Research Program for Progressive Osseous Heteroplasia
at the University of Pennsylvania Research
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In people with POH, heterotopic bone typically begins within the adipose (fat) tissue beneath the skin. At the University of Pennsylvania in Philadelphia, our goal for POH research is to determine the cellular mechanisms though which the extra bone formation (heterotopic ossification; HO) in POH occurs and use this knowledge to identify specific treatment targets for preventing the initiation and progression of HO in POH.

The Penn POH research program has accomplished important milestones including the discovery that inactivating mutations in the *GNAS* gene cause POH. In people who have POH, *GNAS* inactivating mutations cause heterotopic bone that often arises within the subcutaneous fat layer (adipose tissue) of the skin. Our research has determined that mesenchymal stem cells (MSCs) – progenitor cells that reside in many tissues and that have the potential to differentiate into cell lineages such as bone, cartilage, and fat (adipose) cells – more readily become bone cells (osteoblasts) than fat cells (adipocytes) when they carry a *Gnas* mutation, even under conditions that would normally promote differentiation to fat cells. In other words, our work indicates that progenitor cells in the tissues of people with POH have lost their normal control mechanisms for cell differentiation, leading to osteoblast differentiation and bone formation in places where bone does not normally form.

A genetically-engineered mouse with *Gnas* inactivation mimics POH HO bone formation, providing an excellent model system our lab research studies to determine how the HO first begins to form in POH. In recent studies, we have used these POH mice to learn that even before HO begins to form, the adipose tissue where HO eventually forms becomes severely depleted indicating a shift in the tissue identity and that multiple types of cells within the tissue are altered by *Gnas* inactivation.

To examine how the tissue and cells change during HO development over the past year, we first conducted studies to examine adipose tissue sensitivity to the HO-forming effects of *Gnas* inactivation in our POH mouse model. We found that not all depots of adipose tissue have the same sensitivity to the mutation, with the fat tissue below the skin being most responsive. This determination allowed us to refine and optimize our experiment design for our single cell sequencing studies. Single cell RNA sequencing (scRNAseq) is a high-throughput technology that allows us to examine the gene expression information in each individual cell of a tissue through RNA sequencing analyses. While the progenitor cells that will form HO remain a major interest, this approach also lets us examine the other cell populations within the adipose tissue to examine the interactions among these cells and their influence on the progression to HO formation. By examining the gene expression patterns of the populations of cells in adipose tissue over time, we can construct a history of their changes and fates, and thereby identify the cells and cell activities in multiple types of cells that are important for the formation of HO.

We are very excited about these studies and continue to be highly committed to our work on POH; we thank all of the members of the POHA, the Italian POHA, and the Rice Family for your support as we continue to progress toward identifying treatments and cures.

Best regards,
Eileen M. Shore
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