Antitumor Effect of Mesenchymal Stem Cells from Murine in Breast Cancer in Female Rats

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A B S T R A C T

The aim of this study was to evaluate the effect of mesenchymal stem cells as a treatment against breast cancer via modulation TGF-β.

Mesenchymal stem cells (MSCs) are recruited to the stroma of cancers. They interact with cancer cells to promote invasion and metastasis or to suppress tumor growth. The unique tumor-homing capacity of MSCs makes them a promising vehicle to deliver various anticancer agents.

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1. INTRODUCTION:

Breast cancer is the most common malignancy among females throughout the world. Combined therapeutic modalities including surgery, chemotherapy, radiotherapy, endocrine and targeted therapies are the mainstay of treatment. However, they may lead to unsatisfactory outcomes, mainly due to the difficulty in accessing tumor sites, the dispersed nature of the disease and the toxicity of the treatment (Liu et al., 2015).

MSCs were originally isolated from bone marrow, and later from adipose tissues and many other organs. These cells are capable of self-renewal and differentiation into bone, fat, or cartilage cells under appropriate conditions (Zhao and Liu et al.,2014). it is also involved in tissue remodeling after injury and chronic inflammation. Tumors look like chronic wounds. The recruited MSCs and their derivative cancer-associated fibroblasts interact with cancer cells to promote invasion and metastasis. On the other hand, the unique tumor-homing capacity of MSCs renders them a promising vehicle for adequate and specific delivery of various anticancer agents. (Zhao and Liu et al., 2014)

TGF-beta signaling emerged as a variety of key functions in tissue development, homeostasis and regeneration. Underlining its importance, dysfunction of the pathway is linked to severe diseases. Although first described as a secreted polypeptide hormone capable of rendering recipient cells malignant, transforming growth factor-beta was

subsequently found to be able to inhibit cell growth, affect differentiation, and induce cell death as well. In fact, TGF-beta can bring about disparate responses depending on the cell type or on the cellular context (Massague., 2012).

2. MATERIALS AND METHODS:

- 7,12- di- methyl benz (a) anthracene (DMBA) (Sigma USA) Company.
- Estradiol accelerate the development of 7,12 dimethyl benz[a] anthracene (DMBA)-induced mammary tumors.

Experimental animals:

- 40 Virgin female Swiss albino rats of 4 weeks age, with body weight range of 80-100g were used in the present work. Rats were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt).
- *Bone marrow derived mesenchymal stem cells:*
- Mesenchymal stem cells were isolated from rats bone marrow brought from clinical biochemistry department, faculty of medicine, Cairo University.

Methods:

Preparation of 7,12- di- methyl benz (a) anthracene (DMBA):

- 7,12- di- methyl benz (a) anthracene (DMBA) was dissolved in sesame oil in such a way that each ml was containing 5 mg of DMBA and was administered at the dose rat of 50 mg /kg body weight intra-peritoneal (i.p). After dissolving, the DMBA was used within 20 min. The injection was given along the ventral mid-line of the animal, half way between the third and fourth pair of mammary glands two doses at 40, 48 days of age. (Fisher et al ., 1992)
- *Isolation, propagation, identification and labeling of bone marrow-derived MSCs from rats*

Isolation and propagation of BM- derived MSCs from rats

- Bone marrow was harvested by flushing the tibiae and femurs of 6-week-old male rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine serum (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and re-suspended in complete culture medium supplemented with 1% penicillin streptomycin (GIBCO/BRL). Cells were incubated at 37°C in 5% humidified CO2 for 12-14 days as primary culture. Media was changed every 2-3 days (Huang et al., 2003).

Identification of BM- derived MSCs from rat

- Cells were identified as being MSCs by their morphology, adherence, by detection of CD 90, CD 105, CD 34 which is one of the surface markers of rat mesenchymal stem cells in MSC culture and their power to differentiate into osteocytes and chondrocytes (Alhadlaq and Mao., 2004).
- Differentiation into osteocytes was achieved by adding 1-1000 nM dexamethasone, 0.25 mM ascorbic acid, and 1-10 mM beta-glycerophosphate to the medium. Differentiation of MSCs into osteoblasts was achieved through morphological changes, Alzarin red staining of differentiated osteoblasts. Differentiation into chondrocyte was achieved by adding 500 ng/ml bone morphogenic protein-2 (BMP-2; R&D Systems, USA) and 10 ng/ml transforming growth factor b3 (TGFb3) (Peprotech, London) for 3 weeks (Seo et al.,2009). In vitro differentiation into chondrocytes was confirmed by morphological changes, Alcian blue staining of differentiated

chondrocytes. Flow cytometric analysis of cultured MSCs surface markers was done.

