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# Effect of focal ischemia on long noncoding RNAs

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## Abstract

**Background and Purpose—**Long noncoding RNAs (lncRNAs) play a significant role in cellular physiology. We evaluated the effect of focal ischemia on the expression of 8,314 lncRNAs in rat cerebral cortex using microarrays.

**Methods—**Ischemia was induced by transient middle cerebral artery occlusion. Genomic and transcriptomic correlates of the stroke-responsive lncRNAs and the transcription factor binding sties in their promoters were evaluated with bioinformatics.

**Results—**359 lncRNAs were upregulated (>2-fold) and 84 were downregulated (<0.5 fold) at 3h to 12h of reperfusion following MCAO compared to sham. 62 stroke-responsive lncRNAs showed >90% sequence homology with exons of protein-coding genes. Promoters of stroke-responsive lncRNA genes and their homologous protein-coding genes showed highly overlapping transcription factor binding sites. Despite presence of ORFs, lncRNAs did not form any product when subjected to *in vitro* translation.

**Conclusions—**Stroke significantly alters cerebral lncRNA expression profiles.

### Keywords

Stroke; Noncoding RNA; Transcription Factor; exon mimicry

Greater than 90% of the mammalian genome transcribes into noncoding (nc) RNAs that play critical roles in cellular homeostasis.[1] We recently showed that stroke alters cerebral microRNA (miRNA) and piRNA profiles in rats.[2, 3] In addition to these small ncRNAs, the genome transcribes many long ncRNAs (lncRNAs; >200 nucleotides in length) that control protein targeting to genomic loci, epigenetic silencing and serve as scaffolds for multiple proteins (reviewed by Rinn *et al, 2012)*.[4] lncRNAs are thought to play a role in the pathophysiology of Alzheimer's disease and brain cancer.[5, 6] We currently evaluated the effect of focal ischemia on cortical lncRNA expression profiles.

## Methods

See online Supplement file for detailed methods.

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**Disclosures:** None

**Conflict of Interest:** None

Transient MCAO (1h) was induced in adult, male spontaneously hypertensive rats with intraluminal suture under isoflurane anesthesia. RNA extracted from the ipsilateral cortex was analyzed using Arraystar lncRNA expression microarrays. Data was analyzed with GeneSpring software. The annotated, full-length lncRNA sequences (RefSeq database) were queried against rat Baylor 3.4/rn4 genome assembly to identify homology to exons of annotated protein-coding genes. Promoters of stroke-responsive lncRNAs and the homologous protein-coding genes were analyzed using Pattern Search & Analysis tool (Genomatix GmbH).[7] DNA sequences of 3 lncRNAs were cloned and subjected to in vitro transcription and translation.

## Results

The distribution of lncRNA expression in the rat cerebral cortex ranged from 7 to 670,000 units (Supplementary Table 1). Following transient MCAO, 443 of the 8,314 lncRNAs analyzed changed significantly (359 upregulated by ≥2-fold and 84 downregluated by ≤0.5 fold) at all reperfusion time points evaluated (3h, 6h and 12h) compared to sham (Table 1 and Supplementary Table 2).

61 stroke-responsive lncRNAs showed >90% sequence similarity to the exons of protein-coding genes (exon mimicry), a majority of which are involved in transcription and translation (Table 2; Supplementary Tables 3 and 4). In all cases, the genomic loci of the paired stroke-responsive lncRNA and their homologous protein-coding genes were on different chromosomes. 10 TF families showed >100 hits each in the promoters of the 48 out of 61 stroke-responsive lncRNAs (Table 3 and Supplementary Table 5). These TF families also showed similar binding-site probabilities in the homologous protein-coding gene promoters. The ETSF TF family showed the highest overrepresentation in 37/48 lncRNA-gene mimic pairs (Table 3; Supplementary Table 6). Full names of the protein-coding genes that lncRNAs mimicked are given in Supplementary Table 7.

One or more ORFs in 60 stroke-responsive lncRNAs showed a partial, but strong conservation with their protein-coding functional homologs (Supplementary Table 8). They showed a substantially higher number of mutations than the homologous protein-coding genes, but regions that correspond to exon junctions remained non-mutated indicating a possibility that they might undergo splicing and translation to truncated proteins. However, none of the 3 stroke-responsive lncRNAs tested (XR_005672 with 4 ORFs homologous to Eno1, XR_007365 with 3 ORFs homologous to Eef1a1 and XR_005605 with 2 ORFs homologous to RpL3) formed any proteins when tested by in vitro transcription/translation.

## Discussion

The role of ncRNAs in ischemic brain damage is currently unknown. Recent studies showed that cerebral profiles of miRNAs and piRNAs change extensively and modulate brain damage after stroke. The present study shows that ischemia also influences lncRNAs and bioinformatics showed a >90% sequence homology between many stroke-responsive lncRNA genes and protein-coding genes located on different chromosomes indicating that these lncRNAs might be pseudogenes. Previous studies showed that pseudogenes produce truncated proteins which suppress the activity of the homologous proteins. This was shown for endothelial nitric oxide synthase in multiple species.[8, 9] Despite the presence of multiple ORFs with high degree of homology with protein-coding genes, stroke-responsive lncRNAs tested experimentally failed to form proteins. This could be explained by the observations that the lncRNA genes show multiple, small ORFs in contrast to the single, continuous ORFs of the protein-coding genes, and the start and stop codons of the lncRNA ORFs did not match those of the protein-coding gene ORFs indicating codon incompatibility. The

