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Research Report

Computational identification of conserved transcription factor binding sites upstream of genes induced in rat brain by transient focal ischemic stroke

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ABSTRACT

Microarray analysis has been used to understand how gene regulation plays a critical role in neuronal injury, survival and repair following ischemic stroke. To identify the transcriptional regulatory elements responsible for ischemia-induced gene expression, we examined gene expression profiles of rat brains following focal ischemia and performed computational analysis of consensus transcription factor binding sites (TFBS) in the genes of the dataset. In this study, rats were sacrificed 24 h after middle cerebral artery occlusion (MCAO) stroke and gene transcription in brain tissues following ischemia/reperfusion was examined using Affymetrix GeneChip technology. The CONserved transcription FACtor binding site (CONFAC) software package was used to identify over-represented TFBS in the upstream promoter regions of ischemia-induced genes compared to control datasets. CONFAC identified 12 TFBS that were statistically over-represented from our dataset of ischemia-induced genes, including three members of the Ets-1 family of transcription factors (TFs). Microarray results showed that mRNA for Ets-1 was increased following tMCAO but not pMCAO. Immunohistochemical analysis of Ets-1 protein in rat brains following MCAO showed that Ets-1 was highly expressed in neurons in the brain of sham control animals. Ets-1 protein expression was virtually abolished in injured neurons of the ischemic brain but was unchanged in peri-infarct brain areas. These data indicate that TFs, including Ets-1, may influence neuronal injury following ischemia. These findings could provide important insights into the mechanisms that lead to brain injury and could provide avenues for the development of novel therapies.

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1. Introduction

Ischemic stroke occurs when the blood supply to the brain is obstructed. The neuronal death that ensues results from the induction of genes associated with a number of cellular functions including apoptosis, inflammation and oxidative stress. Currently tissue plasminogen activator (t-PA) is the only approved treatment for ischemic stroke. Unfortunately, t-PA has a limited time window for therapeutic use, and only 3–5% of stroke patients arriving at the hospital will qualify for treatment (Fisher et al., 2009). Thus, there is a strong need to understand the molecular mechanisms associated with ischemic stroke so that more effective modes of treatment can be investigated.

It is well established that the transcription of new genes plays a major role in the delayed neuronal injury that occurs in the ischemic penumbra following stroke. A number of laboratories, including ours, have used high throughput microarray analysis to understand how the genome transcriptionally responds to ischemic challenge (Lu et al., 2003; Kury et al., 2004; Xu et al., 2005; Ford et al., 2006; Sarabi et al., 2008;

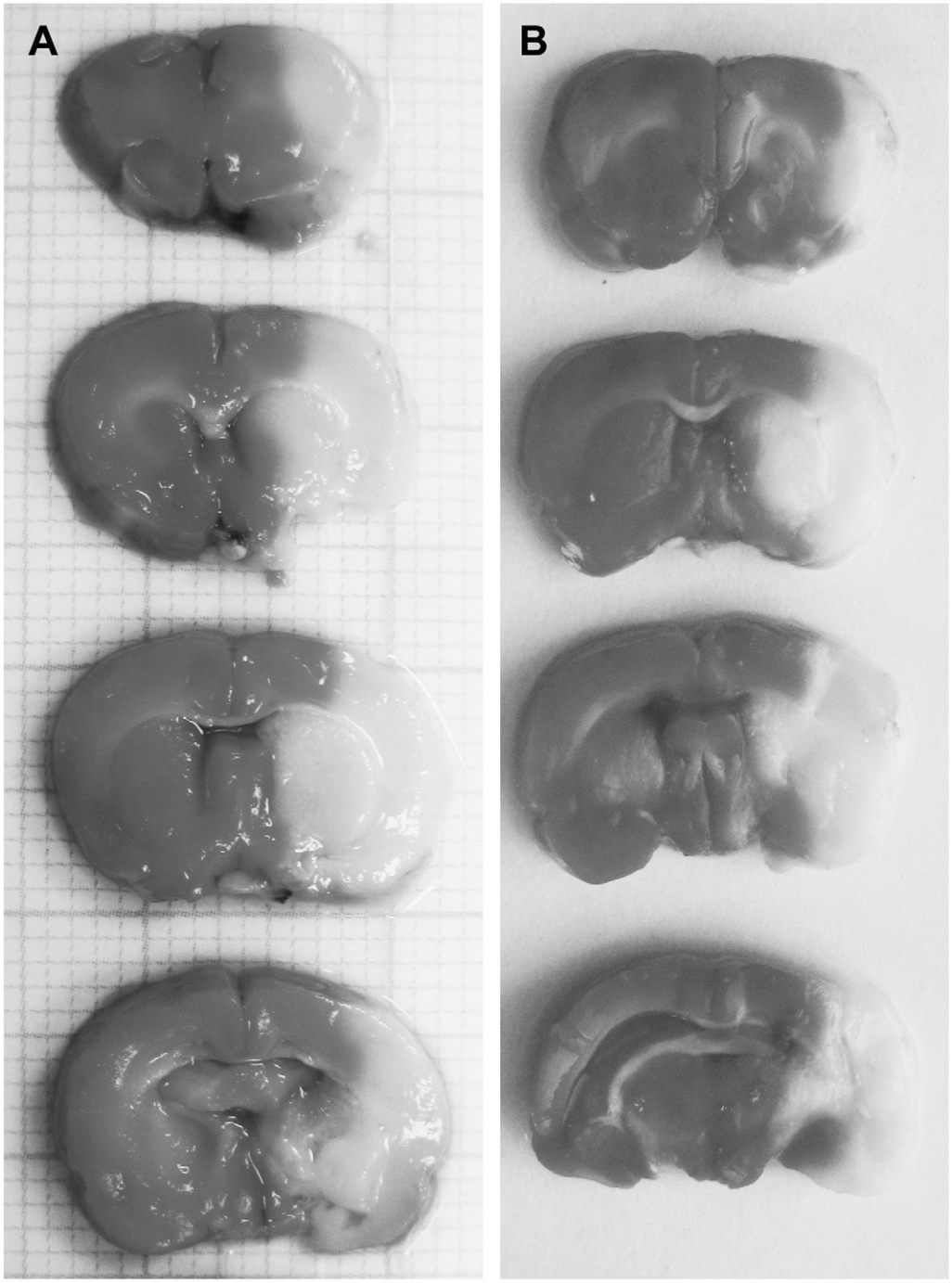

Fig. 1 – Brain injury associated with tMCAO model. Representative triphenyl tetrazolium chloride (TTC) staining of normal (dark areas) and ischemia-injured brain regions (white, indicate infarct) following pMCAO (A) and tMCAO (B).