Non-Invasive Molecular Imaging of Heterotopic Ossification Before Mineralization

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Introduction: Heterotopic ossification (HO) is a frequent and problematic complication of total joint arthroplasty. Additional causes of heterotopic ossification include soft-tissue trauma, central nervous system injury, vasculopathies, arthropathies, and inheritance (2). To define the molecular and cellular events involved in heterotopic ossification (HO) we developed a model for HO which involves the delivery of BMP2 through AdBMP2 transduced cells to the muscle of the mouse hindlimb so that we can reproducibly induce rapid heterotopic bone at a targeted location (4). Within 24 hours post-injection, we observed the appearance of multinocular cells between the muscle fibers, that had the unique characteristics of brown adipocytes (3). Endothelial cell (EC) replication was simultaneous with the appearance of brown adipocytes. By days 3-4, we observed a rapid influx of cells expressing vascular smooth muscle alpha actin (SMA). These “mesenchymal-like” cells also express the myeloid marker, CD68 (5). We confirmed the myeloid origin of these cells using a Cre/lox system, in which a myeloid-specific promoter driving Cre recombinase, irreversibly unblocked expression of β-galactosidase only in cells of myeloid origin, and showed specific activity in the newly formed chondrocytes (5). In this study, we describe tracking the recruitment and engraftment of the stem cells into the site of new bone formation, a process that requires the extravasation of stem cells through the endothelium of newly formed vessels to the site of new bone formation. We have noted that integrin expression on stem cells is important in the final tight binding of stem cells to the endothelium during this process by using a dual-labeled RGD peptide for molecular imaging of this process. This permits the identification of the site of the developing HO prior to deposition of osteoid, providing a powerful tool for developing compounds that would block this process.

Materials and Methods: Transduction of cells with adenovirus: MRC5 cells (fetal lung cells, ATCC) were transduced with (2500vp/cell) of Ad5F35BMP2 or Ad5F35control and then injected into the hind limb quadriceps muscle of NOD-SCID mice (n=8/group). Animals were euthanized 6 weeks after injection of the transduced cells and resultant bone formation analyzed by microCT and histology.

Dual Modality Imaging

Using the BMP-2 model of heterotopic bone, we employed anatomical and molecular imaging techniques to assess the progressive changes of early disease. In these initial studies we employed CT for bone imaging, CT with Fenestra for vascular contrast, and PET imaging of 61Cu-DOTA RGD-Cy5.5 for assessing early stem cell recruitment and engraftment. Figure 1 shows the lack of mineralization deposits in the muscle, while Fenestra contrast shows hypertrophied and angiogenic vessels that are predominant in the muscle inoculated with BMP2 producing cells. PET imaging with the RGD agent (right side) shows the blue “blush” internal to the surrounding vessels in the limb injected with AdSBMP2-transduced cells (left panel) while none occurs at the control site (center panel). These results show that angiogenesis occurs prior to bone deposition and may play a role in “niche” preparation enabling the trafficking of progenitor cells.

Discussion: We have developed a rodent model for HO that has already allowed us to better understand the biologic events that result in heterotopic bone formation. The process involves genesis of new blood vessels in the injured area, and the systemic release from the bone marrow of large numbers of circulating myeloid lineage stem cells. Certain cell adhesion surface molecules that are expressed by the new vessels in the injured area, and function to bind to circulating stem cells, and assist in their traversing the vessel wall to infiltrate into the tissues. Targeting of key molecules in this early process of stem cell engraftment prior to cell differentiation, and matrix deposition, could greatly advance current treatment for HO. The data presented here, identifies the expression of these molecular targets on the recruiting stem cells, and demonstrates the specific identification of early HO through the use of a novel non-invasive live animal imaging modality designed to target this process. This technology is likely to emerge as a clinically invaluable tool to identify the process of heterotopic ossification in its early stages and will allow the earlier and more selective application of therapies.


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