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Role of toll-like receptor signaling pathway in a rat model of spinal cord injury: a transcriptomic analysis

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Abstract. Spinal cord injury (SCI) is a debilitating condition characterized by primary and secondary damage to the spinal cord tissue encompassing the site of injury. In this study, we analyzed transcriptomic data from Rattus norvegicus with SCI induced by aneurysm clip impact-compression at seventh thoracic vertebra to explore the main alterations in pathways expression. RNA was extracted from the specific SCI region after 2 weeks and hybridized on Affymetrix GeneChip arrays. Differential gene expression analysis identified 5213 DEGs among which 25 showing a fold change < -2 or > 2. Over-representation analysis was performed using the 25 DEGs above mentioned and revealed the toll-like receptor (TLR) signaling pathway signaling pathway (rno04620) as the only significantly enriched pathway (q-value < 0.05). Further examination of the regulation of this pathway unveiled upregulation of MyD88, *Tlr2*, and *Tlr4*, activating NF-κB and MAPK pathways, leading to proinflammatory cytokine synthesis and cellular apoptosis. Upregulation of *Cd80* and *Cd86* indicated T-cell activation, while IFN-β downstream regulation showed increased expression of JAK-STAT signaling pathway genes. This transcriptomic perspective highlights the dysregulation of the TLR signaling pathway in SCI at 2 weeks and emphasizes its potential role in the pathology.

Keywords: spinal cord injury, toll-like receptor signaling pathway, transcriptomic analysis, microarray.

INTRODUCTION

Spinal cord injury (SCI) is a traumatic event that can cause primary and secondary damage to the spinal cord tissue surrounding the lesion site (Ahuja et al., 2017). The primary injury is caused by the initial impact, which can lead to the rupture of nerve fibres. This damage is often irreversible and results in the loss of both motor and sensory capabilities below the site of injury (Eckert & Martin, 2017; Ortega et al., 2023). The secondary injury occurs within a short period after the initial impact causing progressive damage to the surrounding spinal cord tissue at the site of injury (Alizadeh et al., 2019). Approximately 500.000 individuals experience a SCI annually, resulting in a higher

risk of premature mortality ("Spinal Cord Injury (SCI) Facts and Figures at a Glance," 2016). An important overview in the SCI was obtained using animal models to replicate this pathological condition (Cheriyan et al., 2014) (Sharif-Alhoseini et al., 2017). In this publication we chosen to explore the pathways that can play a role in the ongoing of the SCI through 2 group of samples of rats. We compared the genes expression of sham group and SCI group after 2 weeks from the damage to highlights gene that can act an important role in the disease.

MATERIALS AND METHODS

Dataset selection

All the data analyzed in this study were obtained from the Gene Expression Omnibus repository (Barrett et al., 2013). The dataset with the accession number GSE45006 was downloaded. This dataset contains information regarding samples of Rattus norvegicus belonging to either the sham group and group related to 2 weeks after SCI induced by aneurysm clip impact-compression at seventh thoracic vertebra. The specific region of the SCI was isolated to extract RNA. Subsequently, the extracted RNA underwent processing and hybridization on Affymetrix GeneChip arrays.

Bioinformatic analysis

The analysis conducted to explore the differentially expressed genes (DEGs) was performed using R (R Core Team) with the limma package (Ritchie et al., 2015) from Bioconductor (Gentleman et al., 2004). A background correction, followed by quantile normalization, was applied to the transcriptomic data. The normalized data were then subjected to a principal components analysis (PCA). Transcriptomic profiles of our groups were then compared to highlights the DEGs with associated the information about fold change and q-value. DEGs were filtered for fold change and those with fold change < -2 or > 2 was used for the over-representation analysis (ORA) of the pathways, performed through the package clusterProfiler (Wu et al., 2021). List and information about pathways used for the analysis were retrieved using the KEGG database (Kanehisa & Goto, 2000).

RESULT

The analysis started considering the 31099 transcripts included in the array. The matrix used for the study was composed by the transcripts on the rows and the 8 samples (4 related to sham group and 4 SCI) on the columns. All the data mentioned were used to perform the different steps of background correction and normalization and then, from this data, was possible to carry out the PCA analysis. The results of PCA analysis are showed in Figure 1.

