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SnoRNA microarray analysis reveals changes in H/ACA and C/D RNA levels caused by dyskerin ablation in mouse liver

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Synopsis

Small nucleolar RNAs (snoRNAs) are key components of ribonucleoprotein particles (snoRNPs) involved in modifying specific residues of ribosomal and other RNAs by pseudouridylation (H/ACA snoRNAs) or methylation (C/D snoRNAs). They are encoded within the introns of host genes, which tend to be genes whose products are involved in ribosome biogenesis or function. Though snoRNPs are abundant and ubiquitous and their components highly conserved, information concerning their expression during development or how their expression is altered in diseased states is sparse. To facilitate these studies we have developed a snoRNA microarray platform for the analysis of the abundance of snoRNAs in different RNA samples. Here we show that the microarray is sensitive and specific for the detection of snoRNAs. A mouse snoRNA microarray was used to monitor changes in abundance of snoRNAs after ablation of dyskerin, an H/ACA RNA protein component, from mouse liver, which causes a decrease in ribosome production. H/ACA snoRNAs were decreased in abundance in these livers while, unexpectedly, C/D snoRNAs were increased. The increase in C/D snoRNAs corresponded with an increase in the abundance of the mRNAs transcribed from snoRNA host genes, suggesting the increase may be part of a cellular response to defective ribosome synthesis.

Keywords

H/ACA RNA; dyskerin ablation; ribosome biogenesis

INTRODUCTION

Small nucleolar ribonucleoproteins (snoRNPs) are highly conserved ribonucleoprotein complexes essential for ribosome biogenesis and also important for mRNA splicing, telomere maintenance and possibly other functions [1]. There are 2 classes of snoRNPs, 1) H/ACA RNPs which catalyze the formation of pseudouridine (Ψ) from specific uridines in newly synthesized ribosomal RNA and spliceosomal snRNAs [2] and 2) C/D RNPs which

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catalyze the methylation of specific residues in these RNAs [1,3]. In both cases the snoRNP complexes consist of a small nucleolar RNA (snoRNA) guide RNA and 4 proteins. The C/D snoRNAs are associated with fibrillarin, Nop56, Nop58, and 15.5K/NHPX proteins and the H/ACA snoRNAs are associated with dyskerin (the pseudouridine synthase), NOP10, NHP2 and GAR1. The latter 4 proteins are also associated with telomerase RNA, which contains an H/ACA domain [4],[5], and mutations in the gene encoding dyskerin are responsible for X-linked dyskeratosis congenita, a disease whose major cause is defective telomere maintenance [6].

The organization of snoRNA genes in eukaryotes is variable. In vertebrates snoRNAs are mainly intron-encoded and their expression is coupled to the transcription of the host gene. Most host genes encode proteins involved in ribosome biogenesis or function, providing coordinated expression of RNA and protein components of the ribosome machinery [7,8]. Some snoRNAs are encoded in introns of genes whose exons do not contain open reading frames and are apparently dedicated solely to the production of snoRNAs [9].

The process of RNA modification by snoRNAs and, indeed, the proteins associated with snoRNAs are highly conserved in evolution [1,3], suggesting their role in cellular metabolism is essential. Despite this, very little is known of their role in development or in disease states. This is partly because of the difficulty in identifying snoRNAs using bioinformatics and partly because of the lack of any obvious model of how snoRNAs may influence gene. Recently the importance of RNA metabolism in general and ribosome biogenesis in particular have come into focus as important pathways in the control of growth and cell division [10,11], and genetic perturbation of ribosome biogenesis has been found to be the cause of several inherited diseases [12]. Defects in snoRNP production, through mutations in snoRNA genes or in genes encoding their associated proteins could have a profound influence on cell metabolism and proliferation. To facilitate our studies on snoRNA regulation we have developed a microarray platform for the simultaneous analysis of all known snoRNAs. To characterize and validate this approach we have used a mouse model in which the H/ACA associated protein dyskerin is specifically ablated from mouse hepatocytes [13]. In this model we have found that, although dyskerin is essential for cell division, hepatocytes lacking dyskerin can survive for weeks, allowing the study of the effects of dyskerin knockout. In this report we show, using microarray analysis, that dyskerin ablation leads to an overall decrease in the abundance of H/ACA snoRNAs. Surprisingly the abundance of C/D snoRNAs increases. This increase is partly correlated with increased mRNA levels of snoRNA host genes.