C) Labeling of stem cells with PKH26

- In the current work, undifferentiated MSCs were labeled with PKH26 according to the manufacturer's recommendations (Sigma, Saint Louis, Missouri, USA). Cells were injected intravenously into rat tail vein. After one month, heart tissue was examined with a fluorescence microscope to detect the cells stained with PKH26.

Experimental design of experimental animals:

- The present study was carried out on 40 virgin female rats. DMBA was administrated as 50mg DMBA/kg body weight. (Fisher et al ., 1992)
- At the beginning of the experiment rats were divided into 4 main groups:
- Group (1) control: Was served as negative control and orally received saline.
- Group (2) DMBA**:** Was rats were injected i.p. with DMBA (50 mg/kg) one does.
- Group (3) MSC: rats were injected i.p. with MSC cells 10^6/rat.
- Group (4) DMBA+ MSC: rats were injected with DMBA i.p. then injected i.p. with MSC cells 10^6/rat.

Collection of samples:

- At the end of the treatment period, animals were fasted overnight prior to dissection under light ether anesthesia. Blood was drawn from the vena cava and centrifuged at 3000*g* for 10 min. immediately after blood collection, mammary gland tissue was excised and one portion was used for the histopathological examination. The rest of the tissue was homogenized in 0.25 M ice cold isotonic sucrose to be used for the estimation of the assessed parameters.

Experimental parameter:

- Serum from each group was assayed for using enzyme-linked immunosorbent catalog no. MBS728582. TGF-β level using rat TGF-β sandwich ELISA kit purchased from My BioSource, Inc. (USA).
- *Histopathological examination:* After sacrificing the rats, Mammary gland tissue was rapidly dissected and excised, rinsed in saline solution, and cut into suitable pieces which were fixed in neutral buffered formalin (10%) for 24 h according to the method adopted by (Banchroft et al., 1996) and examined by light microscope for histopathological investigation.

Statistical analysis:

All mean values are reported as the mean ± standard deviation (SD). Data were analyzed using a one-way analysis of variance (ANOVA). The level of significance between mean values was set at $p < 0.05$ and $p < 0.01$ (significant and highly significant, respectively). All statistical analyses were performed using SPSS software (version 20.0).

3. RESULTS:

Analysis of TGF-β using elissa technique revealed significant elevated level of TGF-β, of female rats treated with DMBA. MSC administration significantly reduced the elevated level of TGF-β in the DMBA+ MSC.

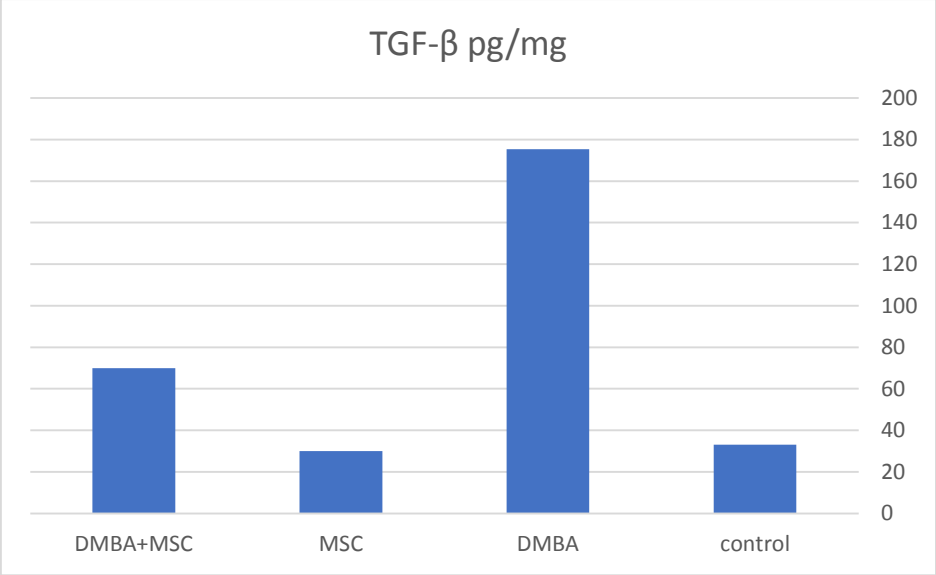

Fig (1): Effect of MSC treatment on TGF-β level in the serum of female rats include mammary gland carcinoma.

