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Original Article

Effects of aflatoxin B1 on DNA damage and P53gene amplification in hepatocyte-like cells differentiated from mesenchymal stem cells and CD34$^+$ cells obtained from human umbilical cord blood

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Abstract

Background: Differentiation of mesenchymal stem cells (MSCs) to hepatocyte-like cells could be associated with development of liver function factors. The impact of differentiation-dependent changes on DNA integrity is not well understood. In this study, hepatocytes and their progenitor stem cells were treated with aflatoxin B1 (AFB1) and amplification of selected genes linked to DNA damage was examined.

Methods: MSCs and CD34$^+$ cells isolated from umbilical cord blood (UCB) were treated with AFB1 (0, 2.5, 10 and 20 μM) in selective media supporting the hepatocyte differentiation. After 24 htreatment the DNA damage (Comet assay) and amplification rates of P53 and β-globin genes were measured using real time polymerase chain reaction (QPCR).

Results: The results show that AFB1 treatments resulted in a concentration- dependent increase in the DNA damage and suppression of the specific gene amplification. The extent of DNA damage was significantly greater in hepatocytes differentiated from MSCs when compared to those obtained from CD34$^+$ cells. The effects of AFB1 on the rate of selected gene amplification in QPCR showed that the lesions (expressed as lesions/10 kb) in P53 and β-globin genes was significantly greater in hepatocytes derived from MSCs as compared to the cells derived from CD34$^+$ cells.

Conclusions: These data together with the results of cytochrome P450 (CYP3A4) expression in the cells suggest that the non-differentiated stem cells are probably less vulnerable to genotoxic agents as compared to hepatocytes differentiated from them.

Keywords: Aflatoxin B1; Hepatocytes; Stem cells; Real time PCR; DNA damage, CYP3A4

Introduction

Mesenchymal stem cells (MSCs) are multipotent stem cells present in adult bone marrow and other tissues which can replicate as undifferentiated cells and that have the potential to differentiate into lineages of mesenchymal tissues. Adult stem cells have been localized in several tissues including mesenchymal, neural, gastrointestinal, hepatic, gonadal and hematopoietic (Pittenger et al., 1999; Gage, 2000; Potten, 1998; Alison and Sarraf, 1998; Margolis and Spradling, 1995; Conrad et al., 2008; Weissman, 2000).

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The effect of chromosome instability on the maintenance and differentiation of stem cells during in vitro and in vivo culture has been reviewed (Teven et al., 2011). Stem cells repopulate tissues after injury while also renewing themselves, but this makes them vulnerable to genotoxic damage. Mohrin et al. (2010) and Milyavsky et al. (2010) showed that mouse and human hematopoietic stem cells make opposing decisions about whether to die or to persist in response to DNA damage (Lane and Scadden, 2010).

Sources of DNA damage include endogenous events, such as those derived from metabolic processes and DNA replication (i.e. free oxygen radicals), and external stimuli, such as environmental agents, mutagenic chemicals and chemotherapic agents. Many of these lesions create structural alterations in the DNA and can modify or eliminate the ability to correctly execute gene transcription. Other lesions induce potentially harmful mutations in the genome, which affect the survival of daughter cells after mitosis (Sancar et al., 2004).

All cell types within the same organism are equipped with DNA repair systems and signaling network for adaptive responses and the relative ability and even necessity to repair DNA lesions might vary depending on the specific cell type, developmental stage, which can change along the life time. As a consequence, distinct cell types, at different developmental stages, might adopt different responses when challenged by genotoxic agents.

It has been reported that a prolonged culture of embryonic stem (ES) cells is associated with chromosomal instability (Buzzard et al., 2004; Yang et al., 2008). These changes were mainly observed in chromosomes 12 and 17, which may predispose the whole genome to chromosome instability and increase the potential of tumor progression (Clark et al., 2004; Draper et al., 2004). More recently Moon and co-workers demonstrated that the preservation of normal chromosomes in human ESc is indispensible for safe transplantation of their derivatives for therapeutic use (Moon et al., 2011).

