fig. 2B).

3.3. MSCs retain their stem cell characteristics after treatment of chemotherapeutic agents

In order to prove that whether or not hMSC are resistant to the genotoxic effects of chemotherapeutic substances, we investigated the characteristics of hMSC after treatment with cisplatin and camptothecin in vitro. We used 3 μM cisplatin or 0.005 μM camptothecin for 2 h every day and repeated four times. This treatment represented a dose corresponding to clinically relevant serum concentrations. Following a slow growth period of 10 days after the end of treatment of cisplatin and camptothecin, treated hMSCs resumed growth nearly to untreated cells (Fig. 3A). Flow cytometry analysis also showed an identical phenotype with CD31−, CD34−, CD105−, CD166− and HLA-DR−, CD11a−, CD45−, glycophorin-A−, Flk-1−, CD29− CD44+ (Fig. 3B) before and after treatment. Upon induction of differentiation, cisplatin and camptothecin treated hMSCs showed signs of osteogenic and adipogenic differentiation (Fig. 3C). These data indicated that hMSC can keep their stem cell characteristics after genotoxic damage.

3.4. Endogenous expression level of p73α protein in MSCs in response to cisplatin

As our data demonstrated that hMSCs were more resistant to cisplatin than K562 cells. To examine whether the different response of these cells to cisplatin could correlate with different expression levels of p73 protein, MSCs and K562 cells were treated with different concentration of cisplatin for 24 h and the p73 protein expression was investigated by western blot. We found that in K562 cells p73α was easily detected under the steady-state, its expression level was gradually induced by treatment with increasing concentration of cisplatin in a dose-dependent manner. However, the endogenous expression of p73α in MSCs was undetectable, whereas the treatment of cisplatin with an apoptosis inducing dose could not result in a remarkable accumulation of p73 in MSCs (Fig. 4). These results showed that there exist a clear correlation between the degree of resistance to cisplatin and the expression state of p73 protein in MSCs.

3.5. Retrovirus-mediated over-expression of p73α in hMSC

In order to introduce ectopic p73α into in vitro cultured hMSC, a retrovirus vector pMSCNeo was used as described in the methods. The stable and high level of p73α expression in stem cells was achieved through a specifically designed 5′-long terminal repeat (LTR) from the murine stem cell PCMV virus. After selection by G418, cells stably expressing p73α were harvested and p73α protein expression level was measured. Equal amounts of protein extracts were analyzed by western blot analysis using the anti-HA monoclonal antibody 12CA5. Cells not infected or infected with backbone vector were used as negative control (Fig. 5). As shown in the figure, transduced hMSCs readily expressed the ectopic HA-tagged p73 protein while the negative control did not show any protein expression (Fig. 5).

3.6. Over-expression of p73α increased the sensitivity of hMSCs to cisplatin

In order to investigate whether p73α over-expression predispose MSC to apoptosis induced by DNA-damaging agent cisplatin, we performed cell survival assay experiment. Here we used 10 μM cisplatin because this dosage did not induce hMSCs apoptosis but can cause most cisplatin-sensitive tumor cells death in our previous experiment (far beyond the IC90 of most cisplatin-sensitive tumor cells). We found that the cell number of MSCs transfected with p73α alone or treated with 10 μM cisplatin was slightly decreased, compared to the control. However, when cells were both infected with p73α and treated by 10 μM cisplatin, there was a markedly decrease in the cell viability shown in Fig. 6. The viability was nearly up to fourfold lower than that of cells infected with the vector. It is worth to be noted that among p73 family members, p73β alone can induce a strong apoptosis response in several cell types. However, in our experiment systems, we could not observe the obvious cell death when hMSCs were stably infected with wt-type p73α. It is probably that the protein level of ectopic p73α was not enough to induces cells death. Similar results are seen in 72 h (Fig. 6).

3.7. Apoptosis assay

To determine whether apoptosis contributes to the reduced viability of hMSC, we performed TUNEL assay. As we expected, in situ detection and quantitation of apoptosis in both p73α transfected and cisplatin treated hMSCs revealed a marked increase in apoptotic positive cells (Fig. 7A). This demonstrated that the decreased viability was due to a significantly increased cell apoptosis. On the other hand, no significant cell death was detected in hMSCs infected with p73α or induced by cisplatin individually compared with the control cells.

With few known exceptions, the terminal apoptotic programme of mammalian cells depends on the activation of intracellular caspases and their modification of protein substrates within the nucleus and cytoplasm. To examine this further, we then determined caspase 3 activity. We found that when cells were both
Fig. 1. In vitro phenotypic characteristics of hMSCs. (A) Phenotype of cultured hMSCs; (B) undifferentiated hMSCs cultured in non-inducing medium show a fibroblast-like morphology; (C) results of RT-PCR show expression of osteopontin (330 bp, Lane 2) and lipoprotein lipase (298 bp, Lane 4), uninduced cells serve as control (Lanes 1 and 3); (D and E) in vitro differentiation of hMSCs to osteocytes and adipocytes von Kossa staining of cells before (left) and after (right) osteogenic induction (D) and Oil red O staining of hMSCs (E).

