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Discordant expression of miR-103/7 and pantothenate kinase host genes in mouse

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Abstract

miR-103 and miR-107, microRNAs hosted by pantothenate kinase genes, are proposed to regulate cellular lipid metabolism. microRNA-mediated regulation is complex, potentially affecting expression of the host gene, related enzymes within the same pathway, or apparently distinct targets. Using qRT-PCR, we demonstrate that miR-103 and miR-107 expression does not correlate with expression of host pantothenate kinase genes in mouse tissues. The miR-103/7 family thus provides an intriguing model for dissecting microRNA transcription, processing and coordinated function within host genes.

Keywords

intronic microRNA; pantothenate kinase

1. Introduction

Mammalian microRNAs (miRNAs) are post-transcriptional regulators that bind to complementary regions generally within 3′ untranslated regions (UTRs) of messenger RNA transcripts (mRNAs), forming double-stranded RNA complexes that ultimately result in gene silencing. Approximately 37% of mammalian miRNAs are located within the introns of protein-coding genes [1]. Initially, intronic miRNAs were predicted to be co-transcribed with their host transcript because many miRNAs and their host genes have been shown to have similar expression patterns [2]. However, not all miRNA expression correlates with host gene expression [3,4]. miRNAs thus likely represent an integral part of a complex regulatory network controlling many key cellular pathways, including metabolism. Differential miRNA expression may be governed by post-transcriptional regulation [5], independent promoters [6] or disparate transcript stabilities [7].

miR-103 and miR-107 are highly conserved miRNAs that map to intron 5 of three distinct pantothenate kinase (*Pank*) genes. *Pank2* and *Pank3* host the precursor miRNA (pre-miRNA)

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sequences miR-103-2 and miR-103-1, respectively, which are processed into miR-103. *Pank1* encodes miR-107, which differs from miR-103 by a single nucleotide. Pantothenate kinase is the rate limiting enzyme in the biosynthesis of coenzyme A, a cofactor that is involved in over 100 metabolic reactions [8]. In addition, *PANK2* is the causative gene in pantothenate kinase-associated neurodegeneration (PKAN), which is characterized by dystonia, brain iron accumulation and neuroaxonal spheroid deposition [9].

The conservation of miR-103/7 within pantothenate kinase genes suggests a role for these miRNAs in metabolism and neurodegeneration. miR-103 and miR-107 are both ubiquitously expressed, with relative abundance in the brain [3,10]. However, differential expression of miR-103 and miR-107 has been reported in adipogenesis [11], models of diabetes [12] and following glucose treatment in a pancreatic β cell line [13], as well as in neurological disease [14,15] and cancer [16-20]. Functionally, miR-103/7 are predicted to target many enzymes that are important in regulating metabolism [21]. Moreover, experimentally validated miR-103/7 targets include β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) [15], granulin (GRN) [22] and hypoxia inducible factor-1β (HIF-1β) [16], which have all been implicated in neurodegeneration.

Microarray analyses of miRNA and mRNA profiles in human tissues previously revealed a low correlation between the ubiquitously expressed miR-103/7 family and their host genes [3,4]. However, in cell culture, correlated expression has been observed. For example, there is similar induction of miR-103/7, *Pank1*, *Pank2* and *Pank3* expression during 3T3-L1 cell adipogenesis [11]. Also, p53 can induce expression of *PANK1* and miR-107; presumably, through a p53 element located ~1kb upstream of the *PANK1* transcriptional start site [16]. Finally, studies in a pancreatic cancer cell line suggest that miR-107 expression can be regulated through epigenetic silencing of the *PANK1* promoter [20].

However, evidence is also surfacing for independent transcription of miR-107 and *Pank1*. In particular, Monteys et al. recently described an independent miR-107 promoter that is sufficient to drive miR-107 expression in a promoterless plasmid [6]. In another study, Corcoran et al. also predicted miR-107 to have an independent promoter, based on RNA polymerase II chromatin immunoprecipitation experiments [23]. Interestingly, however, miR-103-1 and miR-103-2 are not predicted to have promoters independent of *Pank3* and *Pank2*, respectively [23].

In this short report, we present the relative expression of the pantothenate kinase genes and their intronic miRNAs in various mouse tissues and discuss the relationship of these expression levels to the regulation and function of these miRNAs.

2. Material and Methods

qRT-PCR

Total RNA was harvested from three 100 day old C.D2 Es-Hba wildtype mice using RNAqueous-Micro kit (Ambion, Inc.) for retinal tissue and RNA-STAT60 (Tel-Test, Inc.) for all other tissue.

To quantify *Pank1*, *Pank2* and *Pank3* mRNA levels, cDNA was synthesized from 50ng total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Inc.) according to the manufacturer's instructions. For PCR, cDNA was diluted using RNase free water, mixed with primer/probe sets for *Pank1* (Mm00458408_m1), *Pank2* (Mm00463258_m1), *Pank3* (Mm00461298_m1) or *Gusb* (Mm03003537_s1) and 2× PCR Universal Master Mix (Applied Biosystems, Inc.). PCR reactions were performed in triplicate on an ABI Prism 7000 Sequence Detections System following the manufacturer's directions.

To quantify miRNAs, cDNA was reverse transcribed from 4ng total RNA using primers from miR-103 (assay ID 439), miR-107 (assay ID 443) or snoRNA234 (assay ID 1234) TaqMan MicroRNA Assays and reagents from the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems, Inc.). The resulting cDNA was amplified by PCR using primer/probe sets supplied with the TaqMan MicroRNA Assays and 2x TaqMan Universal PCR Master Mix (Applied Biosystems, Inc.). PCR reactions were performed in triplicate on an ABI Prism 7000 Sequence Detections System following the manufacturer's directions.