The effect of epigenetic modifications on MSC multipotency and differentiation potential has also been reported. According to these studies epigenetic modifications associated with the potency of stem cells may be important factors in reprogramming the cells and alter their potency to less differentiated state (Teven et al., 2011). Stem cells obtained from different sources are a target for spontaneous and induced mutations which may be rooted in the host body or caused during cell separation and differentiation. Regardless the sources of mutations, the changes in DNA bases may persist and pose genetic instability in cells to be used for cell therapy. DNA damage is probably an important mechanism behind the loss of adult stem cells over time. The differences in the rate of DNA damage (Comet assay) in adult stem cells from different sources, raised questions about the type and the fate of the DNA damages which occur in cells during differentiation. DNA damage-induced mutations in actively transcribed genes in stem cells can lead to increase in expression of growth-controlling genes which are particularly vulnerable to transcription-associated mutagenesis (Hendriks et al., 2008).

Recently, we showed that the differentiated cells express adequate levels of major phase-I and phase-II xenobiotic metabolizing enzymes (Ghaderi et al., 2011; Allameh et al., 2009). In this line we showed that differentiation-dependent expression of cytochrome P4503A4 (CYP3A4), measured in hepatocyte-like cells was correlated with DNA damages which was induced by aflatoxin B1 (AFB1) (Ghaderi et al., 2011). Moreover, we demonstrated that non-differentiated cells express lower levels of some of the phase-II drug metabolizing enzymes such as glutathione and glutathione S-transferases (GSTs), but increased in levels during hepatogenic differentiation (Allameh et al., 2009).

The emerging tendency of stem cells to undergo differentiation under stress conditions may contribute to pathological events, such as accelerated tissue aging or reduced tissue regeneration. However, the response of the stem cells undergoing hepatic differentiation to

DNA damage induced by a chemical hepatocarcinogen i.e., AFB1 during differentiation is not well understood. In the present study, two methods were used to compare the effect of differentiation on susceptibilities of stem cells before and after differentiation to a genotoxic agent. The DNA damage was estimated using conventional Comet assay, then specific QPCR assays (Kovalenko and Santo, 2009), were used to investigate the effect of induced DNA damage on expression of genes involved in DNA amplification in target genes namely, P53 and β-globin in hepatocyte-like cells differentiated from two multipotent stem cells namely, MSCs and $CD34^+$ cells.

Materials and Methods

Chemicals and reagents

Bovine serum albumin (BSA), Ficoll-Hypaque, stem cell factor (SCF), thrombopoietin (TPO), fibronectin, 3-[4,5,dimethylthiazol-2,-yl]-2,5 diphenyltetrazolium bromide (MTT), normal melting point (NMP) agarose, low melting point (LMP) agarose, ethidium bromide, Tris base, disodium ethylene diamine tetra-acetate (Na_2EDTA), dimethyl sulfoxide (DMSO), Triton X-100 and aflatoxin B1 (AFB1) were purchased from Sigma-Aldrich (USA). Hepatocyte growth factor (HGF), dexamethasone (DEX) and oncostatin-M (OSM) were obtained from Chemicon (USA). Fetal bovine serum (FBS), low-glucose Dulbecco's Modified Eagle Medium (DMEM), trypsin-EDTA, L-glutamine and penicillin-streptomycin were supplied by Invitrogen-Gibco (USA). Hydroxyl ethyl starch (HES) was obtained from Fresenius (Germany). The CD34 separation microbead kit was purchased from Miltenyi Biotec (Germany). Genomic–tip kit (Qiagen, Valencia, CA, USA) was used to extract DNA from the cells. DNA amplification kit; TaKaRa LA Taq kit was used for amplification of DNA in QPCR.

Isolation of mononuclear cells (MNCs)

The UCB sample used in this study was obtained from the umbilical cord of one full-term infant with the informed consent of the mother. The sample was depleted of red blood cells by HES sedimentation: 0.8% HES in 0.9% NaCl was added directly to the collection bag in a ratio of one part HES to five parts blood. The HES-NaCl mixture was then gently layered over Ficoll-Hypaque and centrifuged for 20-30 min at 450 x *g* at room temperature (RT). Low-density UCB-MNCs were collected from the interface layer and washed with PBS. The cells were recovered by centrifugation for 10 min at 400x *g* at RT.

