Advancements in Suppression of Osteosarcoma Tumorigenicity: A Prospective Look

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ABSTRACT

Bone morphogenetic proteins (BMPs) promote differentiation of stem cells into bone cells. Results from our pilot studies indicate these proteins are also capable of inducing the differentiation of stem-like cells that initiate and propagate osteosarcoma, a rare, highly malignant primary bone tumor affecting primarily children and adolescents. Our plans to evaluate the use of BMP as adjuvant therapy to suppress bone tumor while facilitating skeletal reconstruction are reviewed.

Keywords: BMP-2; Osteosarcoma; Cancer Stem Cells; Aldehyde Dehydrogenase

1. Introduction

Osteosarcoma (OSA) is an aggressive primary bone cancer primarily affecting children and adolescents, having significant impact on quality of life and survival. Classic OSA is a rare (0.2% of all malignant tumors) and highly malignant tumor, with an estimated incidence of 3 cases/million population/year [1]. While surgical amputation of an affected limb may contribute to improved survival, patients suffer psychosocial and ambulatory consequences that can be particularly troubling to the adolescent population most affected by this disease [2]. Alternatively, regional resection of symptomatic bone tumors may be successfully performed if adequate stabilization of the structural support impaired by tumor resection can be achieved by bony fusion. Unfortunately, these patients also typically undergo radiation therapy, which negatively impacts bony fusion. Even without radiation, bony fusion can be difficult to achieve with standard fusion/fxation techniques.

While the use of recombinant human bone morphogenetic proteins (BMPs) has been approved by the FDA to augment spinal fusion and recalcitrant long-bone nonunions, the use of BMP-2 is contraindicated in surgery for bone/spinal tumors due to concerns that this anabolic growth factor may contribute to tumor cell proliferation. Even though surgeons are eager to explore the feasibility of using BMP to facilitate skeletal reconstruction in bone tumor cases, studies to elucidate or support this application are relatively limited.

The inherent complexity of these tumors, especially in the context of the growth-factor-rich bone microenvironment, indicates the need for additional studies to allay this critical concern before clinical use of BMP for OSA treatment can be implemented. Recent evidence suggests the existence of cancer stem cells that are capable of reconstituting tumors hierarchically, postulating a new paradigm for the origin of cancer and its propagation [3-6]. Our early work also identified such tumor-initiating cells in OSA, which possess the abilities to form spherical colonies in an anti-adhesive environment, the plasticity to differentiate into multiple lineages, significant high-level expression of the 3 master genes Oct3/4A, Nanog, and Sox-2 that regulate stem cell characteristics, and an overwhelming propensity to reconstitute tumor subcutaneously and orthotopically (Figure 1), even with as few as 100 cells (Table 1) [7,8]. All of these characteristics have been referred to as stem cell attributes in cancer cells, and found to be highly correlated with high-level activity of aldehyde dehydrogenase (ALDH), an enzyme recently found to be highly active in stem and progenitor cells due to its link to retinoid metabolism that participates in terminal differentiation as well as self-renewal of certain cell types including various cancer stem cells [8-14].

Intriguingly, the results from our work indicate that BMP-2 is effective for inducing tumor-initiating cells-associated with high levels of ALDH—namely ALDH³ cells—in the primary bone tumor, to express osteogenic phenotypes without stimulating proliferation, thereby drastically restricting tumor formation and expansion.
Cell proliferation was significantly inhibited at 48 hours when ALDH<sup>br</sup> cells were treated with 300 ng/mL BMP-2. Expression of key stem cell marker genes Oct3/4A, Nanog, and Sox-2 were all significantly lowered, and expression of phosphorylated Smad proteins 1, 5, 8 were significantly elevated in BMP-2-treated ALDH<sup>br</sup> cells compared to controls (data not shown). This was followed by upregulated expression of transcriptional factor RUNX2 that is essential for osteoblastic differentiation and skeletal morphogenesis, as well as type I collagen as an early marker of bone turnover, suggesting that BMP-2 and skeletal morphogenesis, as well as type I collagen as an early marker of bone turnover, suggesting that BMP-2 and BALDH<sup>br</sup> progeny may induce osteogenic differentiation of the targeted cells [15]. Subsequently, development of tumors formed by inoculated ALDH<sup>br</sup> cells dramatically ceased when 30 μg BMP-2 was administered at the lesion site in all experimental animals, with few Ki-67-positive cells present. Systemic as well as local skeletal consequences were also evaluated to ensure overall safety and efficacy of BMP-2. When human OSA cells were injected into the tibia medullary cavity via intercondylar eminence to develop human OSA orthotopically in mice as previously reported, either with or without concurrent BMP-2 application. (a) Twenty days after injection, bone tumor formation was noted with radiographic evidence of osteolysis at the site injected with untreated OSA cells. In contrast, no tumor was observed in the contralateral limb where cells were transplanted along with BMP-2; (b) Intact bone structure with no malignant cells was observed in histology sections of limb injected with BMP-2; whereas (c) malignant cells were dispersed over the entire intervened site along with fragmented bone matrix at the contralateral limb site injected with untreated tumor cells. Induced osteogenesis was contributed by both ALDH<sup>br</sup> cells and their ALDH<sup>lo</sup> progenies; (d) The 3-dimensionally rendered micro-CT illustrates that BMP-2 induced ALDH<sup>lo</sup> cells to form well-structured bone nodule with an outer cortical shell containing inner cancellous bone; (e) Hematoxylin and eosin staining further confirmed the morphological appearance of bone matrix deposition and marrow substances in de novo bone tissue; (f, g) Tissue nodules of much smaller size were retrieved from the contra-lateral side where the same amount of ALDH<sup>br</sup> cells were injected with BMP-2 (Figures 2(f) and (g)).

Using BMP-2 to inhibit tumorigenesis of ALDH<sup>br</sup> cells thus appears to give great promise for suppressing or even autogenously eliminating bone tumors. We hypothesize that BMP-2 induction reduces the tumorigenic capacity of OSA while promoting osseous reposition of the intervened skeleton to facilitate surgical reconstruction after tumor resection. In particular, BMP-2 restricts the...
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REFERENCES


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Figure 3. Schematic illustration of postulated hierarchical impact of BMP-2 in the heterogeneous tumor population of OSA.

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