MATERIALS AND METHODS

Generation of *Dkc1* conditional knockout mice

The generation of the *Dkc1*^{lox} allele in C57BL/6 mice has been previously described [14]. Mice carrying the *Mx1-Cre* transgene were obtained from the Jackson laboratory (Bar Harbor, ME). The Mx1 promoter is silent in healthy mice, but can be induced to high levels of transcription by administration of interferon alpha, interferon beta, or synthetic double-stranded RNA. Female *Dkc1*$^{lox/lox}$ mice were bred to *Mx1-Cre* mice and male *Dkc1*^{lox}*Tg:Mx1-Cre* mice were selected. These are referred to as knockout (KO) mice. The *Dkc1*^{lox} mice were used as controls, and are referred to as wild-type (WT). For induction of the *Mx1-Cre* transgene, mice (5 w) were injected with polyI:C (250 ug. i.p.) every other day for a total of 3 injections. Mice were sacrificed 4 w after the last injection. All experiments on mice were performed under the strict guidelines of the National Institutes of Health and the Institutional Animal Use and Care Committee at Washington University.

RNA and protein analysis

RNA samples from washed livers from 9 w old WT and KO mice were isolated using Trizol (Invitrogen, Carlsbad, CA). Total RNA concentration and purity was obtained from an absorbance ratio at 260nm and 280nm. Total RNA quality was then determined by Agilent 2100 bioanalyzer (Agilent Technologies) according to manufacturer's recommendations. RNA was used for microarray analysis, qPCR analysis and northern blot. For northern blots, RNA (50 μg) was electrophoresed in 1.5% (w/v) agarose–formaldehyde gels and blotted to Hybond-N^+ membranes (GE Healthcare) overnight. Oligonucleotides (10 pmol) were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (Fisher, Pittsburg, PA). The temperature of hybridization and washing was determined by the Tm of each probe. The snoRNA probes used in northern blot were

SNO1104 5'CAA GGT TGG CTT CCC CAC GAC GCA GTC3'

SNO1045 5'GAA AGA GGT CCA CCC CAG TCT 3'

SNO1023 5'TGT TTT TCA CTC TGC CCC TTC T3'

SNO1099 5'AGC CAG TGA ATA AGG TCA GCA GTT3'

U39/U55 5'CAA GGT TGG CTT CCC CAC GAC GCA GTC3'

U30 5'TCC AAG TCT CAA CAG CAA TCA TCA GC3'

SNO1020 5'GTC TCT CTC GGG CGC TGT GCC CAG3'

5S 5'GGT CTC CCA TCC AAG TAC TAA CCA GGC CCG ACC CTG CTT AG3'

5.8S 5'GCG TTC GAA GTG TCG ATG ATC AAT GTG TCC TGC AAT TCA C3'

Nuclear protein extraction was performed using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL). 20μg protein was resolved by SDS-PAGE analysis and transferred to Hybond-ECL nitrocellulose membrane (GE healthcare, Piscataway, NJ). Antibodies were detected by ECL plus™ western blotting detection reagents (GE healthcare) and visualized by autoradiography. Primary antibodies used in this study were polyclonal antiserum to dyskerin; anti-TATA box binding protein (1:2000; Abcam, Cambridge, MA). The secondary antibody was either horseradish-peroxidase–conjugated goat anti-mouse (1:10000; Abcam) or horseradish-peroxidase–conjugated goat anti-rabbit (1:10000; Abcam).