Separation of $CD34^+$ cells

MNCs (10^8 cells) were suspended in a final volume of 300 µl column buffer (PBS, pH 7.2, supplemented with 0.5% BSA and 2 mM EDTA). Then, 100 µl Fc receptor blocking reagent (human immunoglobulin G; IgG) and 100 µl CD34 microbeads were added to the cell suspension and the mixture was incubated for 30 min at 6°C. The cells were washed in column buffer prior to centrifugation (10 min, 300x *g* at RT). The cell pellet was then resuspended in column buffer and the separation column to be used (Miltenyi Biotec., Germany) was selected according to the number of MNCs. The cell suspension was applied to a pre-filled column, placed in a magnetic field and allowed to pass through the column. Unlabeled cells passed through the column ($CD34^-$ fraction) while magnetically labeled cells were retained ($CD34^+$ fraction). The target $CD34^+$ cells were recovered by removing the magnetic field and flushing the column with the buffer. In order to increase the purity of the cell preparation, the magnetic separation step was repeated using a new column. The isolated $CD34^+$ cells were cultured and expanded in low-glucose DMEM supplemented with 20% FBS, 2 mM L-glutamine, 100 µg/ml streptomycin, 100 U/ml penicillin, 100 ng/ml SCF and 100 ng/ml TPO in a 24-well plate and incubated at 37°C in a fully humidified atmosphere with 5% CO_2.

Isolation and processing of MSCs

The isolated $CD34^-$ fractions were seeded in 25 cm^2 culture flasks at a density of 3×10^5 cells/cm^2 in culture medium containing low-glucose DMEM supplemented with 20% FBS, 2 mM L-glutamine, 100 µg/ml streptomycin

and 100 U/ml penicillin. Cell cultures were placed at 37 °C in a humidified 5% CO_2 incubator. The non-adherent cells were removed by replacing the culture medium every 3 days, so that only fibroblast-like adherent cells remained. On reaching confluence, cultures were resuspended in 0.25% trypsin-EDTA and plated in 25 cm^2 culture flasks at a density of 1×10^4 cells/cm^2.

Induction of hepatocyte differentiation

Cultured MSCs and $CD34^+$ cells at the third passage (2×10^4 cells/cm^2) were seeded on a fibronectin (5 µg/ml)-coated 24-well tissue culture plate and incubated for 48 h at 37°C in a humidified 5% CO_2 incubator. Hepatocyte differentiation and characterization was performed using a two-step protocol employing HGF, DEX and OSM, as described in our recent publication (Kazemnejad et al., 2009). Briefly, the cells were cultured in a culture medium consisting of low-glucose DMEM supplemented with 20% FBS, 20 ng/ml HGF, and 0.1 µM DEX for 7 days. Then, OSM (10 ng/ml) was added to the culture medium and maintained for 14 days in order to promote hepatocyte differentiation. Differentiation of the cells was confirmed by showing the differentiation potential of the progenitor cells to adipocyte and oesteogenic cells. Also the hepatocyte-like cells were characterized *by* detection of different liver-specific markers. Expression of albumin, alpha fetoprotein, alpha 1-antitrypsin, cytokeratins (8, 18, and 19), tryptophan 2, 3-dioxygenase and cytochrome 3A4 were considered markers of hepatocyte-like cell differentiation as described in our previous publications (Kazemnejad et al., 2009).

AFB1 treatment and Comet assay

The AFB1 concentrations (AFB1; 0, 2.5, 10 and 20 µM) used for DNA damage studies were selected based on an MTT viability assay. Cells seeded in 24-well plates were treated with AFB1 or DMSO (solvent) for 24 h at 37°C and then harvested by trypsin treatment followed by centrifugation (450 x *g* at 0°C). The supernatant was discarded and the cells were suspended in 140 µl 37°C low melting point (LMP) agarose (0.75% agarose in PBS buffer, free of Ca^{2+} and Mg^{2+}). The sample was spread on an agarose-coated frosted slide (1% NMP agarose) and covered with a coverslip. NMP agarose-coated slides were prepared as described and a single cell gel electrophoresis (comet) assay was performed under alkaline conditions, as described in our recent publication (Ghaderi et al., 2011).

Estimation of relative gene amplification and DNA lesions using QPCR

The basis of DNA lesion assay used in this study is that the DNA strand cannot serve as a template for QPCR amplification because the damage effectively blocks DNA synthesis by Taq polymerase. Hence using long fragment real-time QPCR, the percentage of template molecules containing blocking lesions within a defined amplicon can be determined. QPCR designed to show the presence of DNA lesions which cause reduction in the amount of PCR products relative to an unmodified template by inhibiting Taq polymerase function was used to compare the differences in DNA damage in cell types. For this purpose, DNA was isolated from different cell types and used as template for amplification of a 7 kb fragment from the transcribed P53 gene and 17.7 kb fragment of non-transcribed β-globin gene. The oligonucleotides used as specific primers for each gene, together with annealing temperature and product size are as shown in Table-1. Typical amplification mixture comprised of mM $MgCl_2$, 0.5 µM of each primer, and 2 µl of 10× Light Cycler FastStart DNA Master SYBR Green I in a final volume of 20 µl. Different assay conditions were adopted for PCR assay for the genes amplified. The assay conditions are summarized in Table 2.

