Effect of Mesenchymal Stem Cells on Transforming Growth Factor Beta Level in Hepatocellular Carcinoma Induced Rat Model

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Abstract

Background: Transforming Growth Factor-Beta (TGF-β) signaling pathway has been recognized as a key driver in cancer, where its activation affects cell proliferation, angiogenesis, invasion and metastasis. Mesenchymal Stem Cells (MSCs) are emerging as vehicles for cancer gene therapy due to their inherent migratory abilities toward tumors. The aim of this study was to evaluate the effect of bone marrow derived-MSCs (BM-MSCs) on TGF-β level in experimental Hepatocellular Carcinoma (HCC) in rat.

Material and Methods: This study involved thirty female white albino rats divided equally into: Control group, HCC group induced by Diethyl-Nitroseamine (DENA) and carbon tetrachloride (CCl₄) and HCC group treated with BM-MSCs. TGF-β protein level in rat liver tissues and serum levels of Alpha Fetoprotein (AFP) were assessed by ELISA. Also, serum levels of ALT and albumin were estimated by colorimetric methods.

Results: TGF-β protein level in rat liver tissues was significantly increased in untreated HCC rat group compared to the control group. While a significant decrease was shown in HCC group treated with BM-MSCs when compared to the untreated HCC group. Also, the serum levels of AFP and ALT were significantly increased in untreated HCC group compared to the control group, whereas there was a significant decrease in their levels in BM-MSCs treated groups as compared to the untreated HCC group.

Conclusion: Down-regulation of TGF-β levels could be achieved by administration of BM-MSCs in chemically induced HCC rats. From these findings, targeting the over-expressed TGF-β signaling pathway is a potential therapeutic strategy for HCC.

Key Words: HCC – TGF-β – BM-MSCs.

Introduction

HEPATOCELLULAR Carcinoma (HCC) arises in patients as a consequence of long-standing preexisting liver illnesses including viral hepatitis, alcohol abuse, or metabolic disease. It is one of the most aggressive human cancers with a high frequency of post-surgical recurrence [1]. HCC seems to develop through a multistep process, involving multiple genetic hits, occurring over decades of chronic liver disease that ultimately leads to malignant transformation in hepatocytes [2].

Multiple signaling pathways that affect cell proliferation, angiogenesis, invasion, and metastasis are dysregulated in HCC. Among these, the most frequently reported pathways involve growth factors, such as transforming growth factor beta (TGF-β), Insulin like Growth Factor (IGF), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF) and Hepatocyte Growth Factor (HGF) [3].

TGF-β signaling has been increasingly recognized as a key driver in cancer. Unlike its tumor suppressor function in normal tissue, TGF-β activation causes tumor promotion in cancer tissue. The switch from tumor suppression to promotion is not well understood, but intrinsic and extrinsic factors seem to play important roles. The loss of cell polarity and acquisition of motile properties during Epithelial-Mesenchymal Transition (EMT) are considered crucial intrinsic changes of the tumor cells [4]. These tumor promotion changes mediated by TGF-β signaling are also accompanied by extrinsic factors originating from the tumor microenvironment, such as angiogenesis, inflammation, and fibroblast activation [5].

Stem cell-based therapy has been proposed as a promising alternative approach for end-stage liver diseases. In liver damage, MSCs differentiate into hepatocytes, stimulate the regeneration of
endogenous parenchymal cells, migrate to damaged sites, and enhance fibrous matrix degradation (anti-fibrotic effects) \[7\]. It has been demonstrated that BM-MSCs inhibit the activation of Smad transcription factors that are induced by TGF-\(\beta\), stimulating recovery of liver fibrosis \[8\]. In the study presented here, we evaluated the effect of BM-MSCs on TGF-\(\beta\) level in experimental hepatocellular carcinoma in rat.

**Material and Methods**

This study was conducted at the Unit of Biochemistry and Molecular Biology in Medical Biochemistry Department, Faculty of Medicine, Cairo University, in the period from April 2014 to October 2015. This study included thirty female adult albino rats, inbred strain (Cux 1: HEL 1) of matched age and weight. Rats were maintained according to the standard guidelines of Institutional Animal Care and Use Committee and after Institutional Review Board approval.

I- Isolation, propagation and identification of bone marrow-derived MSCs from rats:

A- Isolation and propagation of BM-derived MSCs from rats:

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (FBS, GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin streptomycin (GIBCO/BRL). Cells were incubated at 37\(^\circ\)C in 5% humidified CO\(_2\) for 12-14 days as primary culture. Media was changed every 2-3 days.

When large colonies developed (80-90% confluence), cultures were washed twice with Phosphate Buffer Saline (PBS) and the cells were trypsinized with 0.25% trypsin in 1mM EDTA (GIBCO/BRL) for 5min at 37\(^\circ\)C. After centrifugation, cells were resuspended with serum-supplemented medium and incubated in 50cm\(^2\) culture flasks (Falcon). The resulting cultures were referred to as first passage cultures \[9\]. On day 14, the adherent colonies of cells were trypsinized, and counted.

