Sympathetic Adrenergic Signaling Mediates Aberrant Musculoskeletal Repair After TBI

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BACKGROUND

- Traumatic brain injuries (TBI) are common and debilitating for military personnel.
- Following TBI, reported association with extremity bone formation within soft tissue, called neurogenic heterotopic ossification (NHO), and increased fracture healing rates[1,2].
- NHO causes severe pain and dysfunction, as bone often forms at the joints.
- TBI increases peripheral neovasculogenesis in fractures from increased adrenergic signaling[3].
- Knowledge gap: few NHO transcriptomics datasets to date.

LEARNING OBJECTIVES

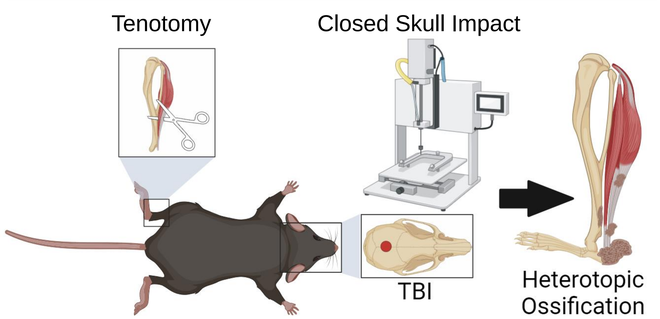
Recognize that TBI increases the risk of pathological musculoskeletal repair, including heterotopic ossification.

Understand how TBI-induced adrenergic signaling influences injury site cellular behavior: pro-angiogenic and osteogenic pathways.

Evaluate the role of sympathetic innervation in aberrant musculoskeletal healing after TBI.

Assess adrenergic signaling as a therapeutic target preventing neurogenic heterotopic ossification.

INJURY MODEL AND METHODS



- TriNetX Cohort Analysis
- Murine model of NHO
- Micro-CT imaging
- scRNA-Seq
- Immunofluorescent Histology
- Adrb1/2 Knockout Mice
- Retrograde trans-synaptic tracing

Citations

1. Sullivan, M. P., Torres, S. J., Mehta, S., & Ahn, J. (2013). Heterotopic ossification after central nervous system trauma: A current review. Bone & joint research, 2(3), 51–57. https://doi.org/10.1302/2046-3758.23.2000152
2. Wang, L., Yao, X., Xiao, L., Tang, X., Ding, H., Zhang, H., & Yuan, J. (2014). The effects of spinal cord injury on bone healing in patients with femoral fractures. The journal of spinal cord medicine, 37(4), 414–419. https://doi.org/10.1179/2045772313Y.0000000155
3. Jahn, D., Knapstein, P. R., Otto, E., Köhli, P., Sevecke, J., Graef, F., Graffmann, C., Fuchs, M., Jiang, S., Rickert, M., Erdmann, C., Appelt, J., Revend, L., Küttner, Q., Witte, J., Rahman, A., Duda, G., Xie, W., Donat, A., Schinke, T., ... Keller, J. (2024). Increased β2-adrenergic signaling promotes fracture healing through callus neovascularization in mice. Science translational medicine, 16(743), eadk9129. https://doi.org/10.1126/scitranslmed.adk9129

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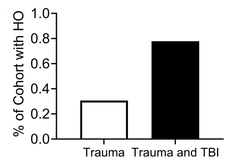
RESULTS

1. TriNetX Query highlights an increased risk of HO in trauma patients with concurrent HO.

A

	Patients in Cohort	Patients with HO	% of Cohort
Trauma and TBI	950,726	7,410	0.779
Trauma	952,260	2,914	0.306

B



C

Risk Difference	95% CI	z	p	Risk Ratio	95% CI	Odds Ratio	95% CI
0.437	(0.453, 0.494)	44.452	<0.0001	2.547	(2.44,2.658)	2.559	(2.452, 2.671)

TriNetX, LLC de-identified clinical database was used to compile two cohorts. Trauma included all patients with trauma to the extremities without head, spinal cord, or nerve injury. Trauma and TBI included all patients with extremity trauma with concurrent TBI. Cohorts were matched by age, male sex, external causes of morbidity, and various injuries that may confer an increased risk of HO (location of injury, concurrent burn wound, pre-existing musculoskeletal disease, etc.). A) Percentage of patients with a diagnosis of NHO following the injury. B) Graphical representation of percentage of cohort with HO. C) Risk difference and ratio analyses.

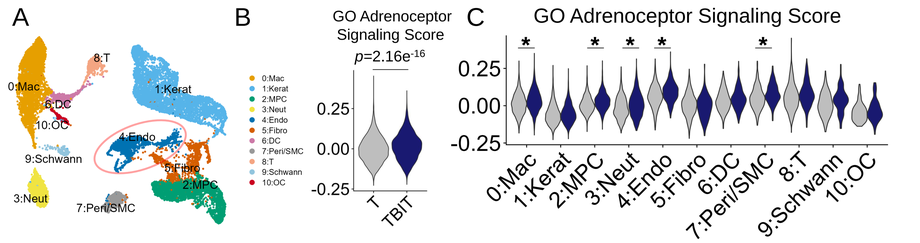
2. scRNA-seq of NHO Model highlights increased osteogenic and angiogenesis gene expression.

