GROWTH FACTOR-ASSOCIATED DIFFERENCES IN MOUSE OSTEOSARCOMA CELL LINES: IMPLICATIONS FOR METASTATIC POTENTIAL

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INTRODUCTION

Osteosarcoma (OS) is the most common primary malignancy of bone. One-third of OS patients succumb to overwhelming pulmonary metastatic disease that is refractory to chemotherapy and surgery. Recent research suggests that bone morphogenetic protein (BMP) expression correlates positively with the incidence of OS pulmonary metastases and negatively with disease-free survival. Other reports indicate that OS patients with high serum vascular endothelial growth factor (VEGF) concentrations are more likely to develop metastases. K7M2 and K12 are related cell lines derived from a spontaneously occurring OS in a Balb-C laboratory mouse. K7M2 displays aggressive metastatic potential in vivo, whereas K12 is much less metastatic.1

The current study was designed to test two hypotheses: (1) OS cell lines with differing metastatic potentials have different BMP and VEGF expression patterns and (2) A BMP antagonist, Noggin, will alter OS cell growth in vitro.

METHODS

K7M2 and K12 cell lines were provided by Lee Helman, MD, and Chand Khanna, DVM, PhD, of the National Cancer Institute. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to compare the expression patterns of mRNA for BMPs 2, 4, and 7 in K7M2 and K12 cells. The enzyme-linked immunosorbent assay (ELISA) was used to compare production of BMP2 and VEGF by K7M2 and K12 cells. Finally, time-lapse video microscopy was used to observe K7M2 and K12 cells in culture for 96 hours with and without Noggin (167 ng/mL). Individual frames obtained at 5-minute intervals were combined to create videos of the cells’ activity in condensed time. After 96 hours of culture, cells were trypsinized, stained with trypan blue, and counted to quantify cell death.

RESULTS

RT-PCR results showed that the highly metastatic K7M2 cells expressed high levels of mRNA for BMP2 and -4 but not BMP7. The less metastatic K12 cells expressed no BMP2 or -7 and less BMP4 than K7M2 (Table 1). The results of ELISA comparing BMP2 production confirmed the RT-PCR results by illustrating that K7M2 cells produced an average of 887 pg of BMP2/10^6 cells/day whereas the K12 cells produced no detectable BMP2 (Table 1). ELISA results for VEGF showed that highly metastatic K7M2 cells produced an average of 478 pg/10^6 cells/day, and less metastatic K12 cells produced 191 pg/10^6 cells/day. This represents a 250% difference in VEGF production (Table 1). Time-lapse video microscopy revealed that the K7M2 control cells (without Noggin) were extremely large and motile. At the end of the 96-hour experiment, the fields of view were ~50% confluent. We observed no evidence of cell death. K7M2 cells treated with Noggin were remarkably less motile and appeared qualitatively smaller than the control cells. We observed a low rate of cell death, and the fields of view were ~50% confluent at the end of the 96-hour experiment. K12 control cells (without Noggin) were much smaller than the K7M2 cells (without Noggin) and exhibited fewer and shorter cytoplasmic projections. They were also less motile than the K7M2 cells without Noggin treatment. After 96 hours, the fields of view were ~95% confluent, and we observed no cell death. Noggin-treated K12 cells showed high rates of cell death throughout the 96-hour study period. At the conclusion of the experiment, approximately one-third of the cells appeared to be dead, and the fields of view were ~40% confluent. The control and Noggin-treated K12 cells exhibited no major differences in motility or morphology. The results of trypan blue staining showed that all the control K7M2 cells survived whereas 11.54% of the Noggin-treated K7M2 cells died during the experiment. Control K12 cells exhibited 3.85% cell death, whereas the death rate for Noggin-treated K12 cells was 28.57% (Figure 1).

DISCUSSION

The frequency with which OS metastasizes to the lung presents the greatest obstacle to disease-free survival by OS patients. Treatments that inhibit metastasis-associated factors in OS could extend the lives of these patients. The differences in metastatic potential between K7M2 and K12 make them powerful tools with which to investigate factors that confer metastatic potential. Here we describe differences in VEGF and BMP expression or production by highly metastatic (K7M2) and less metastatic (K12) OS cell lines. These results support previous research suggesting that BMPs and VEGF may be metastasis-associated factors in OS. We observed changes in the motility, morphology, and viability of OS cells treated with Noggin. This finding supports the feasibility of using natural inhibitors to metastasis-associated factors as a possible means to eradicate OS pulmonary metastases.

Table 1: VEGF and BMP expression profiles in K7M2 and K12. Summary of the RT-PCR and ELISA data. Note that VEGF, BMP2, and BMP4 were expressed or produced in greater amounts in the highly metastatic K7M2 cells than in the less metastatic K12 cells.

<table>
<thead>
<tr>
<th>Factor</th>
<th>K7M2</th>
<th>K12</th>
<th>Test Type</th>
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<tbody>
<tr>
<td>VEGF</td>
<td>++++</td>
<td>+</td>
<td>ELISA</td>
</tr>
<tr>
<td>BMP2</td>
<td>++++</td>
<td>-</td>
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<tr>
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<td>++++</td>
<td>+</td>
<td>PCR</td>
</tr>
<tr>
<td>BMP7</td>
<td>-</td>
<td>-</td>
<td>PCR</td>
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Figure 1: Cell death in treated and nontreated OS cells. Note that Noggin-treated groups displayed more dead cells than control groups.

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REFERENCES