B- Identification of BM-derived MSCs from rats:

Cells were identified as being MSCs by their morphology, adherence, and their power to differentiate into osteocytes \[10\] and chondrocytes \[11\].

II- Preparation of experimental animal model:

Experimental design:

Animals were divided into 3 groups:

- **Group 1:** Ten rats used as negative control group (normal healthy rats).
- **Group 2:** Ten rats with experimental HCC (pathological control) induced by a single intraperitoneal dose of DENA at a dose of 200mg/kg body weight followed by subcutaneous injections of CCI\(_4\) at a dose of 3mL/kg body weight (twice/week) for 6 months \[12,13\]. Then, HCC was confirmed after histopathological examination of two sacrificed rats.
- **Group 3:** Ten HCC rats injected with MSCs (3X 10\(^6\) cells intravenously, once) then sacrifice was done after 4 weeks \[14\].

Venous blood was collected from the retro-orbital vein from rats of all groups. At the planned time, animals were sacrificed by cervical dislocations, and liver tissues were harvested for assessment of the following:

1- TGF-\(\beta\) protein level in rat liver tissue by ELISA (eBioscience, San Diego, CA, USA) according to the manufacturer’s recommendations.

2- Alpha fetoprotein serum levels by ELISA (Uscn, Life science Inc., UK) according to the manufacturer’s recommendations.

3- Serum ALT and albumin levels by the routine laboratory colorimetric method.

**Statistical analysis:**

The data was coded and entered using the statistical package SPSS version 22. Data was summarized using mean \(\pm\) SD for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test \[15\]. \(p\)-values less than 0.05 were considered as statistically significant.

**Results**

Regarding the serum levels of AFP and ALT, a significant increase was found in HCC group compared to the control group, while there was a significant decrease in MSCs treated groups compared to the HCC group. As for the serum albumin levels, there was a significant decrease in HCC group and MSCs treated group compared to the control group. Whereas, there was a significant
increase in its level in HCC + MSCs group compared to the HCC group (Table 1).

As regards TGF-β protein level in rat liver tissues, there was a significant increase in TGF-β levels in HCC group compared to the control group. However, there was a significant decrease in TGF-β levels in MSCs treated group compared to the HCC group (Table 2) & Fig. (1).

Table (1): Comparison between serum levels of AFP, ALT and Albumin in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>HCC</th>
<th>HCC + MSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (ng/mL)</td>
<td>0.40±0.13</td>
<td>1.70±0.35*</td>
<td>0.65±0.20#</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>19.24±2.12</td>
<td>71.74±7.94*</td>
<td>53.02±10.54*#</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>5.20±0.34</td>
<td>2.21±0.75*</td>
<td>3.24±0.30*#</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD.
*: Statistically significant compared to corresponding value in control group (p<0.05).
#: Statistically significant compared to corresponding value in HCC group (p<0.05).

Table (2): TGF-β levels (mean ± SD ng/ml), in rat liver of all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.54±3.66</td>
</tr>
<tr>
<td>HCC</td>
<td>131.98±34.83*</td>
</tr>
<tr>
<td>HCC + MSCs</td>
<td>75.34±9.57#</td>
</tr>
</tbody>
</table>

*: Statistically significant compared to corresponding value in control group (p<0.05).
#: Statistically significant compared to corresponding value in HCC group (p<0.05).

Discussion

The TGF-β pathway exerts a dynamic effect on cancer cells. Early in carcinogenesis process, TGF-β suppresses tumors and arrests cell growth [16]; in later and advanced tumor stages, TGF-β potentiates EMT, angiogenesis, tumor progression, invasion, and metastasis [17,18]. Recently, with the advances in understanding the molecular biology of HCC, new therapeutic strategies to treat HCC have emerged [6]. The present study was conducted to evaluate the effects of BM-MSCs on TGF-β levels in experimental HCC in rat.

The results of the current study revealed that the TGF-β protein levels in rat liver tissues were significantly increased in untreated HCC group of animal model compared to the control group. Whereas, the HCC group treated with MSCs showed a significant decrease in the TGF-β levels in comparison with the HCC group.

This is in agreement with Chen et al., [19] and Ji et al., [20]. They demonstrated that TGF-β1 was mainly localized in the cytoplasm of HCC cells. A significant difference was observed between TGF-β1 expression levels in HCC and matched normal peri-tumor tissues. TGF-β1 expression was related to tumor grade and pathological stage, but not to tumor size. These results showed that the expression of TGF-β1 increased with the increase of tumor grade.