A) Micro-CT imaging nine weeks after tenotomy or TBI with concurrent tenotomy resulted in increased HO volumes (p*<0.05). B) Single-cell RNA sequencing UMAP from tenotomy injury site seven days after injury identified differences in cell types in mesenchymal progenitor cells and fibroblasts. C) Marker gene expression for each cluster. D) Monocle 2.0 trajectory analysis of MPC and fibroblast clusters showed three distinct branches (left, top), distribution of cells split by cell type (top, middle), distribution split by injury (top, right), and mesenchymal progenitor cell, chondrogenic (*Sox9*), and osteogenic (*Runx2*) gene expression (bottom). E) Angiogenesis score using Seurat Module Score from list of GO Angiogenesis term with t-test between condition (grey p<0.05 increased in tenotomy, black p<0.05 increased in TBI/T).

3. NHO has increased neovasculogenesis three weeks post-injury compared to tenotomy injury.

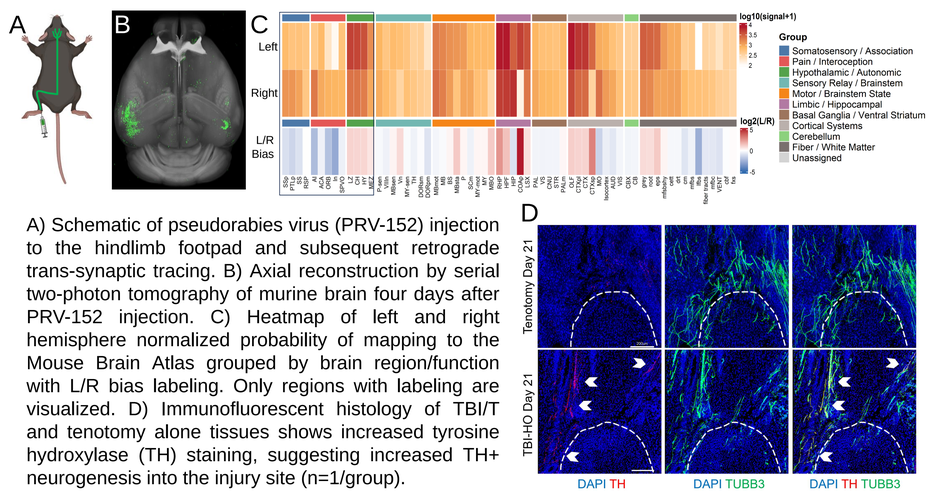
A) Schematic of hindlimb histology section. B) Confocal microscopy tile scan of hindlimb in both conditions (scale bar=500 um). C) Graphs of area of Endomucin stain or TUBB3 stain normalized by quantified area of tissue (p*<0.01, n=4 biological replicates/group).

4. scRNA-seq of NHO tissue and bone marrow highlight adrenergic signaling gene expression. Genetic ablation of adrenergic signaling reduces HO volume.



A) UMAP of tissue injury site highlighting endothelial cell cluster. B) GO Adrenoceptor signaling score comparing T to TBIT and C) for all clusters between conditions (p*<0.05). D) Micro-CT imaging nine weeks after TBI with concurrent tenotomy in wildtype and Adrb1/2 null mice resulted in significant reduction of tendon-associated HO (p***<0.001).

5. PRV retrograde tracing labels somatosensory processing, pain and interoceptive integration, and autonomic regulation neuroanatomy. TBI may alter sympathetic nerve abundance.



A) Schematic of pseudorabies virus (PRV-152) injection to the hindlimb footpad and subsequent retrograde trans-synaptic tracing. B) Axial reconstruction by serial two-photon tomography of murine brain four days after PRV-152 injection. C) Heatmap of left and right hemisphere normalized probability of mapping to the Mouse Brain Atlas grouped by brain region/function with L/R bias labeling. Only regions with labeling are visualized. D) Immunofluorescent histology of TBI/T and tenotomy alone tissues shows increased tyrosine hydroxylase (TH) staining, suggesting increased TH+ neurogenesis into the injury site (n=1/group).

CONCLUSIONS

- Concurrent TBI increases the risk of neurogenic HO in trauma patients.
- TBI with concurrent Achilles' tenotomy increases HO compared to tenotomy alone
- TBI drives MPCs away from fibroblast fate and towards osteogenic/chondrogenic cell fate.
- scRNA-seq analyses highlighted endothelial cell growth signaling.
- TBI+T had more blood vessels after injury than tenotomy, which may impact bone formation.
- Tissue injury site endothelial cells have adrenergic signaling transcriptome signature which may increase neovasculogenic signal.
- Sympathetic nerves innervate tendon regions at baseline and increase in density after injury.
- Genetic ablation of adrenergic signaling attenuates TBI-induced NHO formation and aberrant wound healing, supporting a role for sympathetic signaling in TBI-induced NHO pathogenesis.

NIAMS National Institute of Arthritis and Musculoskeletal and Skin Diseases NATIONAL INSTITUTES OF HEALTH

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