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LETTER

Up-regulation of Long Non-coding RNA *TUG1* in Hibernating Thirteen-lined Ground Squirrels

Jacques J. Frigault [a], Daneck Lang-Ouellette [b], Pier Morin Jr. [*,c]

Department of Chemistry and Biochemistry, Faculty of Sciences, Université de Moncton, Moncton E1A 3E9, Canada

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Abstract Mammalian **hibernation** is associated with multiple physiological, biochemical, and molecular changes that allow animals to endure colder temperatures. We hypothesize that long **non-coding RNAs** (**lncRNAs**), a group of non-coding transcripts with diverse functions, are differentially expressed during **hibernation**. In this study, expression levels of **lncRNAs** *H19* and *TUG1* were assessed via qRT-PCR in liver, heart, and skeletal muscle tissues of the hibernating thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). *TUG1* transcript levels were significantly elevated 1.94-fold in skeletal muscle of hibernating animals when compared with euthermic animals. Furthermore, transcript levels of *HSF2* also increased 2.44-fold in the skeletal muscle in hibernating animals. *HSF2* encodes a transcription factor that can be negatively regulated by *TUG1* levels and that influences heat shock protein expression. Thus, these observations support the differential expression of the *TUG1–HSF2* axis during **hibernation**. To our knowledge, this study provides the first evidence for differential expression of **lncRNAs** in torpid ground squirrels, adding **lncRNAs** as another group of transcripts modulated in this mammalian species during **hibernation**.

Introduction

Many small mammals undergo hibernation when confronted with unfavorable environmental conditions such as cold temperatures. Hibernation is characterized by a marked reduction in metabolism, prolonged periods where body temperatures (Tb) are significantly reduced (Tb ~ 4 °C), a substantial reduction in heart rate, as well as resistance to skeletal muscle atrophy [1–4]. Activity of several metabolic pathways are tightly controlled under these conditions notably via reversible protein phosphorylation of key regulatory enzymes [5]. Regulation of ATP-consuming processes such as gene transcription and protein translation is also a common theme observed in mammalian hibernation [6–8]. Molecular levers that are utilized to impact these two processes include, for example, histone deacetylases (HDACs) [9] and microRNAs (miRNAs) [10]. Nevertheless, the complete characterization of molecular players underlying mammalian hibernation is ongoing.

* Corresponding author.
E-mail: pier.morin@umoncton.ca (Morin P Jr).
[a] ORCID: 0000-0001-6907-1788.
[b] ORCID: 0000-0002-8100-2678.
[c] ORCID: 0000-0001-8117-0236.

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Long non-coding RNAs (lncRNAs) are non-coding RNAs (ncRNAs) typically longer than 200 nucleotides that affect diverse cellular functions including gene transcription and protein translation. lncRNAs have been notably shown to impact histone modifications [11], modulate transcription factor–promoter interaction [12], and influence mRNA stability [13]. Interestingly, several lncRNAs were shown to contain miRNA binding sites that could promote miRNA sequestration and subsequent inhibition of miRNA-mediated target recognition and expression [14,15]. Differential expression of lncRNAs have been reported in a variety of conditions and processes relevant to hibernation including fasting and lipid metabolism [16,17]. However, identification of torpor-associated lncRNAs has not been fully explored.

Since lncRNAs are involved in regulating crucial processes impacted during mammalian hibernation, the current study was conducted to evaluate the expression of two lncRNAs, *H19* and *taurine up-regulated gene 1* (*TUG1*), in liver, heart, and skeletal muscle tissues of the hibernating thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). *H19* is one of the earliest lncRNAs identified [18], while *TUG1* can influence the expression and activity of transcription factors relevant to hibernation [19,20]. We report up-regulation of lncRNA *TUG1* levels in the skeletal muscle of hibernating thirteen-lined ground squirrels and discuss the potential significance of this modulation in mammalian hibernation.

