The collagen triple helix repeat-containing 1 (CTHRC1) is transcriptionally modulated in the spinal cords of salamanders following traumatic resection.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

In nature, there are extraordinary creatures that have the ability to regenerate spinal cord tissue following traumatic injuries^{1,2,3,4}. We mined published microarray data^{5,6} to understand in an unbiased fashion how this process transpires, by studying transcription at the systems level in the spinal cord tissue of axolotl salamanders following traumatic cord resection. We identified CTHRC1, encoding the collagen triple helix repeat-containing 1, as among the genes whose expression changes most significantly in the spinal cords of salamanders during the regenerative process. CTHRC1 is likely one of many molecules whose modulation at the level of gene expression, in a complex, multi-step process, is critical for rebuilding the spinal cord.

Keywords: paralysis, spine, spinal cord regeneration, central nervous system regeneration, CTHRC1, collagen triple helix repeat-containing 1.

In humans, the peripheral but not central nervous system has regenerative capacity⁷. Thus, humans cannot regenerate spinal cord tissue following a severing injury. This is the basis by which humans become paralyzed following acute trauma in civilian or military life. To understand the molecular basis of spinal cord regeneration in nature, we studied the transcriptional response to spinal cord trauma in a creature capable of spinal cord regeneration using published microarray data^{5,6} from a severing injury model in the axolotl.

Results

We identified the collagen triple helix repeat-containing 1, encoded by CTHRC1, as among the genes most differentially expressed in spinal cord tissue of the axolotl salamander following a severing injury, studying tissues before injury, and at day 2, day 14, and day 21 post-injury using published microarray data⁵. When sorting each of the genes expressed in the spinal cord at day 2, day 14, and day 21 post-injury based on significance of difference as compared to baseline, CTHRC1 ranked 1 out of 20080 total transcripts (Table 1). Differential expression of CTHRC1 in the spinal cord of the axolotl salamander following severing injury was statistically significant (Table 1; $p=5.60\text{e-}13$).

We studied a second microarray dataset⁶ to validate differential expression of CTHRC1 in the axolotl salamander in response to a severing injury and again observed significant, transcriptome-wide differential expression of CTHRC1 during the spinal cord regenerative response. When sorting each of the genes expressed in the spinal cord at day 1, day 3, and day 7 post-injury based on significance of difference as compared to baseline, CTHRC1 ranked 888 out of 20080 total transcripts (Table 2), equating to 95.6% differential expression. Differential expression of CTHRC1 in the spinal cord of the axolotl salamander following severing injury was again statistically significant (Table 2; $p=1.73\text{e-}11$).

Figure 1: CTHRC1 is transcriptionally induced in the spinal cord of the salamander during spinal cord regeneration.

The mean expression value of CTHRC1 messenger RNA transcript is depicted to the left prior to injury, at 2 days post-injury, at 14 days post-injury and 21 days post-injury.

Discussion

In humans, spinal cord injury resulting in paralysis has traditionally been viewed as a final injury with no chance of recovery. Amphibians including the salamander are capable of regenerating the spinal cord following a traumatic injury, and we used published microarray data to understand how this occurs, hoping that studying how this process occurs in nature can guide humans in their efforts to treat those with spinal cord injuries. We demonstrated here that the collagen triple helix repeat-containing 1 (CTHRC1) is transcriptionally modulated following severing spinal cord injury in a creature capable of spinal cord regeneration, the axolotl salamander, during a period in which the central nervous system regenerates itself. Changes in CTHRC1 expression during regeneration of the salamander spinal cord are likely a major factor in the transcriptional biology of central nervous system regeneration in nature. Future systematic dissection of the most significant transcriptional changes that transpire during spinal cord regeneration in amphibians may perhaps provide insights that inform therapeutic approaches to treating spinal cord injury in humans.

Methods

We used microarray datasets GSE79299 and GSE71934 for this global differential expression analysis of the response to spinal cord injury during spinal cord regeneration in the axolotl salamander. GSE79299 was generated using Affymetrix Ambystoma mexicanum AMBY_002 20k array technology with $n=3$ control samples, $n=3$ at day 2 postinjury, $n=3$ day 14 post-injury, and $n=3$ at day 21 post-injury; analysis was performed using platform GPL15153. GSE71934 was generated using Affymetrix Ambystoma mexicanum AMBY_002 20k array technology with $n=3$ control samples, $n=3$ at day 1 post-injury, $n=3$ at day 3 post-injury, and $n=3$ at day 7 post-injury; analysis was performed using platform GPL15153. The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

References

1. Chernoff, E.A., Sato, K., Corn, A. and Karcavich, R.E., 2002, October. Spinal cord regeneration: intrinsic properties and emerging mechanisms. In *Seminars in cell & developmental biology* (Vol. 13, No. 5, pp. 361-368). Academic Press.
2. Tazaki, A., Tanaka, E.M. and Fei, J.F., 2017. Salamander spinal cord regeneration: the ultimate positive control in vertebrate spinal cord regeneration. *Developmental biology*, 432(1), pp.63-71
3. Sofroniew, M.V., 2018. Dissecting spinal cord regeneration. *Nature*, 557(7705), pp.343-350.
4. Kwon, B.K., Oxland, T.R. and Tetzlaff, W., 2002. Animal models used in spinal cord regeneration research. *Spine*, 27(14), pp.1504-1510.

5. Erickson, J.R., Gearhart, M.D., Honson, D.D., Reid, T.A., Gardner, M.K., Moriarity, B.S. and Echeverri, K., 2016. A novel role for SALL4 during scar-free wound healing in axolotl. *NPJ Regenerative medicine*, 1(1), pp.1-11.
6. Sabin, K., Santos-Ferreira, T., Essig, J., Rudasill, S. and Echeverri, K., 2015. Dynamic membrane depolarization is an early regulator of ependymogial cell response to spinal cord injury in axolotl. *Developmental biology*, 408(1), pp.14-25.
7. Horner, P.J. and Gage, F.H., 2000. Regenerating the damaged central nervous system. *Nature*, 407(6807), pp.963-970.

Table 1: CTHRC1 is differentially expressed in the spinal cord tissue of the axolotl salamander following traumatic injury and during the course of central nervous system regeneration

Rank	probe ID	p-value	F	Gene	Gene name
1	axo00770-f_at	5.60E-13	940.5	CTHRC1	collagen triple helix repeat-containing 1

Table 2: CTHRC1 is differentially expressed in the spinal cord tissue of the axolotl salamander following traumatic injury and during the course of central nervous system regeneration

Rank	probe ID	p-value	F	Gene	Gene name
888	axo00770-f_at	1.73E-11	280.204	CTHRC1	collagen triple helix repeat-containing 1