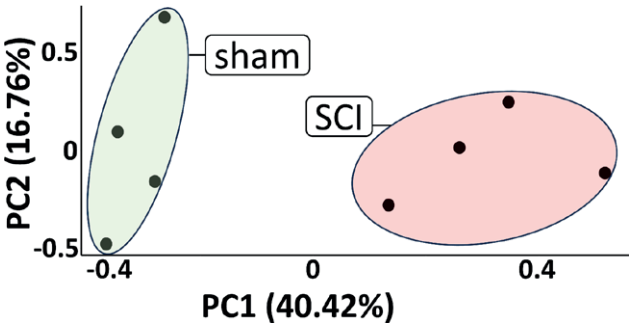

Figure 1. PCA of sham and SCI groups. In the figure, on the axes, are reported the principal component 1 and 2 that respectively describe the 40.42% and 16.76% of the variance. In green are highlighted the area that includes the sham samples and in red the area that includes the SCI samples.

Figure 1 suggests an important difference at transcriptomic level among the samples of each group. To explore this difference and observe which genes results as DEGs we carried out the comparison among sham and SCI. We considered DEGs all those genes that had a q-value, obtained through the Benjamini-Hochberg post-hoc correction of the p-value, <0.05. This investigation results in 5213 DEGs. The 5213 DEGs were further inspected to identify those that exhibited a better fold change in the comparison and, to accomplish this, DEGs

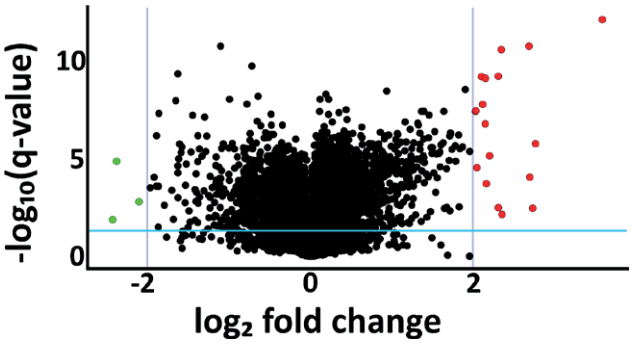

Figure 2. Dot plot distribution of all the transcript after comparison among sham and SCI. The dots represent the transcripts and, upper the horizontal light blue line, are differentially expressed (q-value < 0.05). Vertical blue lines indicate the fold change values of -2 and 2. In red are reported the DEGs upregulated in SCI conditions with a fold change > 2 and in green are reported the DEGs downregulated in SCI conditions with a fold change < -2.