Table 1 The primers and product size used for PCR β-globin and P53 genes.

Gene	Annealing Tm (˚C)	Primer	Product size
β-globin	66	5´-CGA GTA AGA GAC CAT TGT GGC AG-3´ (F) 5´-GCA CTG GCT TAG GAG TTG GAC T-3´(R)	17.7 kb
P53	67	5´-TGA GGA CCT GGT CCT CTG AC-3´ (F) 5´-TGA CGC ACA CCT ATT GCA AG-3´ (R)	7.7 kb

Table 2 PCR condition performed for β-globin and P53 genes

PCR condition	Time	Temperature (˚C)
First denaturation	5 min	95˚C
Denaturation	10 s	98˚C
Annealing	15 min	β-globin
	8 min	P53
Extension	10 min	72˚C

In QPCR the fluorescence of SYBR green reporter signal during the central exponential phase amplification point, baseline subtraction was used to calculate the relative amounts of undamaged template in each reaction. Data were analyzed by using Light Cycler Software. All expression levels were normalized to β-globin (a house keeping gene) in each well. Fold induction was defined as the fold increases for each sample relative to MOCK (MOCK = 1).

Expression of CYP3A4 at mRNA levels

The mRNA expression of CYP3A4 using reverse transcription polymerase chain reaction (RT-PCR) was carried out to estimate CYP3A4 expression in differentiated $CD34^+$, differentiated MSCs , non-differentiated $CD34^+$ and non-differentiated MSCs. The details of this assay are as described in our previous publication (Ghaderi et al., 2011).

Statistical analysis

All statistical analyses were carried out using SPSS software, version 13. Data were analyzed using a nonparametric Kruskal-Wallis test followed by a Mann-Whitney U-test. The results are presented as mean ± SD.

Results

DNA damage in AFB1-treated cells (Comet assay)

The background DNA lesion was always significantly lower in control (untreated) cells than those treated with AFB1 as determined by Arbitrary Units (AU) and % DNA in comet tail. The level of DNA in tail was found to be 11 % in case of non-differentiated MSCs which corresponded to 35.5 AU. The DNA damage response in hepatocytes derived from MSCs exhibited a dose-dependent pattern.

The DNA damage caused by AFB1 was found to be greater in $CD34^+$ cells (3-5 folds) and MSCs (2.5-3.5 fold) when compared to their corresponding controls. The DNA damage caused by AFB1 in non-differentiated $CD34^+$ stem cells was also higher when compared to that measured in non-differentiated MSCs (Fig. 1 and 2). The data obtained from visual scoring was verified by Comet software analysis. Figure-3 is a representative image of the Comet assay showing DNA damage in the comet tail. Based on Comet data the differentiated MSCs were ~1.5-2 times more sensitive to AFB1 toxicity when compared to non-differentiated MSCs. The hepatocytes differentiated from $CD34^+$ were less sensitive to DNA damage as compared

to the non-differentiated $CD34^+$ cells. The DNA damage caused by AFB1 (20 µM) in non-differentiated $CD34^+$ cells was significantly higher (~48%; $P<0.05$) than that measured in hepatocytes derived from them (Fig.3).

