PURPOSE: Acquired hypoparathyroidism is most commonly caused by surgical removal or iatrogenic injury. Permanent hypoparathyroidism occurs in up to 5% of patients undergoing total thyroidectomy or neck dissection, which can result in profound hypocalcemia. If injury is noted intraoperatively the parathyroid gland can be minced and implanted subcutaneously to recover function. Frequently, the manifestations of hypoparathyroidism are only discovered postoperatively when symptoms of calcium and phosphorus dysregulation are noted. Medical management is required to prevent tetany, bone loss, and possibly death. Adipose derived stem cells (ADSCs) are multipotent mesenchymal stem cells which may offer an autologous treatment for this chronic condition. We hypothesize that ADSCs can be converted into functional parathyroid cells in vitro, which could be utilized for delayed subcutaneous implantation and correction of hypoparathyroidism.

METHODS: Adipose tissue was obtained from patients undergoing lipectomy (n = 5). Tissue was digested and the stromal vascular fraction obtained. ADSCs were isolated using magnetic activated cell sorting against CD90 and undertook in vitro directed differentiation over a 26-day period. Differentiated cells were stained for both Calcium Sensing Receptors (CaSR) and Parathyroid Hormone/related protein-Receptors (PTH/PTHrp-R) being verified by microscopy and flow cytometry. The physiologic response of differentiated parathyroid cells to calcium was assessed via Enzyme-Linked Immunosorbent Assay of PTH.

RESULTS: ADSCs were reliably differentiated into parathyroid cells in all patients as verified by both immunofluorescence and flow cytometry against CaSR and PTH/PTHrp-R. Furthermore, differentiated parathyroid cells exhibited a dose and time dependent release of parathyroid hormone following calcium stimulation. PTH secretion was noted at 5 minutes, peaked at 10 minutes, and returned to baseline levels after one hour.

CONCLUSIONS: A century ago, Lahey was the first to perform human parathyroid autotransplantation following a partial thyroidectomy. Failure to acutely recognize an iatrogenic injury makes autotransplantation impossible. We have consistently converted adipose derived stem cells into a parathyroid cell phenotype. Our cells demonstrate appropriate responsiveness to extracellular calcium by the release of parathyroid hormone. They may therefore represent a reliable autologous solution to hypoparathyroidism diagnosed in a delayed setting.

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Characterizing The Contribution Of Circulating Mesenchymal Cells To Pathologic Wound Healing And Heterotopic Ossification

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PURPOSE: Pathologic wound healing after injury represents dysregulation of several cellular components of the physiologic wound niche. Trauma induced heterotopic ossification (tHO) is a highly morbid class of pathologic healing characterized by endochondral formation of de novo osseous lesions in soft tissue. These lesions occur at several anatomic sites and it remains unclear which cell populations form the pre-HO niche. Identification of the specific cells which give rise to tHO is critical to the development of targeted therapeutic options. Similarities between endochondral ossification and tHO suggest a common progenitor, however, it is unclear if this population is entirely local or receives contributions from circulating cells. Here we utilized a parabiotic reporter-based model of tHO to identify and characterize contributions from circulating cells to tHO.

METHODS: A mouse model of parabiosis between wild type mice and reporters carrying the CAG-luc-eGFP L2G85 transgene to examine the presence of circulating (eGFP+) mesenchymal cells at the injury site was generated. These animals carry both luciferase and eGFP reporters allowing for concurrent bioluminescent and histologic analysis of circulating populations. After blood chimerism was confirmed, the wild type parabiotic mouse received a hindlimb Achilles’ tenotomy and dorsal burn. Bioluminescence imaging was used to study the timing and localization of circulating cells. Immunofluorescence was performed to identify common inflammatory, vascular, mesenchymal markers and to characterize their source and contribution to each stage of the tHO anlagen.
RESULTS: Early pre-osseous lesions were highly enriched in eGFP+ circulating cells. These cells primarily co-stained for neutrophil (Ly6G+), macrophage (F4/80+), and trauma-reactive mesenchymal (PDGFRα+) markers consistent with acute inflammation and consequent mesenchymal condensation. Vascular (CD31+) and pericyte (CD105+) staining at the wound site demonstrated minimal co-staining. Bioluminescent signals persisted from one- to eight-weeks post-injury diminishing slightly by twelve weeks. Samples of developing tHO from this period demonstrated presence of eGFP (+) cells in fibroproliferative, cartilaginous, and stromal/marrow populations consistent with joint contribution to early endochondral ossification (SOX9+; OSX+) and to the development of a mature shared marrow space in the definitive osseous lesion.

CONCLUSION: Here we demonstrate the presence of circulating cells at all stages of HO from early-inflammatory populations to the definitive and mature marrow. Importantly, we identify the presence of PDGFRα+ mesenchymal cells in pre-osseous lesions with direct contributions to the endochondral anlagen and (eGFP+/PDGFRα+/SOX9+ or OSX+ cells). These data suggest a contributory role for circulating cells in the pathogenesis of tHO and ultimately identifies a novel therapeutic target, the recruitment of circulating cells, to prevent this disease.

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Subselected Adipocyte Progenitor Niche Cells Show Superior Incorporation and Function in a Mouse Model

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PURPOSE: Previous work from our group has reported that human fat elaborates vascular sprouts when placed in angiogenic media. These vascular sprouts constitute a unique niche for specific cells (Adipocyte Progenitor Niche Cells =APNC) which, when then subjected to adipogenic media, differentiate into adipocytes. This study shows that these subselected adipocytes have superior fat cell function when grafted into a mouse host, as assessed by functional and biochemical parameters that report degree of differentiation and vascularization (levels of human adiponectin in mouse serum and human and mouse PLIN1 and CDH5 mRNA levels in graft).

METHODS: Fat excised from a human panniculectomy specimen, was embedded to obtain vascular sprouts. APNCs were extracted, plated and at confluence exposed to control vehicle (APNC_control) or adipogenic cocktail (insulin, dexamethasone and IBMX) for three days (APNC_differentiated). After 10 days cells were injected subcutaneously into the flanks of NOD mice. The following groups were compared: 1) APNC_differentiated, 2) lipoaspirate from the same specimen, 3) APNC_control and 4) ADSCs prepared from the stromovascular fraction. Grafts were assayed for volume by micro-CT, and functional integration by human serum adiponectin. In addition, immunohistochemistry and RT-PCR for both human and mouse cadherin5 (CDH5, endothelium) and perilipin 1 (PLIN1, adipose) were performed on grafts, and compared to human and mouse adipose tissue samples.

RESULTS: Grafts formed from APNC_differentiated produced higher levels of adiponectin per unit volume compared to grafts formed from liposuctioned tissue. Grafts formed from APNC_differentiated were vascularized by host vessels to the same extent as endogenous mouse fat, and contained the same levels of perilipin as human fat. APNC_control, but not ADSCs were able to differentiate in-vivo to a lesser degree, as assessed by detection of human adiponectin in mouse serum.

CONCLUSIONS: APNCs may represent more elegant way to retrieve the balanced niche of cells needed for adipocyte tissue engineering. In this pilot work, we show that APNCs produce a superior graft in terms of host incorporation and adipocyte function. Although processing time is an issue, if these cells prove to be more effective than other cells extracted from fat tissue, then they could be used to improve fat graft survival and/ or deliver tissue regenerative stimuli in altered wound states. Further work will look to compare APNC_differnetiated and APNC_control to the function of SVF and ADSC isolates (important control groups which are already used to ‘supercharge fat grafts) after they have been differentiated through the same media exchanges as APNC_differentiated.