function of the lncRNAs in ischemic pathophysiology is still elusive, but they might stabilize the mRNAs. In support, a previous study showed that RNAs of pseudogenes that transcribe at a high rate influence the translation of the mRNAs of the homologous protein-coding genes.[10] Knocking-out a specific pseudogene homologous to Makorin-1 gene led to the destabilization of the Makorin-1 transcription leading to bone deformities that were reversed when the pseudogene was reintroduced.[10] As many of the stroke-responsive lncRNAs are homologous to protein-coding genes involved in ribosomal complex formation, splicing, translation initiation and nuclear import of mRNAs, they might stabilize those mRNAs to restore the protein synthesis inhibited during the acute phase after stroke. The stroke-responsive lncRNAs might also control chromatin modifications, transcription factor activity and apoptosis as demonstrated previously.[11–13] Further studies are needed to show the significance of lncRNAs to post-stroke functional outcome.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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**Table 1**

lncRNAs altered maximally after stroke

| lncRNA | 3h | 6h | 12h | Chr | lncRNA | 3h | 6h | 12h | Chr |
|---|---|---|---|---|---|---|---|---|---|
| MRAK077719 | 47.8 | 36.2 | 20.9 | 2 | BC086373 | 0.1 | 0.1 | 0.1 | X |
| XR_009313 | 25.2 | 53.7 | 45.7 | 1 | MRAK049859 | 0.2 | 0.2 | 0.2 | 3 |
| XR_007365 | 23.2 | 93.2 | 101.8 | 2 | BC103647 | 0.3 | 0.3 | 0.2 | 11 |
| MRAK159688 | 20.2 | 13.0 | 28.9 | 6 | MRAK078348 | 0.3 | 0.4 | 0.3 | 7 |
| MRAK079854 | 20.1 | 21.5 | 13.0 | 8 | uc.236+ | 0.3 | 0.3 | 0.5 | 16 |
| MRAK049735 | 12.7 | 18.4 | 22.1 | 4 | MRAK040488 | 0.3 | 0.3 | 0.5 | 15 |
| MRAK078894 | 12.6 | 14.1 | 10.5 | 6 | MRAK047500 | 0.3 | 0.3 | 0.4 | 2 |
| XR_006073 | 12.5 | 33.0 | 42.2 | 6 | uc.308- | 0.3 | 0.5 | 0.5 | 1 |
| XR_008501 | 12.1 | 32.4 | 35.8 | 3 | MRAK033792 | 0.3 | 0.5 | 0.5 | 4 |
| U77626 | 11.1 | 34.6 | 28.1 | 15 | uc.228+ | 0.3 | 0.4 | 0.6 | 4 |
| XR_009083 | 10.8 | 22.1 | 21.9 | 14 | MRAK051099 | 0.3 | 0.5 | 0.3 | 10 |
| XR_007044 | 10.6 | 27.9 | 30.9 | 17 | MRAK053030 | 0.3 | 0.4 | 0.4 | 8 |
| XR_006491 | 9.9 | 28.8 | 29.0 | 3 | uc.408+ | 0.3 | 0.3 | 0.4 | 19 |
| XR_009527 | 9.9 | 28.5 | 26.3 | 2 | BC158779 | 0.3 | 0.4 | 0.3 | 10 |
| XR_006222 | 8.8 | 22.2 | 32.7 | X | S39217 | 0.4 | 0.3 | 0.3 | 3 |

Values are mean fold changes in the ischemic groups over sham group (≥2-fold, n=3/group; $p<0.05$ by ANOVA). 3h, 6h and 12h are reperfusion time points after transient MCAO. lncRNA IDs are listed per Refseq annotations. Chr is chromosome from which lncRNA is transcribed.

## Table 2

Representative stroke-induced lncRNAs that showed homology to protein-coding genes

| LNC ID | Mimicked Gene | Chr# of lncRNA gene | Chr# of mRNA gene | Sequence Similarity (%) |
|---|---|---|---|---|
| MRAK034983 | Sav1 | 4 | 6 | 96.6 |
| XR_007384 | RpL21 | 5 | 12 | 96.3 |
| XR_005722 | Actg1 | 1 | 10 | 96.1 |
| XR_009394 | RpL19 | 1 | 10 | 96.1 |
| XR_007963 | RpL31 | 7 | 9 | 96.0 |
| XR_009083 | RpL21 | 14 | 12 | 95.9 |
| XR_008876 | RpL27a | 1 | 1 | 95.9 |
| XR_005592 | Ppia | 16 | 14 | 95.8 |
| XR_009459 | Ppid | 10 | 2 | 95.8 |

Exon mimicry was determined with BLAT program of the UCSC genome browser.

**Table 3**

Top TF families that showed binding sites in the lncRNA and protein-coding gene promoters

| Family | # of lncRNA promoters bound | # of sites in lncRNA promoters | # of protein-coding gene promoters overrepresented |
|---|---|---|---|
| ETSF | 48 | 258 | 36 |
| HOMF | 47 | 357 | 37 |
| SORY | 47 | 272 | 27 |
| HOXF | 45 | 263 | 25 |
| CREB | 46 | 156 | 22 |
| FKHD | 45 | 267 | 22 |

Total lncRNA promoter representation is the combined number of binding sites for each TF family on all 48 promoters.