Osteosarcoma treatment using the different bone growth factors


Osteosarcoma is an extremely aggressive primary bone tumor characterized by rapid growth and highly metastatic potential. Pro-angiogenic factors are related to poor prognosis and disease progression. There are important factors which can be mediators for bone formation and repair. The aim of this study was to evaluate the association of cell derived from human osteosarcoma cells (MG63) with the bone growth factors (FGF, VEGF, rBMP2). MG63 were plated and cultured in DMEM-H supplemented with 10% bovine fetal serum, antibiotics (1%) and sodium pyruvate (1%) for 24 hours. The cells were treated with FGF (20nM), VEGF (20nM) e rBMP2 (1µg) for 48 hours. After 48 hours, the cells were fixed with 4% of paraformaldehyde and stained with Von Kossa. All the treatments showed decrease in the osteogenic potential in the MG63 cells. However, the best treatment was the rBMP2, which showed a high therapeutic potential for osteosarcoma in vitro and it can represent a new alternative to complement the current clinical treatments.

Keywords osteosarcoma, rBMP2, bone growth factors.

1. Introduction

Osteosarcoma is a primary bone tumor that occurs with most incidence in childhood and adolescence, representing 5% of malignancies in this group [1,2]. Although rare, is the most common bone cancer and main death cause by cancer in children, with the incidence peak corresponding to the period of rapid skeletal bone growth [3].

Osteosarcoma is locally invasive and potentially metastatic, which makes it particularly difficult to treat, being the metastatic disease the most common cause of patient’s death. Metastases occur early and the lung is the preferentially affected organ, surrounding 90% of the cases [4,5].

The regions most affected by the tumor are areas of rapid bone growth as distal femur, proximal tibia and proximal humerus. In adults, there is a prevalence in the axial skeleton and in areas that were previously irradiated or that have underlying abnormalities such as Paget's disease [6].

Histologically, the osteosarcoma is malignant mesenchymal cells which appear in the extended and polygonal shapes and produce an osteoid matrix. The osteoid matrix is a distinguishing feature of osteosarcomas, non-osteogenic bone tumors do not produce this matrix [7,8].

The prognosis of osteosarcoma depends on many factors including age, gender, localized tumor or metastatic tumor site (axial or appendicular skeleton), surgical margins, tumor volume and necrosis after preoperative chemotherapy, type of treatment chosen, serum phosphatase alkaline and tumor subtypes [9].

Osteosarcoma arises from mesenchymal stem cells or osteoprogenitor cells due to a disruption in the osteoblast differentiation pathway [10, 11]. Chemotherapy combination along with limb-sparing surgery has been the main treatment for osteosarcoma [12].

Multimodality treatments have markedly improved the prognosis for patients with osteosarcoma and life expectancy is now 10 years for 50-70% of patients [13]. However, currently, osteosarcoma is the second leading cause of cancer-related death for children and young adults [14].

Bone morphogenetic proteins are members of the transforming growth factor (TGF)-β superfamily, functionally induce bone and cartilage formation and are considered multifunctional cytokines [15]. Therefore, they can represent an important role in the treatment of osteosarcoma due to their inhibitory effects in the tumorigenesis [16, 17].

Tumor cell differentiation correlates with the prognosis and growth factor plays an important role in malignant bone tumor development. FGF is involved in proliferation, differentiation and cell migration of the skeletal tissues [18, 19].

Angiogenesis is essential for tumor growth and metastasis formation. The vascular endothelial growth factor (VEGF) is an important regulator of this process. The activation of the VEGF receptor pathway triggers a signaling process that promote endothelial cell growth, migration and maintenance of pre-existing vasculature. Due to its role in angiogenesis, this receptor has become an important focus in the development of antiangiogenic drugs [20].

Although the last two decades have been promising in the neoadjuvant treatment of osteosarcoma, and even that new therapeutic strategies give options and information to prolong survival and maintain a functional member without pain and without metastases, the expectations are still rare. So our goal was to test the therapeutic potential of several bone growth factors such as bone morphogenetic protein type 2 (rBMP2), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) in the osteosarcoma treatment.
2. Material and Methods

2.1 Cell Culture
The osteosarcoma cell line MG-63 from ATCC (USA) was used and cultivated in DMEM-H containing 10% fetal bovine serum (FBS), 0.1 mg/mL streptomycin/penicillin and 1mM sodium pyruvate. Cells were cultured in a humidified incubator at 37° C and 5% CO2.

2.2 Osteosarcoma treatment
After confluence, the MG63 cells were detached with trypsin/EDTA and subsequently replated in 12 wells plates. After 24 hours, cells were treated with different growth factors: FGF (20nM), VEGF (20nM) and rBMP2 (1µ) separately in duplicates. After 48 hours of treatment, the medium were removed, cells were washed with PBS and fixed with 4 % paraformaldehyde for 24 hours. Cells was examined using a light microscope (Nikon Eclipse E-800).

2.3 Von Kossa Staining
MG63 cell were washed in distilled water and stained with 5% silver nitrate solution, in the dark, for 30 minutes. They were washed in distilled water and exposed to a 100W lamp for 60 minutes (Von Kossa staining), and then quickly washed with 5% sodium thiosulfate, counter-stained with Harris Hematoxylin, subject to the diaphanisation process and mounted in SP 15 Permount. Cells were examined using a inverted microscope (NIKON Eclipse TS100, Nikon Instruments Inc., Brazil), coupled to a NIKON image capture system.