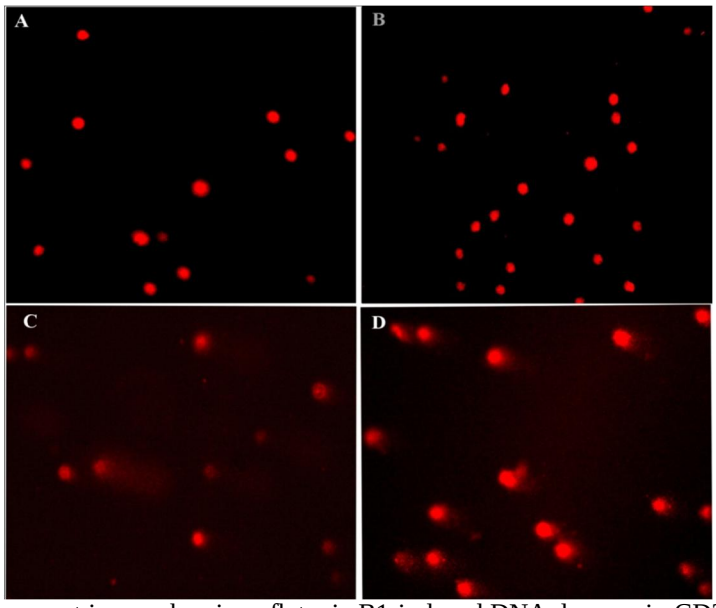

Figure 1 Representative comet image showing aflatoxin B1-induced DNA damage in $CD34^+$ and hepatocyte-like cells. The cells were exposed to AFB1 for 24 h prior to comet assay. Details are as described in methods. A: Non-differentiated-untreated $CD34^+$ stem cells (Control), B: Differentiated –untreated hepatocytes- like cells, C: $CD34^+$ stem cells treated with AFB1 for 24 h, and D: Hepatocytes treated with AFB1 for 24 h.

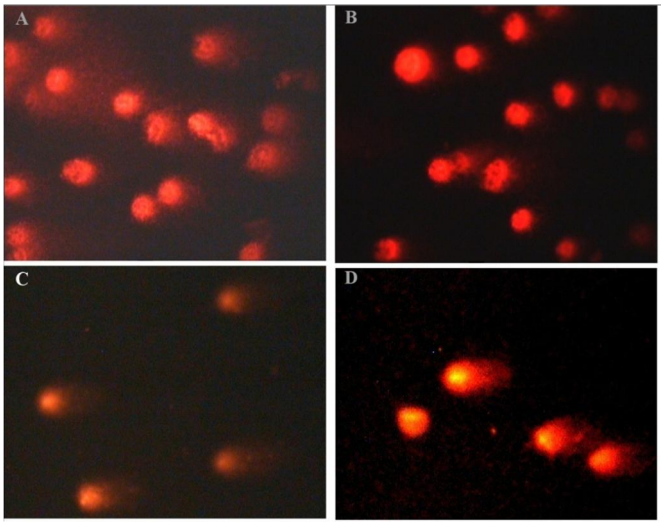

Figure 2 Representative comet image showing aflatoxin B1-induced DNA damage in mesenchymal stem cells (MSCs) and hepatocyte-like cells. The cells were exposed to AFB1 for 24 h prior to comet assay. Details are as described in methods. A: Non-differentiated-untreated MSCs (Control), B: Differentiated –untreated hepatocytes, C: MSCs treated with AFB1 for 24 h, and D: Hepatocytes treated with AFB1 for 24 h.

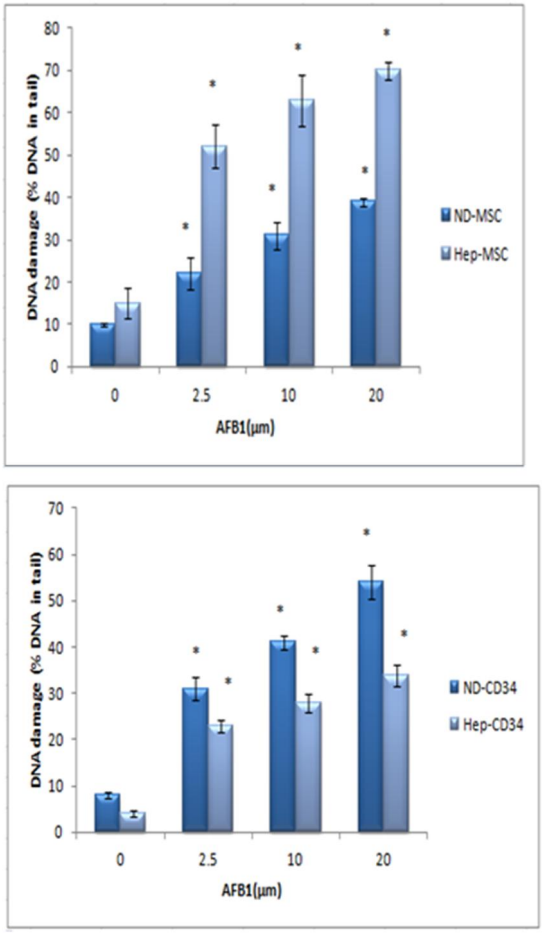

Figure 3 The rate of DNA damage (Comet assay) induced by aflatoxin B1 in hepatocyte-like cells and their progenitor stem cells. A: compares the DNA damage in mesenchymal stem cells with hepatocyte-like cells.B: compares the DNA damage in CD34+ stem cells with hepatocyte-like cells. DNA damage is measured by measuring the DNA in comet tail and expressed as percentage DNA in tail.