Li et al., [21] evaluated the effect of MSCs, in vivo, on tumor growth and metastasis of HCC. They reported that the serum concentration of TGF-β was elevated, and tumor-derived TGF-β could activate smads to accelerate the proliferation and malignant progression of HCC. The expression of TGF-β1 was significantly down-regulated in the MSCs-treated HCC group compared to the control group. The MSCs enhanced tumor growth but significantly inhibited the invasiveness and metastasis of HCC, possibly through down-regulation of TGF-β1. These findings suggested that MSCs could be useful in controlling metastatic recurrence of HCC.

Likewise, a study was performed by Jang et al., [7] to investigate the effect of BM-MSCs on hepatic fibrosis in thioacetamide-induced cirrhotic rat model. They explained the fundamental mechanism of attenuation of hepatic fibrosis caused by BM-MSCs treatment. They found that administration of MSCs at 2 X 10^6 cells into thioacetamide-induced cirrhotic rat livers, allowed recovery from induced fibrosis at 4 weeks after BM-MSCs treat-
ment. Also, they showed that BM-MSCs have recovered liver functions, which likely correlates with the downregulation of TGF-β 1/Smad signaling pathways.

On the other hand, Han et al., [22] investigated the effect of MSCs, in vitro, on HepG2 cells and in vivo, on induced HCC animal model by subcutaneous implantation of HCC cells (alone or mixed with MSCs) in armpit areas of nude mice. Tumor growth was evaluated by measuring the length and width of the tumor mass. After sacrifice, tumor masses were weighed and analyzed by histology. Results showed that MSCs in inflammatory microenvironment may persistently promote the development of chemoresistance in HCC cells during tumor growth. One mechanism underlying MSC-promoted development of chemoresistance in HCC cells is via their over-expression of TGF-β in response to inflammatory stimuli in the tumor microenvironment.

In conclusion, administration of BM-MSCs could decrease the TGF-β levels in chemically induced HCC rats. These data suggest that TGF-β signaling pathway could be used as a therapeutic target in HCC.

References


الملخص العربي

بعد سرطان الخلايا الكبدية أحد أشهر الأورام السرطانية في العالم فهو يحتل المركز السادس بين السرطانات الأكثر انتشاراً، بينما يحتل المركز الثالث بين السرطانات التي تؤدي إلى الوفاة. وحتى وقتنا الحاضر، فإنه لا يوجد علاج نهائي لحالات سرطان الخلايا الكبدية. وفي الحالات المتاخرة من المرض يصبح الاستئصال الجراحي أو زراعة الكبد أو العلاج الكيميائي أمراً غير ممكن. وهو ما يجعل استحداث طرق جديدة للعلاج أمراً ضرورياً. تعتبر عملية تسرطن الكبد عملية معقدة ومتنوعة الخطوات، تتنتج عن مجموعة من التغيرات الجينية.

بعد طريق إشارات معامل تحول النمو بيتا أحد أهم مسببات السرطان، حيث يؤدي تعطيل هذا الطريق إلى تكاثر الخلايا وتكون أوعية دموية جيدة، كما يساعد على انتشار الخلايا السرطانية.

لقد برز استخدام الخلايا الجذعية كناقل في العلاج الجيني للسرطان نتيجة لقدرته على الهجرة إلى موقع السرطان، كما وجد أن تلك الخلايا تلعب دوراً مهماً في علاج وإعادة بناء أنواع مختلفة من الأنسجة والأعضاء.

الهدف من العمل: تأثير الخلايا الجذعية المستخلصة من نخاع العظم على مستوى معامل تحول النمو بيتا في سرطان الكبد.

المواد والطريقة: لقد تم إدراج خمسون من إناث الجرذان في هذه الدراسة وتم تقسيمها إلى ثلاث مجموعات كالأتي:

المجموعة الأولى: تشتمل عشرة جرذان وتمثل المجموعة الضابطة.

المجموعة الثانية: تشتمل عشرة جرذان مصابة بمرض سرطان الكبد.

المجموعة الثالثة: تشتمل عشرة جرذان مصابة بمرض سرطان الكبد وقد تم إعطاءهم خلايا جذعية مستخلصة من نخاع العظم عن طريق الوريد.

وقد تم سحب عينات الدم بعد إدخال الجرذان وإجراءات القياس:

- مستوى معامل تحول النمو بيتا في نسيج الكبد عن طريق ELISA.
- مستوى معامل تحول النمو بيتا في الدم.
- مستوى AFP, ALT and albumin.

وقد وجد أن الخلايا الجذعية المستخلصة من نخاع العظم لها القدرة على تقليل مستوى معامل تحول النمو بيتا في سرطان الكبد في الجرذان. ومن هذه النتائج يمكن اعتبار إشارات معامل تحول النمو بيتا هدفًا لعلاج سرطان الكبد. ونوصي بمزيد من الدراسات للخلايا الجذعية لتحديد دورها في السرطان.