Microarray construction

The probes for all known mouse snoRNAs compiled from the RNAdb (http://jsm-research.imb.uq.edu.au/rnadb/) were designed using Agilent earray software (https://earray.chem.agilent.com/earray/) using Agilents “Base Composition Methodology”. Probes were antisense because we hybridized labeled RNA to the arrays. Our customized microarray consists of the 145 snoRNA 60mer oligonucleotide probes, 40 *maize* control 60mer oligonucleotide probes (see below) and the Agilent-provided control probes. The customized microarray was manufactured on an Agilent custom 8×15K microarray, and each designed oligonucleotide probe was replicated at random for an average of 81 times per array.

To test the specificity of the microarray, we used a series of model sequences with varying degrees of similarity to the probes. We designed two set of probes with 0–5 nucleotide mismatches for each of two control maize RNAs. RNA oligonucleotides, complementary to the perfect match sequences, were designed, synthesized by Integrated DNA technologies and added to the mouse RNA before labeling.

Hybridization

Four total RNAs, (two KO and two WT) from liver tissue, were chemically labeled at two input masses (10ug and 1.5ug) with Kreatech ULS RNA labeling kit (Kreatech Diagnostics). RNA was mixed with the maize control RNA oligonucleotides (1ug each), Kreatech 10× labeling buffer (2ul), and Kreatech cy3-ULS reagent (2ul). The reactions were incubated at 85°C for 15 minutes in the dark and placed on ice for 3 minutes. Labeled RNAs were purified with Kreapure gel columns according to manufacturer's protocol. The labeled RNAs were then quantitated on a NanoDrop fluorometer at A260 to determine RNA concentration and A530 for cy3 dye incorporation.

Labeled RNAs (volume reduced to 7.5ul via speedvac) were mixed with Kreablock (12.5ul), Agilent 10× Block (5ul), and Agilent 2× Gene Expression Hi-RPM Buffer (25ul). The hybridization solution was heated to 70°C for 5 minutes and allowed to cool to room temperature. 45ul of each hybridization solution was then added to the customized mouse snoRNA microarrays and hybridized for 18 h at 65°C. The Agilent hybridization oven rotator was set at 10rpm. Arrays were washed according Agilent Gene Expression wash protocol.

Microarray analysis

Slides were scanned on a Molecular Devices Genepix 4000B microarray scanner to detect Cy3 fluorescence. Laser power was kept constant and PMT voltage was established for all 8 arrays on the glass such that the high-input hybridizations had less than 1% signal saturation. Images were quantitated with Genepix Pro, v6.1 (Molecular Devices).

Raw, background-subtracted intensity data was imported into GeneSpring (Agilent) in order to derive the mean value of replicate spots. This data was then exported and imported into Partek Genomic Suite (St. Louis) and quantile normalized and log-transformed. 2-way ANOVA analysis was done on the snoRNA derived elements, RNA mass and genotype as variables. A false discovery rate of 5% was applied to identify genes differentially expressed in livers from KO vs WT mice.

Quantitative RT-PCR

The SYBRGREEN master mix (Applied Biosystems; Foster City, CA) was used to follow RNA amplification in real time. Reverse transcription and PCR reactions were performed according to the manufacturer's protocols of the SuperScript III first strand synthesis system for RT-PCR kit (Invitrogen; Carlsbad, CA). The primers were specifically designed by Primer Express 3.0 software (Applied Biosystems) (Supplementary Table 1). 28S and β-actin were used as internal positive controls. Fluorescence was quantitated using an Applied Biosystems 7500 Fast Real-Time PCR system.