The effects of AFB1 on gene amplification in hepatocyte-like cells and their progenitor cells

Figure 4, shows that effect of different concentrations of AFB1 on β-globin expression in hepatocyte-like cells and their progenitor non-differentiated stem cells. The results show that the rate of lesion in β-globin gene in both the cells is increasing depending on the AFB1 concentration in the media. When the amount of lesion is compared in both the cells it is clear that the AFB1-induced lesions are significantly greater in differentiated cell (hepatocytes) as compared to non-differentiated stem cells (Fig.4 section A). However, the amount of β-globin gene lesion in hepatocytes derived from $CD34^+$ cells was relatively lower when compared to non-differentiated $CD34^+$ stem cells (Fig. 4, section B).

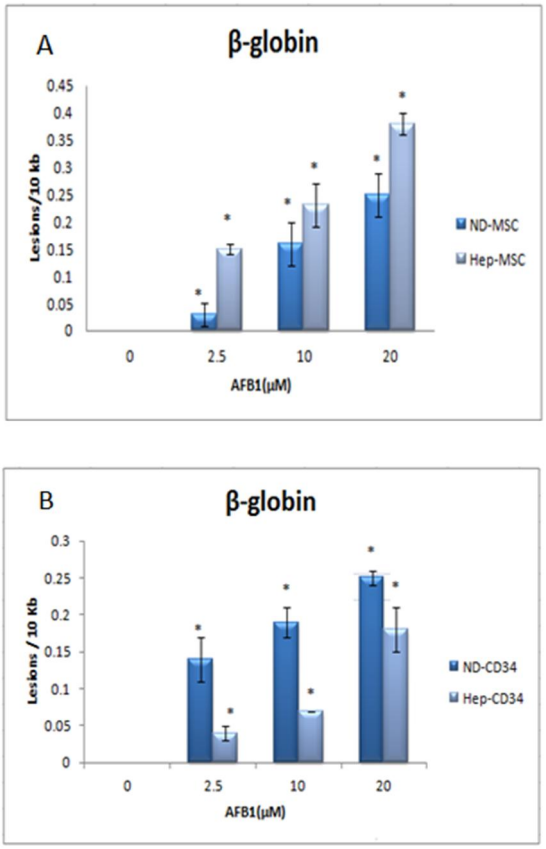

Figure 4 Comparison of the rate of lesions in β-globin lesion induced by aflatoxin B1 in hepatocyte-like cells and their progenitor stem cells. A: compares the DNA damage in mesenchymal stem cells with hepatocyte-like cells. B: compares the DNA damage in CD34+ stem cells with hepatocyte-like cells. The DNA lesion is determined by relative amplification rate and expressed as lesions/10 kb.

Figure 5 summarizes the data obtained by QPCR showing AFB1-induced P53 gene lesions in hepatocyte-like cells after differentiation and in their undifferentiated stem cells. Section A (Fig. 5) clearly shows that the rate of lesions to P53 gene is

significantly higher as compared to the MSCs before differentiation. Section B (Fig. 5) compares the rate of P53 amplification in presence of AFB1 in $CD34^+$ cells and hepatocytes obtained from them. It is obvious that the hepatocytes after differentiation have lower levels of lesions as compared to their progenitor stem cells.