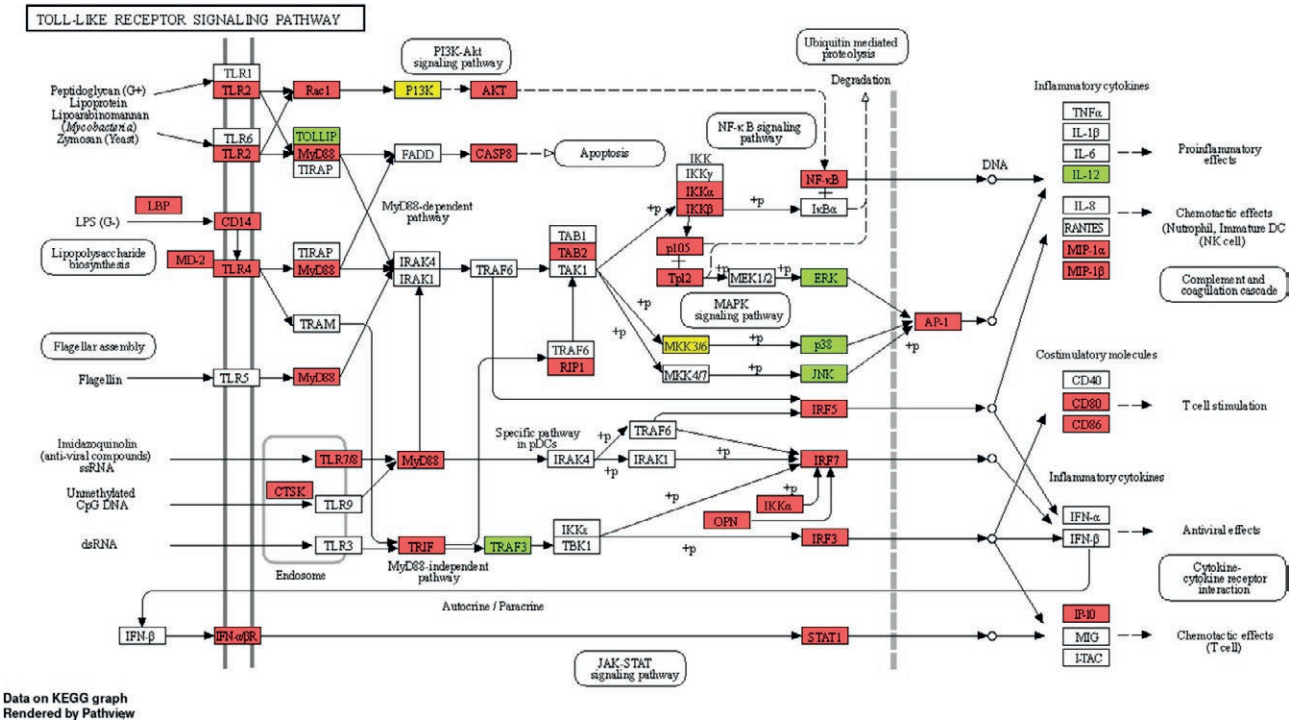

Figure 3. Toll-like receptor signaling pathway. In the pathway are highlighted in red the transcripts obtained from upregulated DEGs in SCI condition and in green the downregulated in SCI condition.

with fold change >2 or <-2 were filtered out. Data related to fold change and q-value of the transcripts are reported in Figure 2.

Figure 2 shows that 25 DEGs have a fold change > 2 o < -2. These DEGs with extreme fold change were used to perform the ORA. The aim was to discover some pathway that can result as over-represented to give us information about some possible biological focus altered in SCI condition. Results of ORA indicate that the only one pathway resulted enriched is "rno04620" related to toll-like receptor (TLR) signaling pathway with a q-value of 0.02. In Figure 3 are reported the pathway with the up and down regulated DEGs resulted involved. All the DEGs reported in the Figure 3 are not filtered for fold change to better understand every change in SCI condition.

As reported in Figure 3, it is possible to see that a significant portion of the pathway is altered. The pathway involves a total of 94 genes out of which 44 resulted DEGs. Among these DEGs, 37 genes are upregulated in the SCI condition, while 10 genes are upregulated.

DISCUSSION

The presence of just 1 pathway from ORA can indicates an important involvement of TLR signaling pathway in this stage of SCI. Is reported that TLR signaling pathways can be divided into two distinct categories: a MyD88-dependent and a MyD88-independent pathway. MyD88-dependent pathway triggers the rapid activation of NF-κB and MAPK, leading to the synthesis of proinflammatory cytokines. MyD88-independent pathway is linked to the initiation of IFN- and IFN-inducible gene expression (Kawai & Akira, 2007; Uematsu & Akira, 2007). As showed in the results, MyD88 is upregulated and this upregulation appears to be a consequence of upstream upregulation of *Tlr2* and *Trl4*. Among the downstream effects, there is an upregulation of *Casp8*, which leads to cellular apoptosis, as well as an upregulation of NF-kB. Following our results, we can also observe that, downstream of the pathway, there is an increase in the expression of *Cd80* and *Cd86* the products of which are known for their activation of T cells (Halliday et al., 2020). In addition to what has been reported so far, the regulation downstream of IFN-β shows an increase in regulation of genes involved in the JAK-STAT signaling pathways. Several genes in this pathway have already been associated with SCI (Heiman et al., 2014; Kigerl & Popovich, 2009; Li et al., 2021) but this study highlights the overall dysregulation of the pathway from a transcriptomic perspective. Significance of our discussion is reinforced by the fact that TLR sign-

aling pathway it is the only pathway found to be over-represented. Future investigations are required to ascertain whether the observed gene expression pattern in this analysis is maintained at various temporal stages or how it may potentially evolve.

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