Fig. 2. hMSCs are resistant to cisplatin as compared with K562 cells. hMSCs and K562 cells were treated with different concentration of cisplatin (A) or camptothecin (B) for 72 h, then the percentage of surviving cells relative to untreated controls was determined by XTT analysis. Data are presented as mean values ± the standard deviation (SD) of three independent experiments.
Fig. 3. hMSCs retain their stem cell characteristics after DNA damage substances treatment in vitro. hMSCs were treated with 3 μM cisplatin or 0.005 μM camptothecin for 2 h in vitro, and treatment was repeated four times every 24 h. (A) Flow cytometry showed the phenotype for selected markers in treated and untreated hMSCs. (B) Treated or untreated hMSCs were incubated in differentiation-inducing media for adipogenic or osteogenic differentiation or cultivated in growth medium, resulting in typical signs of differentiation, that is, lipid droplets stained by Oil red or calcium deposits stained by von Kossa, respectively.

expressed exogenous p73α and treated with 10 μM cisplatin, the activity of caspase 3 in MSCs was increased to a substantially higher level than in cells treated with cisplatin or expressed p73α alone (Fig. 7B). Hoechst staining also confirmed that apoptosis contributed to the reduction of hMSCs. Untreated hMSCs showed normal nuclear morphology while in contrast (Fig. 7C), after treated with 10 μM cisplatin, p73α over-expressed hMSCs exhibited numerous apoptotic nuclei, that is condensed and fragmented nuclei (Fig. 7D). Taken together, these findings show that over-expression of p73α sensitize hMSCs to the induction of DNA damage agent and the decreased viability was mediated at least in part by apoptosis.

Fig. 4. Endogenous expression level of p73α protein in MSCs in response to cisplatin. hMSCs and K562 cells were treated with different concentration of cisplatin for 24 h and whole cell lysates were subjected to Western blot analysis. Actin was used as a loading control.

Fig. 5. Expression of the ectopic HA-tagged p73α protein in human marrow mesenchymal stem cells. Cells were infected with or without retrovirus vector containing human wt-p73, after selection by G418, cells stably express p73α were obtained and Western blot analysis was performed using anti-HA antibody 12CA5 with actin as a loading control.
Fig. 6. Over-expression of p73α increase the sensitivity of MSC to cisplatin. Early passage of p73α or vector transformed human marrow MSCs were treated with or without 10 μM cisplatin. XTT assays were performed 48 and 72 h later in triplicate. Error bars equal one standard error.

3.8. p73α and cisplatin coordinate to induce the expression of Bax in hMSCs

As previous studies reported, p73 can transactivate some p53 down-stream genes to induce apoptosis (Flores et al., 2002). Since our work has demonstrated that p73 can cooperate with cisplatin to induce hMSCs apoptosis, we next examined which important genes might be involved in this progress. We used several selected antibodies to analyze the expression level of some endogenous p53-responsive elements following exogenous p73α expression, exposure to cisplatin or both. Western blot analysis showed that endogenous p53 was maintained at low levels in MSC under physical conditions, and could be slightly induced when treated with 10 μM cisplatin. The over-expression of p73α in MSC did not change the level of p53 protein. Moreover, the expression of p53 protein induced by cisplatin was not increased by over-expression of p73α, suggesting that the increased apoptosis is not mediated by p53 over-production (Fig. 8).

The cyclin-dependent kinase inhibitor p21 is a down-stream target of p53 and an important regulator of cell cycle progression and apoptosis. Similar to p53, p21 protein was kept low in MSCs physically and was enhanced by 10 μM cisplatin or exogenous p73α. In addition, when MSCs were infected with p73α and treated by cisplatin together, p21 was further induced (Fig. 8). Next, we examined the expression of Bax, a pro-apoptotic p53 target gene. We found that Bax was not or slightly activated by 10 μM cisplatin or p73α, respectively. However, a higher level of Bax was detected following the treatment of both cisplatin and the expression of exogenous p73α (Fig. 8).