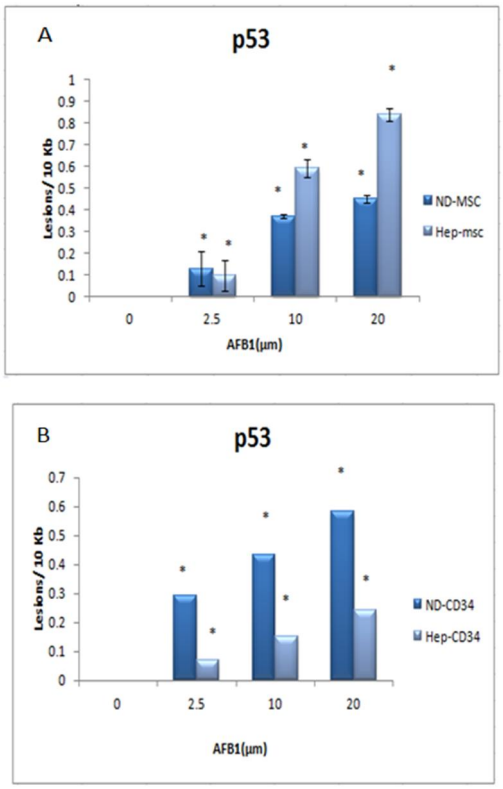

Figure 5 Comparison of the rate of lesions in P53 lesion induced by aflatoxin B1 in hepatocyte-like cells and their progenitor stem cells. A: compares the DNA damage in mesenchymal stem cells with hepatocyte-like cells. B: compares the DNA damage in CD34+ stem cells with hepatocyte-like cells. The DNA lesion is determined by relative amplification rate and expressed as lesions/10 kb.

Comparison of CYP3A4 expression in hepatocyte-like cells and their progenitor stem cells

The results of RT-PCR showed that hepatocytes considerable levels of CYP3A4 at mRNA levels when compared to the undifferentiated MSCs and $CD34^+$ stem cells. The undifferentiated stem cells express very little amounts of CY3A4 specific mRNA (Fig.6).

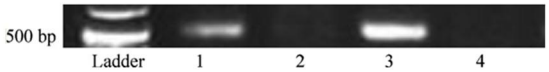

Figure 6 CYP3A4 expression in hepatocyte-like cells and their progenitor stem cells The mRNA expression of CYP3A4 using reverse transcription polymerase chain reaction (RT-PCR) estimated in cells collected 14 days of differentiated CD34+ (lane 1), differentiated MSCs (lane 3), non-differentiated CD34+ (lane 2), and non-differentiated MSCs (lane 4).

Discussion

In this study for the first time we report the effect of a genotoxic agent in increasing the lesions to specific target genes and DNA damage in multipotent stem cells during in vitro differentiation. The results showed that differentiation of the stem cells to hepatocyte-like cells is associated with increased susceptibilities of the cells to DNA damaging agents. The process of differentiation of multipotent stem cells to a specific cell line such as hepatocytes-like cells could always be associated with the DNA damage responses. This response varies depending on the differentiation stage and the endogenous and exogenous factors responsible for the damage.

In recent years, cellular therapy using human hepatocytes is being evaluated worldwide as an alternative to organ transplantation in patients with liver failure (Reviewed by Allameh and Kazemnejad, 2012). The safety of using these cells may also be linked to the minor or major damages to DNA that occur during cell proliferation and differentiation. The DNA damages may increase the tendency of stem cells to undergo differentiation under stress conditions which may be associated with pathological events, such as accelerated tissue aging or reduced tissue regeneration.

In this regard, cells that proliferate to generate proliferating daughter cells are substantially different from cells that proliferate toward the formation of post-

mitotic, differentiated progenies. As such, tissue progenitors, otherwise defined as to 'differentiation-committed' cells, will necessarily interpret the DNA damage-activated cell cycle checkpoint in relationship to the function of cell cycle arrest as a trigger signal toward their terminal differentiation (Polesskaya and Rudnicki, 2002).

In the present study the cells before and after differentiation to hepatocyte-like cells were treated with a genotoxic agent to find out their relative susceptibilities to DNA damage. The results of this study may provide useful information for selecting an appropriate stem cell for application in cell therapy protocols. The results showed that hepatocytes are more sensitive to AFB1-induced DNA damage as compared to their undifferentiated stem cells, suggesting that differentiation of cells to hepatocytes is associated with increased capacity of the cells to carry out metabolic activation of AFB1. This finding was further confirmed by showing that unlike stem cells the hepatocytes differentiated from them express relatively high levels of CYP3A4 (Fig. 6).

Interestingly, we showed that the hepatocytes differentiated from MSCs are more efficient in metabolic activation of AFB1 when compared to that obtained from $CD34^+$ cells. As a result of this, the rate of DNA damage as well as suppression in the amplification of target genes determined by QPCR was greater in hepatocytes differentiated from MSCs as compared to the cells originated from $CD34^+$ cells.