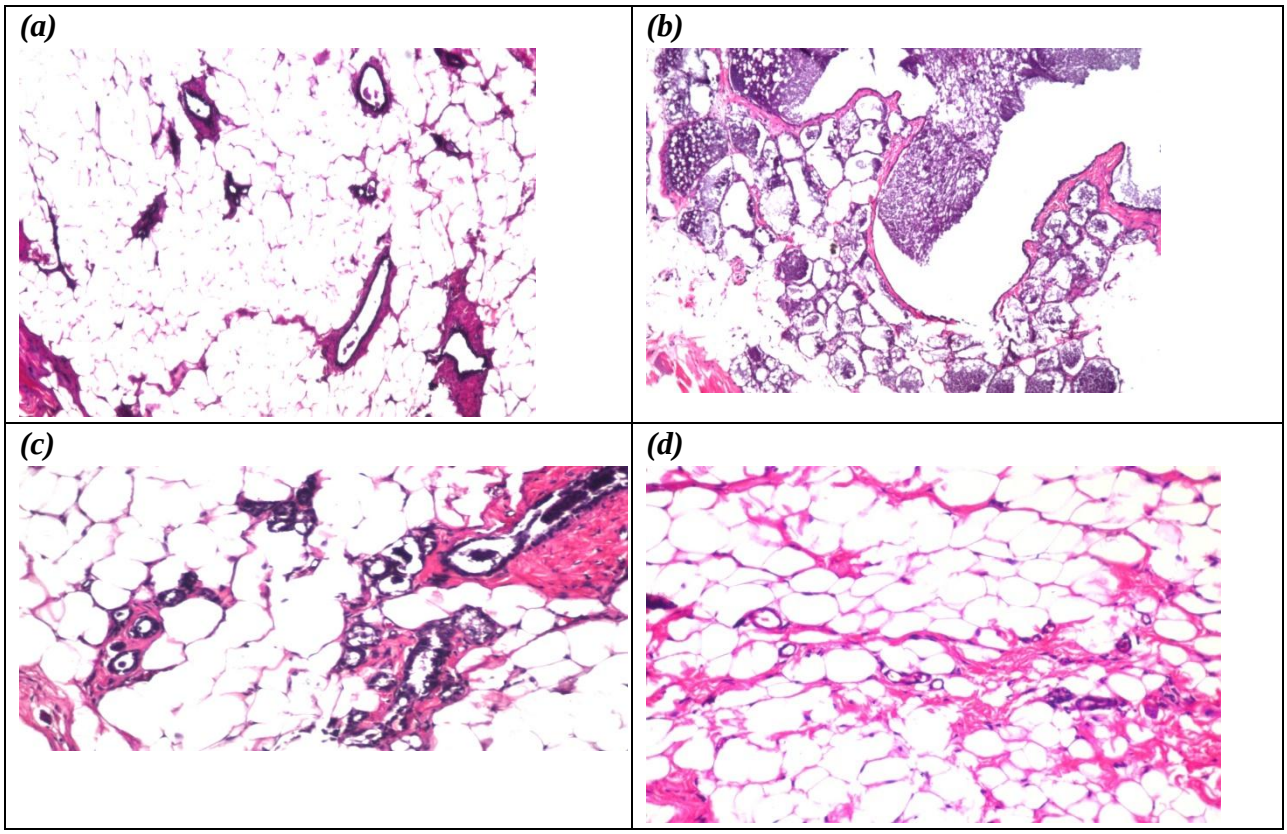

Fig (2): Histopathological findings of mammary gland tissue (a)control was served as negative control and orally received saline**, *(b)*** DMBA group was rats were injected i.p. with DMBA (50 mg/kg), (c) MSC group rats were injected i.p. with MSC cells 10^6/rat, (d) DMBA+ MSC group rats were injected with DMBA i.p. then injected i.p. with MSC cells 10^6/rat.