4. Discussion

In bone marrow microenvironment, there are two types of stem cells; one is haemopoietic stem cells and the other is mesenchymal stem cells. Recently, more and more evidences have demonstrated that MSCs may play crucial roles in supporting haemopoiesis and enhancing the engraftment after bone marrow transplantation (Devine and Hoffman, 2000; Koc et al., 2000). Thus the response
Over-expression exogenous p73 was not induced by chemotherapeutic agents in chemoresistant hMSCs. In the present study, p73 expression was not detected and could not be activated by chemotherapeutic agents in chemoresistant hMSCs. Over-expression exogenous p73 could increase the sensitivity of hMSC to apoptosis induced by cisplatin. The apoptosis assay by TUNEL and caspase-3 enzyme activity confirmed that the increased apoptosis might account for the reduction in cell numbers. It thus suggested that p73 might play an important role in regulating the cellular response to chemotherapy in hMSCs.

In this study, the induction of endogenous p73 expression was not found in the chemotherapeutic agent treated hMSCs. This was correlated with a previous study which showed that radiotherapeutic treatments had no effect on altering the endogenous p73 protein levels in cancer cells (Koivusalo et al., 2002). This was probably because that p73 was induced by different signals and might play different roles in maintaining cell homeostasis or that the response of p73 to DNA damage was highly dependent on the cellular context. Furthermore, other factors such as epigenetic mechanisms might also influence the activation of p73. Down-regulation of p73 expression was found to be significantly correlated with epigenetic promoter methylation or allelic loss (Melino et al., 2002). The methylation-dependent silencing of p73a in clinical cervical cancer samples was previously shown to have significant association with the adverse outcome of the patients following radiotherapy (Liu et al., 2004).

p73, a homologue to the tumor suppressor gene p53, shares a similar configuration with p53 especially in the central sequence-specific DNA binding domain, the amino terminal activation domain, and the carboxyl terminal tetramerization domain (Kaghad et al., 1997). However, in contrast to p53, the p73 gene is expressed as two types of isoforms with opposite effects based on alternate promoter usage and post-transcriptional splicing: full-length transactivating (TA)p73 shows pro-apoptotic effects, while the shorter ∆Np73, which lacks the N-terminal transactivating domain, has an evident anti-apoptotic function (Grob et al., 2001; Yang and McKeon, 2000). Furthermore, several papers demonstrated that p73 may play crucial roles in neuronal development and differentiation suggesting its diversity in function (Moll and Slade, 2004). Similar to p53, the expression of p73 is difficult to detect in mammalian cells and can be activated by DNA damage agents such as chemotherapeutic substances and ionizing radiation or by T-cell receptor activation (Gong et al., 1999; Lissy et al., 2000). To date, the specific role of p73 remains unclear. The inhibition of endogenous p73 by specific siRNA in p53 deficient tumor cells decreased the sensitivity of cells in response to chemotherapeutic agents (Irwin et al., 2003). Over-expression of exogenous p73 in adult somatic cells such as neurons could activate the transcription of p53-responsive genes and inhibit cell growth by inducing cell apoptosis (Liang et al., 2006). The activation of p73 in cancer cell lines can induce p53 downstream genes such as p21, Bax, MDM2, GADD45 and Cyclin G, production of these proteins then activates a signaling cascade that results in cell death via DNA cleavage and nuclear fragmentation. We showed that the expression of p53 was maintained at low levels in MSCs, the treatment with sub-apoptotic dose of cisplatin resulted in an elevated expression of p53 protein, and the endogenous increasing of p53 protein did induce the CDK inhibitors p21 expression. However, the level of pro-apoptotic gene Bax was not altered. This apparent discrepancy was probably due to the fact that cisplatin-induced p53 in hMSC could transactivate cell cycle arrest mediator p21 up-regulation, but the increased quantity of p53 could not provoke the pro-apoptosis factors such as Bax alteration. We hypothesize that this must be a protection mechanism specifically in adult stem cells because that the cell cycle arrest by up-regulation of p53 might give cells sufficient time to proceed damage repair and sustain their stem cell characteristics. However in embryonic stem cells p53 directly participates in the induction of cell apoptosis under the influence of UV to avoid the expansion of potentially abnormal ES cells (Xu et al., 2002). These opposite observations may reflect that the degree of influence of p53 on apoptosis induction is cell type specific. Over-expression of p73a did not further increase the level of endogenous p53 in response to cisplatin, coincident with the results of a previous study (Zhu et al., 2001). However we observed that p73 cooperates with cisplatin to induce a strong elevation of Bax gene expression. This was probably that cisplatin can stabilize the exogenous p73a protein and strengthen the transcription efficiencies of target genes such as Bax. Such an increase could lead to apoptosis by translocating Bax from nucleus to mitochondria, releasing cytochrome C and activating caspase 3 and finally result in apoptosis (Wolter et al., 1997). Taken together, the data presented in our study suggest that the expression of p73a may play important roles in regulating the sensitivity of hMSCs to chemotherapeutic agent-induced apoptosis. The underlying mechanism on how p73 is modulated in hMSCs needs further investigation.

Conflict of interest

The authors declare no conflict of interest.

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