Matrix metalloproteinase 13 is transcriptionally modulated in the spinal cords of salamanders during spinal cord regeneration.

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In nature, there are extraordinary creatures that have the ability to regenerate the spinal cord following a severing injury; these include the axolotl salamander^{1,2,3,4}. To being to attempt to understand the mechanisms by which this transpires, we studied the transcriptional response to spinal cord trauma in a creature capable of spinal cord regeneration using microarray data^{5,6} from a severing injury model in the axolotl. We observed significant differential expression of the matrix metalloproteinase 13, MMP13, during regeneration of the spinal cord following complete transection. MMP13 is likely one actor in a complex multi-step process by which the central nervous system regenerates itself.

Keywords: paralysis, spine, spinal cord regeneration, central nervous system regeneration, MMP13, matrix metalloproteinase 13.

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

7 **Methods**

8 We used microarray dataset GSE71934⁵ and GSE79299⁶ for this global differential expression
9 analysis of the response to spinal cord injury during spinal cord regeneration in the axolotl salamander.
10 GSE71934 was generated using Affymetrix Ambystoma mexicanum AMBY_002 20k array technology
11 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-
12 injury; analysis was performed using platform GPL15153. GSE79299 was generated using Affymetrix
13 Ambystoma mexicanum AMBY_002 20k array technology with $n=3$ control samples, $n=3$ at day 2 post-
14 injury, $n=3$ day 14 post-injury, and $n=3$ at day 21 post-injury; analysis was performed using platform
15 GPL15153. The Benjamini and Hochberg method of p -value adjustment was used for ranking of
16 differential expression but raw p -values were used to assess statistical significance of global differential
17 expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform
18 annotation was used. A statistical test was performed to evaluate whether MMP13 gene expression was
19 significantly between spinal cord tissue at baseline, at day 1 post-injury, day 3 post injury, day 7 post
20 injury, or at baseline, at day 2, day 4, and day 21 post-injury using a one-way analysis of variance
21 (ANOVA) with Dunnett's multiple comparisons test. We used PRISM for all statistical analyses of
22 differential gene expression during regeneration of the salamander spinal cord (Version 8.4.0)(455).

23 **Results**

24 We used published microarray data^{5,6} to discover in an unbiased fashion and at the systems-level
25 genes associated with the regenerative response of the central nervous system.

26 **MMP13 is among the genes whose expression changes most significantly in the spinal cord 27 following complete transection.**

28 We identified matrix metalloproteinase MMP13 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 1, day 3, and day 7 post-injury using published microarray data⁵. 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, MMP13 ranked 144 out of 20080 total transcripts (Table 1).
Differential expression of MMP13 in the spinal cord of the axolotl salamander following severing injury
was statistically significant (Table 1; $p=2.16E-06$).

We studied a second microarray dataset⁶ to validate differential expression of MMP13 in the
axolotl salamander in response to a severing injury and again observed significant, transcriptome-wide
differential expression of MMP13 during the spinal cord regenerative response. 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, MMP13 ranked 83 out of 20080 total transcripts (Table 2).
Differential expression of MMP13 in the spinal cord of the axolotl salamander following severing injury
was again statistically significant (Table 2; $p=1.34E-15$).

MMP13 expression initially increases, then decreases during spinal cord regeneration following spinal cord transection.

We obtained exact mRNA expression levels for MMP13 in the spinal cord prior to injury, and at day 1, day 3 and day 7 following a severing injury to evaluate magnitude and direction of change in expression following regenerative response. At day 1 following spinal cord injury, MMP13 levels increased as compared to baseline; we calculated a 1.47 ± 0.01 fold change in MMP13 transcript levels at day 1 post-injury. At day 3 and day 7 following a severing cord injury, MMP13 levels decreased to levels below that of baseline, with a fold change of 0.83 ± 0.00 at day 3 and 0.68 ± 0.02 at day 7 post-injury. The change in MMP13 gene expression at each of these time points as compared to baseline was statistically significant: $p < 0.0001$ at day 1, $p < 0.0001$ at day 3, and $p < 0.0001$ at day 7.

We studied exact expression changes in MMP13 using a separate microarray data from which we identified transcriptome-wide differential expression of MMP13 and again observed a similar trend in MMP13 modulation in response to a spinal cord injury and during the regenerative response. At day 2, we calculated a 1.89 ± 0.06 fold change in MMP13 expression as compared to baseline; at day 14, MMP13 levels decreased, with a fold change of 0.92 ± 0.05 at day 14, and a 0.85 ± 0.07 fold change at day 21. The change of MMP13 at day 2 was statistically significant; as MMP13 levels decreased close to baseline at day 14 ($p = 0.8698$) and day 21 ($p = 0.5126$), these changes were not statistically significant.

Thus, by studying published microarray data, we discovered the matrix metalloproteinase 13 as among the genes whose expression changes most significantly transcriptome-wide in the spinal cord tissues of the axolotl salamander.

Discussion

Spinal cord injury resulting in paralysis is a final injury in humans with no chance of recovery. We demonstrated here that a member of the matrix metalloproteinases 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 MMP13 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.

References

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Rank	ID	p-value	F	FC	Gene	Gene name
83	axo08186-f_at	1.34E-15	1344.72257	d1: 1.47 ± 0.01 d3: 0.83 ± 0.00 d7: 0.68 ± 0.02	MMP13	matrix metalloproteinase 13 preproprotein [Homo sapiens]

Table 1: Differential expression of MMP13 in the spinal cord of salamanders during regeneration following complete transection.

The rank of differential expression, probe ID, *p*-value with respect to differential expression, F statistic, fold change in gene expression at each time point as compared to baseline (prior to severing injury), gene, and gene name are listed in this chart.

Rank	ID	p-value	F	FC	Gene	Gene name
144	axo08186-f_at	2.16E-06	47.76353	d2: 1.89 ± 0.06 d14: 0.92 ± 0.05 d21: 0.85 ± 0.07	MMP13	matrix metalloproteinase 13 preproprotein [Homo sapiens]

Table 2: Differential expression of MMP13 in the spinal cord of salamanders during regeneration following complete transection.

The rank of differential expression, probe ID, *p*-value with respect to differential expression, F statistic, fold change in gene expression at each time point as compared to baseline (prior to severing injury), gene, and gene name are listed in this chart.

Figure 1: MMP13 expression increases between days 1 and 2 post-injury, but then decreases and remains decreased from day 3 to day 21 post-injury.

The expression of MMP13 in the spinal cord tissue of the axolotl salamander is represented in $n=3$ animals per time point, at baseline, prior to injury (control), and at day 1, day 3, and day 7 post-injury (above), or at baseline, prior to injury (control), and at day 2, day 14, and day 21 post-injury (below). The graph above represents data from GSE71934 while the graph below represents data from GSE72977. The result of a statistical test evaluating significance of difference in MMP13 expression as compared to baseline is marked above each time point as an exact p -value.