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4. DISCUSSION:

Breast cancer is the commonest female cancer and most common cancer in both sexes**.** Breast cancer is a kind of cancer that develops from breast cells. It is the most common type of cancer among women **(**Abdulkareem**.,** 2010).

The current study discovered the changes of expression patterns in tumor promoter gene. MSC have significant carcinoma cell growth suppressing abilities via suppressing promoter gene (TGf-β) (Ganta et al., 2009).

The unmodified MSC have been shown to have anti-tumor effects both in vitro and in vivo studies of cancer. This is attributed to the factors released by MSCs that have antitumor properties reducing the proliferation and breast cancer cells. (MSCs) injected intravenously shown to home to sites of tumorigenesis and potently inhibit tumor growth**.** Direct injection of MSC into subcutaneous melanoma bearing rats induced apoptosis and abrogated tumor growth**.** MSC have been genetically modified mainly to introduce and over express target exogenous genes for expression/secretion of a desired therapeutic factor for targeted treatment of different cancer types (Otsu., 2009).

Henry and Narendra (2006) have shown that 7,12- dimethylbenz (a) anthracene (DMBA) can be used to induce experimental breast carcinomas in rats and that this process involves disruption of tissue redox balance. Polycyclic aromatic hydrocarbons (PAH) such as 7,12-dimethylbenz(a) anthracene (DMBA) have been shown to form free radicals and these compounds play a critical role in carcinogenesis and pathophysiological disturbances may result from oxidative damage. The mammary tumors in rats arise in the epithelium of the terminal end buds, which are comparable structures to the terminal ductal lobular units in the human breast.

It is suggested that MSC dependent inhibition of breast carcinoma cell growth is partially due to secreted proteins. Consequently, TGF-β's complicated biological responses have been proposed to be governed by the different cellular contextual determinants of Smads (Massague, 2012)**,** including a wide-ranging complement of DNA-binding transcription factors, involving p53 and members of the bHLH, Forehead box (e.g., Foxo3a), and zinc finger protein families (e.g., Gli2) in different cells and various biological processes (Massague, 2012). the factors triggering the contextual changes in Smad determinants are also not clear. Here, we unveiled that (1) p53 is the critical Smad partner for TGF-b-induced p21 expression and tumor suppression in premalignant HMECs, whereas Gli2 is the decisive Smad partner for TGF-b-induced PTHrP expression and metastasis promotion in breast cancer cells, and (2) 14-3-3ζ switches TGF-β's function by triggering the contextual change of Smad partners from p53 in premalignant HMECs to Gli2 in breast cancer. Mechanism is an abnormal, tumor-activated secretion of TGF-β by the stromal fibroblasts in the TAT locale (niche) that suppresses normal mammary progenitor expansion by causing a decrease in CD49f and EpCAM expression. Opposite effect on malignant human mammary cells exemplified by an ability of the same fibroblasts (and TGF-β) to promote their growth both in vitro and in vivo.

The TGF-β-induced suppression of their expression in progenitor cells could result in cell death. In breast cancer cells, however, we show that exposure to TGF-β has no effect on the expression of these genes. TGF-β has also been shown to induce proliferation and survival of breast cancer

cells *(*Seoane and Gomis.,2017*)*. These findings establish the biological origin and in situ consequences in TAT regions of human breast tissue of the disparate effects of TGF-b on normal and malignant mammary cells. Sensitivity of normal bipotent progenitors as well as luminal progenitors to inhibition by TGF-β and a consequent effect in situ in TAT regions on both cell types. TGF-β in breast carcinogenesis is postulated to switch from acting initially as a tumor suppressor on premalignant cells to a tumor promoting growth factor during the later stages of tumor progression. The results presented here provide further support for this model by showing that TGF-b acts as a tumor suppressor in situ in histologically normal human mammary epithelial tissue adjacent to breast tumors, while also confirming its ability to augment the growth of established malignant human breast cancer (Seoane and Gomis., 2017*)*.

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