

# lncRNAs – their potential in regulation of hypertension and behavior of ISIAH rats



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## **SUMMARY**

### **Long non-coding RNAs (lncRNAs)**

- central role in the epigenetic control of gene expression
- the control of many biological processes in the body, including the development of cardiovascular diseases and hypertension.
- the understanding of biological functions and interactions is still far from being complete.

We identified the lncRNAs differentially expressed in the hypothalami of hypertensive ISIAH and normotensive WAG rats, and revealed lncRNA-associated differentially expressed genes (DEGs) related to hypertension and behavioral characteristics of ISIAH rats in the open field tests (grooming, vertical activity, hyperactivity, abnormal emotion/affect behavior (including abnormal response to novelty)).

Transcriptome sequencing (RNA-Seq method) => the expression of three lncRNAs (Bc1, RGD1562890, and Snhg4) differs in the hypothalami of hypertensive ISIAH and normotensive WAG rats.

Snhg4: the largest number of co-regulated genes, both associated with hypertension and behavior

These findings may be useful for further understanding the role of lncRNAs in regulating the protein coding genes and modulating processes associated with both hypertension and behavior.

# Experimental scheme

The ISIAH/Icgn and WAG/GSto-Icgn rats (3 months old) were kept under standard conditions

Hypothalami from 3 rats in each group were collected and stored in RNA Later (Qiagen, Chatsworth, CA) at  $-70^{\circ}$  C

RNA-Seq (transcriptome sequencing) was performed (Genoanalytica, Moscow, Russia)

The data obtained were mapped to the RGSC Rnor\_5.0 \ rn5 reference genome.

Cufflinks / Cuffdiff programs were used to reveal the differentially expressed transcripts (p value cutoff = 0.01)

The RNA-Seq data were deposited in the NCBI Short Read Archive database with Accession number PRJNA299102.

The lncRNAs and DEGs expression: Pearson correlation analysis (a correlation coefficient  $>0.7$  was recognized as significant for the identification of the lncRNA-associated genes).

ISIAH rats (Inherited Stress Induced Arterial Hypertension) model the stress-sensitive form of arterial hypertension: elevated blood pressure, characteristic structural and functional changes in target organs, increased stress sensitivity under the influence of mild emotional stress, and specific behavior in an unfamiliar environment. Control are normotensive WAG (Wistar Albino Glaxo) rats.

The hypothalamus is suggested to regulate the manifestation of the above phenotypic characteristics in ISIAH rats.

This work presents the results of identification of differentially expressed lncRNAs in the hypothalami of the rats and reveals the lncRNA-associated protein coding DEGs related to hypertension and behavioral phenotypes of ISIAH rats.

Rat Genome Database (<https://rgd.mcw.edu/>) was used to identify the genes associated with the hypertension and behavioral phenotypes. Atlas of combinatorial transcriptional regulation in mouse and man was used to reveal the DEGs encoding the transcription factor genes.

# Results and Conclusion

Expression of three lncRNAs (**Bc1**, **RGD1562890**, and **Snhg4**) differed in the hypothalami of hypertensive ISIAH and normotensive WAG rats (Table 1).

**Table 1. The lncRNAs differentially expressed in the hypothalami of ISIAH and WAG rats**

lncRNA	Expression level in		log2 (fold_change) ISIAH/WAG	p_value
	ISIAH, RPKM	WAG, RPKM		
Bc1	137705.0	98177.6	0.49	1.85E-02
RGD1562890	247.4	51.4	2.27	7.00E-04
Snhg4	11.2	34.2	-1.61	4.50E-03

The relationship between these lncRNAs and the expression level of protein coding DEGs associated with hypertension and behavioral phenotypes was calculated using the Pearson correlation coefficient.

Among the hypothalamic protein coding DEGs that were associated with hypertension, one gene (**Hyal1**) associated with **RGD1562890** ( $r = -0.73$ ) and 12 DEGs associated with **Snhg4** were found. Among them, the most significant association was found with the **lgfbp2** gene ( $r = 0.97$ ), which is associated with insulin resistance in the development of hypertension.

Among the hypothalamic protein coding DEGs associated with behavioral phenotypes, one gene (**Ercc2**) associated with **Bc1** ( $r = 0.88$ ), two genes (**Evc** and **Foxg1**) associated with **RGD1562890** ( $r = -0.78$  and  $r = 0.83$ ), and 9 genes associated with **Snhg4** were found. Among them, the most significant association was identified with the **lgf2** gene ( $r = 0.88$ ). It should be noted that the **Ercc2** and **Foxg1** genes encode transcription factors, and the **lgf2** gene is associated with cerebrovascular disorders.

The most highly expressed lncRNA (**Bc1**) is likely to influence the behavior of ISIAH rats by regulating the expression of the **Ercc2** gene, which encodes the transcription factor and is associated with abnormal emotion/affect behavior and abnormal response to novelty, but does not affect the regulation of genes directly associated with hypertension.

We can expect that lncRNA **RGD1562890** is involved both in the regulation of processes associated with hypertension and in the regulation of behavior, in particular with hyperactivity, through the regulation of the **Foxg1** gene encoding the transcription factor. The largest number of co-regulated genes, both associated with hypertension and behavior, was found for **Snhg4**.

**These findings may be useful for further understanding the role of lncRNAs in regulating the protein coding genes and modulating processes associated with both hypertension and behavior.**