Results

Amplification of lncRNAs in thirteen-lined ground squirrels

Consensus sequences of *H19* and *TUG1* in mammalian species were generated for primer design. Target lncRNAs were subsequently amplified in liver, heart, and skeletal muscle tissues of ground squirrels via RT-PCR. The products PCR were confirmed by sequencing and the resulting *H19* and *TUG1* sequences were submitted to GenBank (GenBank accession Nos. KT305775 and KT305776). Figure S1 shows the nucleotide sequences of *H19* and *TUG1* aligned with the sequences for the human, mouse and rat lncRNAs. The partial *H19* nucleotide sequence of ground squirrels displayed 75% homology with that of humans over the amplified region, respectively (Figure S1A). Similarly, the partial *TUG1* nucleotide sequence was 86% homologous with that of humans over the amplified fragment. BLAST alignment of the full length human *TUG1* (GenBank accession No. NR_110492.1) and thirteen-lined ground squirrel genome (SpeTri2.0 reference Annotation Release 101) revealed 74% conservation between sequences from humans and thirteen-lined ground squirrels (GenBank accession No. NW_004936523.1, scaffold 00055).

H19 and *TUG1* expression in tissues of hibernating ground squirrels

H19 and *TUG1* transcript levels were examined in liver, heart, and skeletal muscle tissues of euthermic and hibernating ground squirrels. Relative levels of both transcripts were quantified in each tissue using qRT-PCR by normalization against that of α-*tubulin* in each sample. **Figure 1** shows the ratio of normalized *H19* and *TUG1* transcript levels in the three tissues examined. Compared to euthermic animals, expression levels of *TUG1* in the skeletal muscle of hibernating ground squirrels were 1.94 ± 0.17-fold of that from euthermic animals, which represents a significant increase ($P < 0.005$). On the other hand, although there is a trend of increased expression of *H19* in hibernating animals, the changes are not significant due to the huge variations.

HSF2 expression in tissues of hibernating ground squirrels

The transcription factor heat shock factor 2 is encoded by *HSF2*, which is potentially modulated by *TUG1* via miR-144 [21]. We thus measured *HSF2* expression in liver, heart and skeletal muscle tissues of euthermic and hibernating animals using qRT-PCR by normalization against that of α-*tubulin* as above. **Figure 2** shows the ratio of normalized *HSF2* transcript levels in all tissues. *HSF2* levels increased by 2.44 ± 0.22-fold in hibernating versus euthermic skeletal muscle tissues ($P < 0.005$). Compared to euthermic animals, expression levels of *HSF2* in the skeletal muscle of hibernating animals were 2.44 ± 0.22-fold of that from euthermic animals, which represents a significant increase ($P < 0.005$), whereas the expression in heart and liver samples were comparable between euthermic and hibernating animals.

miR-144 expression in skeletal muscle of hibernating ground squirrels

Previous work indicated that *TUG1* can affect *HSF2* expression via miR-144 in glioma cells [21]. Transcript levels of miR-144 were quantified in skeletal muscle tissues of ground squirrel using qRT-PCR. Expression of miR-144 in hibernating animals was 1.31 ± 0.30-fold of that in euthermic animals. However, this change was not statistically significant ($P > 0.05$).

miR-144 binding site in human and ground squirrel *TUG1* sequences

The miR-144 binding site in human *TUG1* has been reported previously [21]. Interestingly, there is 74% homology between full length human *TUG1* sequence and the whole genome shotgun sequence of the ground squirrels (contig043218; GenBank accession No. AGTP01043218.1). Sequence alignment also revealed a conserved (82.6%) miR-144 binding site in the ground squirrel sequence (Figure S2), suggesting there might exist *TUG1*–miR-144 interaction in the ground squirrels as for humans.

Discussion

Differential expression of ncRNAs in animal models of cold adaptation has garnered significant interest in the field over recent years. Modulation in expression of ncRNAs, in particular miRNAs, at low temperatures has been reported in small mammalian hibernators [22,23], cold-hardy insects [24], and freeze-tolerant wood frogs [25]. Unlike miRNAs, lncRNAs have not been explored extensively in models of cold adaptation. Pioneering work in this area has revealed reduced levels of, a natural antisense lncRNA transcript of the gene