2.4 Scanning Electron Microscopy
Cells were grown in 3cm Petri dishes. After confluence, the medium was removed and used for washing with PBS, and then placed in 3% glutaraldehyde. After fixing, the plates were washed in PBS and distilled water, post-fixed in osmium tetroxide (1%) and dehydrated in a progressive ethanol series (70-100%). The material was drying at the critical point apparatus (PCD 020) by using CO2. After drying, the plates received a metal coating with gold by sputtering (EMITECH K550). Finally, the samples were analyzed by scanning electron microscope (LEO 435 VP).

2.5 Flow Cytometry
The cells were washed with PBS and incubated with 1 µg of the antibodies STRO-1, OCT3/4, Ki-67, VEGF, and Caspase-3. Each sample was analyzed by flow cytometry to quantify antibody activity. The analysis was conducted by a FACSCalibur (Becton Dickinson, San Jose, California, USA) and analyzed by the WinMDI 2.9 software. The expression of markers was determined by comparison using an isotype control labeled with FITC fluorochrome non-specific (Alexa Fluor 488).

3. Results

3.1 Osteosarcoma Treatment (inverted microscopy)
The fotodocumentation by inverted microscopy showed cell density decreased in FGF e rBMP2 treatments. In addition, the MG63 cell treatment using rBMP2 showed changes in cell morphology, loss of adhesion to extracellular matrix and cell to cell communication decreased. It showed that the treatment of human osteosarcoma cells with this protein leads to apoptosis.
3.1.2 Von Kossa Staining

The analyses of the osteosarcoma treatment using different bone growth factors by osteogenic potential showed a decreased in osteogenic potential, cell density and calcification areas in all treatments type, being the rBMP2 the best treatment.
3.2 Scanning Electron Microscopy

The treatment analyses by Scanning Electron Microscopy showed apoptosis in the treatment with FGF and rBMP2 such as extracellular matrix degradation (rBMP2).
3.3 Flow Cytometry

The flow cytometry analyzes showed that treatment with FGF MG63 cells induced an increase in the expression of markers Oct3 / 4, Stro-1, Caspase-3 and VEGF and a decreased Ki-67 expression. Treatment with VEGF showed an increased expression of all markers compared to control. RBMP2 treatment induced a decrease in expression of the pluripotency markers (Oct3 / 4), osteogenic potential stem cell tumor (Stro-1), cellular proliferation (Ki-67), angiogenesis (VEGF) and increased the cell apoptosis (Caspase -3).
4. Discussion

Osteosarcoma cells are derived from malignant bone tumors. These osteoblastic cells share some characteristics; however, they have chromosomal abnormalities that lead to abnormal cellular and molecular functions (PAUTKE et al., 2004). Blockage of stem cell differentiation may lead to tumorigenesis [21] and the BMP-2 has been acting as a potent inducer of osteogenic differentiation [22].

In studies related to cancer, tissue cultures have a key role with possible applications in the diagnosis and in the treatment conditioning of several cancer types [23].

We found that treatment of human osteosarcoma cells with rBMP2 was efficient confirmed by testing osteogenic potential and Von Kossa staining, highlighting the differences between the use of rBMP2 and other factors noted by a reduction of calcification area. The rBMP2 use is reported in other studies [11,12] as an important tumorigenesis inhibitor. Recent studies have reported that treatment osteosarcoma using bone marrow stem cells associated with rBMP2 was effective in reducing its osteogenic potential [24], suggesting a high therapeutic potential of the protein.

In our study, the analysis by inverted microscopy and scanning electron microscopy showed that rBMP2 induced tumor cells apoptosis. BMPs are important in cell differentiation, proliferation, morphogenesis, cellular survival and apoptosis [25].

Apoptosis is defined as a cascade of biochemical events that lead to cell death and nuclear fragmentation. The cytotoxic effect of most chemotherapy agents "in vitro" and "in vivo" depend on the induction of apoptosis in susceptible tumor cells [26].

In flow cytometry analysis, the therapeutic activity of the protein was assessed using Caspase-3, Ki-67, Oct3 / 4, VEGF and Stro-1. In a result an increase in the phosphorylated Caspase-3 expression was observed confirming the induction of tumor cells apoptosis, also reported in other studies [24]. A decrease in the expression of cell proliferation (Ki-67), pluripotency (Oct 3/4), angiogenesis (VEGF) and osteogenic potential (Stro-1) markers was also observed.

BMP-2 inhibits embryonic stem cell marker expression; it might prevent tumor formation and growth in vivo [27]. Oct3/4, Nanog and Sox-2 are important embryonic stem cell markers implicated in the tumorigenesis of several cancers. In addition, they are essential transcription factors regulating self-renewal and pluripotency of embryonic stem cells. Recent studies showed that these markers have also been implicated in tumorigenesis [28,29].

Some studies have reported the presence of a small cell subpopulation expressing Stro-1, also known as tumor stem cells that can arise by stem cells transformation [30,31]. The increased expression of VEGF is an important factor involved in solid tumors growth, including osteosarcoma. Therefore, VEGF expression in osteosarcoma is related to a decrease in the survival time and presence of metastases [32,33].

All the treatments showed decrease in the osteogenic potential in MG63 cells. However, we concluded that the best treatment was the rBMP2, which showed a high therapeutic potential for osteosarcoma in vitro, which can represent a new alternative to complement the current clinical treatments.

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References


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