DIFFERENCES IN THE CYTOKINE PROFILES ASSOCIATED WITH PROSTATE CANCER CELL INDUCED OSTEOSCLASTIC AND OSTEOLYTIC LESIONS IN BONE

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Introduction: Prostate adenocarcinoma is associated with the formation of osteoblastic metastases in bone. Although the pathophysiology of the formation of osteoblastic metastases is unknown, it has been hypothesized that cytokines produced by prostate tumors may play a critical role in the development of these lesions. The purpose of this study was to determine if there was a difference in the cytokine profile between prostate cancer cells that induce an osteolytic lesion versus those that form an osteoblastic lesion in bone.

Methods: To test this theory, we isolated PC-3 and LAPC-9 cells into cell suspension from bulk tumors grown subcutaneously in SCID mice. We implanted 1x10^5 cells of each of these tumors respectively into the tibias of SCID mice. These mice were sacrificed at 1, 2, 4, 6, and 8 weeks after implantation and radiographic and histologic analysis was performed on the hindlimbs of these mice. PBS was used as a negative control. PCR analysis was also performed on bulk tumors to screen for selected cytokines.

Results: Radiographs of the PC-3 implanted tibias showed that no lesions formed in the tibias 1 week after PC-3 injection but small osteolytic lesions were present in the tibia 2 weeks after cell implantation. Four weeks after cell implant, there was significant bone destruction and by 8 weeks the proximal tibia in these animals had been destroyed by the osteolytic process. Histologic analysis at week 1 revealed the presence of PC-3 cells within the intramedullary canal. By week 2, the PC-3 cells were firmly established and cortical erosions were evident. At weeks 4, 6, and 8 after cell implantation the proximal tibias were completely eroded with PC-3 cells filling up the space between the remaining proximal and distal ends of the tibias. No areas of new bone formation were noted in any of the PC-3 injected tibias. The PC-3 implanted tibias showed that a few TRAP positive cells were evident at 1 week while TRAP positive cells surrounded the tumor at 2 weeks and were eroding into both cortices of the tibia. However, at 4, 6, and 8 weeks, few TRAP positive cells were evident as the tumor cells completely filled in the space between the proximal and distal ends of the tibia as the bone was destroyed.

The LAPC-9 cells were much slower in expressing their phenotype. There were no osteolytic or osteoblastic lesions noted at 1, 2, or 4 weeks after cell implantation. Evidence of new bone formation was noted by 6 weeks while osteoblastic lesions were clearly visible 8 weeks after cell implantation. Five of six of the LAPC-9 implanted tibias developed osteoblastic lesions by 8 weeks while on one tibia no lesion was noted on radiographs. Histologic analysis showed that the lesions were purely osteoblastic with no areas of osteolysis evident in the tibias that formed a lesion. At 1 week, small pockets of tumor cells could be seen within the intramedullary canal. At 2 weeks, small nodules of bone could be seen surrounding the tumor cells. At 4, 6, and 8 weeks, bone completely filled the intramedullary canal with pockets of tumor cells encased in bone. The LAPC-9 implanted tumors revealed few TRAP positive cells at 1, 2, or 4 weeks. However, more TRAP positive cells were notable at week 6 and week 8 as the new bone that was formed was remodeled.

PCR analysis and immunostaining showed that PC-3 expressed receptor activator for NF-κB ligand (RANKL) and interleukin-1 while LAPC-9 did not. Also, LAPC-9 was shown to express osteoprotegerin and interleukin-6 whereas PC-3 did not. There were also notable differences in the expression of bone morphogenetic protein, transforming growth factor β1, and insulin-like growth factor 1.

Discussion: While the exact mechanism for the formation of osteoblastic lesions in bone remains unknown, these results suggest that cytokines play a critical role in their development. PC-3 cells expressed RANKL which has been shown to induce osteoclastogenesis and is critical for the formation of osteolytic lesions in bone. In contrast, the LAPC-9 cells did not express RANKL, but did express OPG which inhibits osteoclast activation. This inhibition of osteoclast activity may be a critical aspect of the formation of an osteoblastic lesion. The LAPC-9 cells also expressed various BMPs, which are associated with bone formation. However, further study is necessary to define the cytokines that are necessary for the development of osteoblastic lesions in bone.

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