Gusb and snoRNA234 were endogenous controls for the analysis of *Pank* and miRNA expression, respectively. For each tissue, the mean $Ct_{control}$ value was subtracted from the mean $Ct_{experimental}$ value (ΔCt). Spleen was chosen as a reference tissue and the mean ΔCt value for spleen was subtracted from each tissue ΔCt value (ΔΔCt). Relative expression (RE) in each tissue was calculated as $RE = 2^{-\Delta\Delta Ct}$. The mean of the RE for three biological replicates is plotted in Figure 1 and error bars represent the standard error of the mean.

3. Results & Discussion

To determine whether miR-103 and miR-107 are co-expressed with their host genes in mouse, we measured relative miR-103, miR-107, *Pank1*, *Pank2* and *Pank3* expression by qRT-PCR (Figure 1). We detected widespread expression of all pantothenate kinase genes. *Pank2* has highest expression in brain, retina and testis, three tissues that are affected by PKAN [24]. *Pank3*, in contrast, is most abundantly expressed in the small intestine, and *Pank1* has relative abundance in the liver. Notably, the relative expression patterns of *Pank1*, *Pank2* and *Pank3* in testis, brain and liver differ from a previously published report [25]. miR-103 is broadly expressed across mouse tissues with highest relative expression in brain and lung, consistent with previous findings using microarrays [10]. In contrast, miR-107 was most abundant in brain and kidney.

Despite the shared characteristic of ubiquitous expression, miR-103 and miR-107 expression profiles poorly correlate with that of their host genes. For miR-107, this result supports the recent model of transcription from an independent promoter [6]. For miR-103, the discordant expression is likely attributed, at least in part, to its redundant expression from *Pank2* and *Pank3* transcripts. However, post-transcriptional factors may also play a role in controlling the relative abundance of the mature transcript. In support of this concept, Lee et al have demonstrated that regulation at the level of miRNA processing is common and often controls tissue-specific regulation of ubiquitously expressed miRNA precursor transcripts [26].

The miR-103/7 family represents an intriguing model of disease-associated intronic miRNA. They are highly conserved within pantothenate kinase genes; one of which, *PANK2*, is associated with a neurodegenerative disease. Moreover, miR-103/7 likely regulates proteins involved in acetyl-coA metabolism [21], as well as neurodegeneration. However, expression of the mature miRNAs is not synchronized with that of the host genes. These distinct expression patterns are likely due to multiple factors, including the existence of alternate promoters, variable stabilities of the RNA transcripts and regulation of post transcriptional processing. Therefore, the miR-103/7 family provides an intriguing model for dissecting miRNA transcription, processing and coordinated function with host genes.

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Abbreviations

miRNA	microRNA
PANK	pantothenate kinase
PKAN	pantothenate kinase-associated neurodegeneration
precursor microRNA	pre-miRNA
UTRs	untranslated regions

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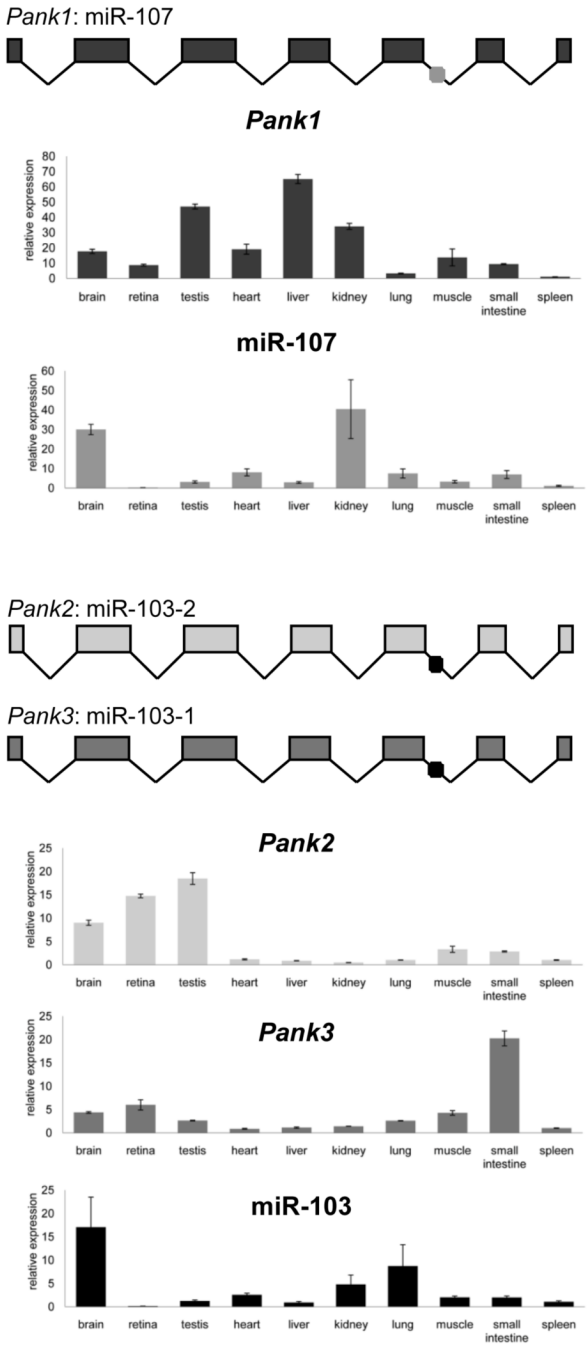

Figure 1.

Relative expression of miR-103, miR-107 and their host genes in mouse tissues. RNA was harvested from mouse tissues (n=3) and analyzed by qRT-PCR. Error bars represent standard error.