Dynamic and cell-type specific Expression of Nrf2 after Traumatic Brain Injury in mice

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Abstract: Objective Oxidative stress has been demonstrated to play a major role in the pathogenesis after traumatic brain injury (TBI). Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important regulator against TBI-induced antioxidative stress. Accumulated studies provide evidence that Nrf2 plays neuroprotective roles through regulating neuron apoptosis and inflammatory response induced by TBI, but the dynamic and cell-type specific expression of Nrf2 is uncertain. Methods In this study, we investigated the cell-type location of Nrf2 accumulation at different time points after TBI by double immunofluorescence staining. Paraffin embedded sections were performed by double-labeling fluorescence with Nrf2 specific antibody and cell type specific antibodies, including NeuN (neurons), IBA1 (microglia), GFAP (astrocytes) and NG2 (oliodendrocyte/pericyte), then the subcellular distribution of Nrf2 was observed, and its cell type-specific location was evaluated at 6h, 12h, 1d, 3d, 7d, 14d and 21d after TBI in adult male C57/BL6 mice. Results In the uninjured mouse, no evident Nrf2 staining was observed in the cortex. Following TBI, significant accumulation of Nrf2 protein in the perilesional cortex. The ratio of Nrf2-positive cells peaked at 1d in the perilesional cortex, then gradually decreased. Analysis on Nrf2 expression profiles showed that cell-type specific expression of Nrf2 was time-dependent in different cells. Nrf2 immunosignaling is overlapped predominantly with NeuN positive cells, but this accumulation was transient, fewer neurons (NeuN(+)) were Nrf2 positive at 3d post injury. Conversely, significant and continuous expression of Nrf2 was detected in microglia, particularly at 3d and 7d post injury, the ratio of Nrf2-positive cells in IBA1 (+) was peaked at 7d. In addition, Nrf2 expressed slightly and stably in astrocytes (GFAP(+)) besides 7d post injury at which most gfap+ cells were positive stained by Nrf2. We also observed that several Nrf2
positive parenchymal cells in the perilesional cortex were positive staining with NG2.

**Conclusion** Our data revealed that Nrf2 expression varies in different cell types in the ipsilateral cortex after TBI. The cell specific location of Nrf2 provides evidence that Nrf2 is involved in the second brain injury after TBI in mice, which also suggests the Nrf2 may regulate distinguished roles in different cells.

**Keywords:** Nuclear factor erythroid 2-related factor 2 (Nrf2); traumatic brain injury