tissue growth factor (CTGF) and myofibroblast alpha smooth muscle actin (α-SMA) protein production by Western blot as well as immunofluorescence and validated at the mRNA level by Quantitative PCR. Activation of the TGF-β pathway was determined by measuring the TGF-β receptor 1 (ALK5) and phosphorylated Smad2/3 levels by using Western blot and immunofluorescence. Luciferase assay was used to measure nAG protein’s capacity to inhibit the three TGF-β isomers. Cell migration was assessed using a scratch assay and finally colocalization of nAG protein with TGF-β receptor 1 was evaluated using confocal microscopy.

RESULTS: Both the Western Blot and the immunofluorescence results revealed that the application of the nAG protein to human scleroderma fibroblasts in the presence of TGF-β successfully inhibited the fibrotic response shown by a decrease in the fibrotic factors such as collagen III, alpha smooth muscle actin (α-SMA), connective tissue growth factor (CTGF) and fibronectin. In addition, immunofluorescence and western blot after 1 hour of treatment with nAG revealed a significant decrease in phosphorylated Smad2/3, and a decrease in the TGF-β receptor 1 (ALK5) inhibiting the TGF-β pathway. Luciferase assay revealed nAG’s inhibition of the canonical TGF-β pathway to be most specific with TGF-β1 isomer reducing activity by 83%. Cell migration was significantly inhibited with nAG protein treatment and confocal microscopy revealed the colocalization of nAG protein with ALK5 receptors.

CONCLUSION: Fibrosis in scleroderma fibroblasts was effectively inhibited when treated with nAG protein, demonstrated by the decrease in ECM using Western Blot and immunofluorescence. Although much about the mechanism of the nAG protein is still unknown, the decrease in pSmad2/3 and ALK5 receptors most potently with TGF-β1 after treatment, inhibition of cell migration and binding of nAG protein with ALK5 receptors suggest that nAG blocks the canonical TGF-β pathway. This research is anticipated to lead to the development of an injectable antifibrotic agent for the treatment of fibrotic disorders such as scleroderma, hypertrophic scars and keloids.

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Somatic MAP2K1 Mutations in Arteriovenous Malformation Constitutively Activate the RAS/MAPK Pathway

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PURPOSE: Arteriovenous malformation (AVM) is a locally destructive congenital vascular anomaly that enlarges over time. We previously reported that AVMs contain somatic mutations in the MAP2K1 gene, and that the mutation is isolated to the endothelial cell (EC). The purpose of this study was to determine the effects of MAP2K1 mutations in ECs on its signaling pathway.

METHODS: Human AVM-ECs were collected during a clinically-indicated procedure. MAP2K1 mutations (K57N) were confirmed in the ECs using droplet digital PCR (ddPCR). We also engineered wild-type endothelial colony forming cells (ECFCs) with the MAP2K1 (K57N) mutation. Patient-derived MAP2K1-AVM ECs and engineered MAP2K1-ECFCs were compared to control ECs: human white adipose (HWAT-ECs), ECFCs + empty vector, and ECFCs + wild-type MAP2K1 vector. Western blot analysis was used to assess cell signaling along the RAS/MAPK pathway (e.g., baseline and phosphorylated MEK1 and ERK1/2). Densitometry quantification was performed with Image Studio™ Lite (Version 5.2).

RESULTS: Human MAP2K1-AVM ECs had consistent baseline MEK1 and ERK1/2 expression with controls; however, MAP2K1-AVM ECs produced 176% more active/phosphorylated ERK1/2 than non-mutant ECs. Similarly, ECFCs engineered to overexpress mutant MAP2K1 demonstrated 433% more phosphorylated ERK1/2 expression than control ECFCs.

CONCLUSIONS: MAP2K1 mutations in both human-derived AVM ECs as well as in engineered ECs activate the RAS/MAPK signaling pathway by increasing phosphorylation of ERK1/2, which is the downstream target of MAP2K1. Pharmacotherapy that inhibits the activating function of the mutation may inhibit the growth of AVMs.

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Systemic Inhibition of Tgfβ Signaling Attenuates Trauma-induced Heterotopic Ossification
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PURPOSE: Heterotopic ossification (HO) can commonly occur after severe trauma, burn injuries, and is a debilitating consequence of the congenital disease fibrodysplasia ossificans progressive (FOP). The etiology remains poorly understood, however, it is presumed that inflammation plays a critical role with several inflammatory cell types being recruited to the site of HO development. While the role of aberrant BMP signaling is established in HO formation, there is recent indication that macrophage secreted transforming growth factor beta 1 (TGFβ1) is also involved through propagation of chondrogenesis and endochondral ossification. Here we explore the effect of Tgfβ1 inhibition utilizing a near clinical Tgfβ1 receptor ligand trap to attenuate HO formation.

METHODS: Bone marrow derived macrophages were isolated and polarized into M2 phenotype in-vitro. Secreted TGFβ was measured in conditioned medium using ELISA. A model of traumatic heterotopic ossification involving a 30% dorsal burn and Achilles tenotomy was utilized in-vivo and 6-week old male C57BL/6 mice were randomized into receiving treatment with the pre-clinical pharmaceutical grade TGFβR-Fc ligand trap (n=10) or PBS control (n=10). This was administered subcutaneously twice weekly for 3 weeks. At 3 weeks, histology samples were collected, decalciﬁed and stained with Safranin O to assess formation of HO anlagen (n=3/group). Volume of mature formed HO was quantified using micro CT analysis and imaging reconstruction at 9 weeks (n=6/group).

RESULTS: Recruited macrophages are a known source of cytokines that influence the inflammatory microenvironment. We therefore initially assessed the secretion of Tgfβ1 in cultured macrophages. Interestingly, we observed that while M0 macrophages secrete only minimal TGFβ1, the regenerative M2 polarized macrophages, which are known to be recruited to inflammatory sites, had a 500-fold increase in Tgfβ1 secretion. Furthermore, this increase in TGFβ1 levels was completely abrogated when the TGFβR-Fc ligand trap was present in the culture media. We next aimed to assess the effect of systemically administered TGFβR-Fc on HO formation. Following treatment for 3 weeks, we observed a substantially attenuated development of HO anlagen in mice treated with TGFβR-Fc with decreased osseous deposition and marrow space formation on histologic examination. Furthermore, the volume of mature HO was signiﬁcantly decreased in the treatment group.

CONCLUSIONS: Heterotopic ossification, from trauma or congenital FOP, severely limits mobility, function and quality of life of affected patients and currently, no prevention strategies exist. In this study, we demonstrate that Tgfβ1 signaling plays a critical role in HO formation and treatment with TGFβR-Fc is effective in attenuating the early stages of ectopic bone deposition resulting in decreased HO volume. This therapeutic approach reveals a novel avenue for the treatment of this condition that may translate into effective clinical applications.

Minimally Processed Adipose-Derived Stem Cells Increase Union Rates in a Murine Model of Irradiated Mandibular Fracture Repair: Enhancing the Translational Application of Cell Based Therapeutics

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PURPOSE: Cell-based therapeutics represent a critically important component of regenerative medicine and tissue engineering methodologies, as these techniques inherently possess the requisite machinery for tissue growth and differentiation. More speciﬁcally, adipose-derived stem cells (ASCs) have been studied extensively throughout the past several decades due to their multipotent potential,