These data clearly show that cell types vary in their response to DNA damage and a distinct cell type, at different developmental stages, might adopt different responses to DNA lesions caused by genotoxic agents. These data are in agreement of our previous report showing that the stem cells derived from human UCB exhibit differential susceptibilities to chemical carcinogen induced DNA damage (Ghaderi et al., 2011). MSCs, like other cell types possess DNA repair system, and regardless of the DNA damaging factors, the repair system could be induced in response to the damages.

Differences in the rate of DNA damage frequency in different stem cell types may partly be attributed to the genome positional and chromosome structure as well as the repair capacity o f the cell types.

Moreover, the relationship between DNA damage and lesions in P53 gene was observed when AFB1-dependent DNA damage resulted in suppression of the gene amplifications in QPCR (Figure 5). Among the several proteins involved in DNA damage response in proliferating cells, P53 plays a crucial role during hepatogenic differentiation of the stem cells. It has been reported that the unique DNA damage response of mouse hematopoietic stem cells (HSPCs) involves the tumor suppressor protein p53 and is lost when stem cells are forced out of quiescence and into the cell cycle by treatment with chemotherapy or cytokines. In this line, not only are quiescent HSPCs poised to resist apoptosis as evidenced by their antiapoptotic gene expression program, but they are also able to repair their DNA by non-homologous end joining (NHEJ). Repair of DNA damage through homologous recombination (which has a lower error rate than NHEJ) requires that cells enter the cell cycle; thus, quiescent stem cells must rely on NHEJ as an alternative. The reliance of quiescent adult tissue stem cells on NHEJ for the repair of DNA damage may in fact be a general phenomenon in mice, given the similar conclusions of a recent study using hair follicle stem cells as a model system (Sotiropoulou et al., 2010).

Comparison of AFB1 induced DNA damage in MSCs before differentiation and hepatocytes differentiated from them measured by QPCR of P53 gene revealed that hepatocytes are more susceptible as compared to their progenitor MSCs in terms of lesions/10 Kb. A lower levels of DNA amplification (P53 gene) in hepatocytes as compared to MSCs imply that more serious damage occur in hepatocytes as compared to MSCs due to AFB1 exposure (Fig. 5). As a result, AFB1-induced DNA

damage, with emphasize to P53 gene may be reflected in cell cycle regulation and normal cell expansion. It has been suggested that the stem cell escape from acute damage, particularly if it involves a decrease in p53 activity, may lead to long-term deleterious effects on stem cell fitness and repopulating ability (Milyavsky et al., 2010). Perhaps, a significant increase in DNA lesion as shown by reduction in DNA amplification in differentiated hepatocytes relays on the extent of metabolic activation of AFB1 mediated by CYP3A4 which is expressed in differentiated hepatocytes (Fig. 6). The QPCR data are inconsistent with the data obtained from Comet assay showing that DNA damage that the hepatocytes differentiated from mesenchymal stem cells are relatively more susceptible to AFB1-induced DNA damage (Fig. 3).

Inhibition in the β-globin amplification in cells pretreated with AFB1 may suggest that genes which are indirectly involved in DNA damage-repair system are also affected by AFB1. Therefore it appears that the differences in the rate of DNA lesions corresponding to the rate of gene amplification in hepatocytes and their progenitor MSCs is a multifactorial mechanism mainly rooted in differences in DNA damage-repair systems in different cell types. The capacity of cells to catalyze metabolic activation of chemical carcinogens *via* cytochrome P450 which is increased during cell differentiation and development is also important. This preliminary data, on one hand shows that non-differentiated stem cells are less vulnerable to DNA damage; on the other hand, the non-differentiated cells are deficient in CYP enzymes the development of which relies on induction of stem cell differentiation.

These findings provide important information on how stem cells respond to DNA lesions and the consequences on cell differentiation and transplantation. Also, this information helps in understanding the role of stem cells in short-term tissue reconstitution and long-term cell and genomic integrity.

Overall results suggest that the hepatogenic differentiation of stem cells is associated with increased expression of cytochrome P450 (CYP3A4). During the hepatogenic differentiation of stem cells the capacity of the cell for metabolic activation of AFB1 is increases which may be responsible for greater levels of induced DNA damage and lesions to specific target genes.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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