RESULTS AND DISCUSSION

Specificity of the snoRNA microarray

The microarray was composed of 60 mers complementary to the snoRNA sequences. Total RNA was uniformly labeled as the probe. SnoRNAs in higher organisms sometimes contain families of related sequences that may differ by one or a few nucleotides. It was therefore important to determine the specificity of the hybridization and the sensitivity to nucleotide changes. For this purpose we included on the array 2 sets of 60mers designed from a *maize* genomic DNA sequence that were not homologous to any mouse sequence. These sets consisted of wild type oligos and oligos with mutations in different positions along the 60mer (Figure 1). Two synthetic RNA oligos, which contained the complement of the wild type probes were spiked into the samples and labeled along with the liver RNA. The results

from this analysis show hybridization efficiency is sensitive to the number, proximity, and location of mismatches within the probe. Hybridization was relatively insensitive to changes close to (within 13 base pairs of) the 3'end of the immobilized oligonucleotide probe, a phenomenon observed previously with a microRNA array [15]. This is the end of the probe that is attached to the glass, leading us to suspect a possible steric effect. We therefore suggest including a non-hybridizing linker sequence at the 3' end of the oligonucleotides when it is desirable to distinguish between highly homologous targets. Of the 145 snoRNA 60 mers used in our microarray only 3 were partially complementary to a second sequence within the mouse transcriptome in which mismatches were confined to the 3'end of the sequence, and none of these alternative sequences have so far been annotated as snoRNAs. We conclude that, based on sequence homology, the specificity of our microarray was suitable for our purpose and the array can effectively differentiate between snoRNAs and other sequences.

Dyskerin ablation in mouse liver

We previously described a line of mice with lox elements inserted into intron 11 and into the 3'UTR [14] of the *Dkc1* gene. These mice were bred to contain the *Mx1-Cre* transgene, which can by induced by interferon or double stranded RNA (PolyI:C) to produce the Cre recombinase protein [16]. Poly I:C treatment of these mice causes efficient excision of exons 12–15 of the *Dkc1* gene, resulting in the loss of detectable full length or truncated dyskerin protein in all tissues we examined. However, after one month, most tissues have returned to near normal levels of dyskerin protein as they have become populated by proliferating cells that escaped the deletion event. In the liver however dyskerin levels remain very low even 4 weeks after the final polyI:C dose [13]. In the livers of 2 $Dkc1^{lox}Tg:Mx1Cre$ (KO) mice, one month after polyI:C injection, dyskerin levels were 10% (KO1) and 40% (KO2) of WT (Figure 2).

Immunohistochemistry of liver tissue showed that for each cell dyskerin ablation was all or none, with some cells having no detectable dyskerin and others, having escaped the deletion, having normal amounts [13]. In 2 control $Dkc1^{lox}$ (WT) animals that did not contain the *Mx1-Cre* transgene there was no reduction in dyskerin levels.

SnoRNA expression profile in the dyskerin knock out liver tissue

Labeled RNAs from KO and WT livers were hybridized to our microarray contained 60mers representing all 145 known mouse snoRNAs. Interestingly, all snoRNA signals were robustly strong compared with those of the Agilent negative control spots (negative control 39.7 ± 7.1, snoRNAs 456.4 ± 174.7), suggesting that all known snoRNAs are expressed in liver. After normalization of the raw data, principal component analysis (PCA), which detects global trends of gene expression patterns in microarray analysis, showed that the gene expression in two WT samples was closely clustered, and that in the two KO samples was well separated from WT (Figure 3). In PCA, the KO1 sample, in which the dyskerin level was lowest was more divergent from WT than the KO2 sample. 59 snoRNAs were detected at a higher expression level in the KO samples than the WT, and 42 snoRNAs were detected at a lower expression level in the KO samples (multiple testing corrected $p<0.05$) (Figure 4). The significantly altered snoRNAs, along with their host genes and target RNAs are listed in Table 1.

Eight differentially expressed snoRNAs were randomly selected to validate microarray results by q-PCR. The relative change of snoRNA expression assayed using microarray analysis and q-PCR were consistent (Figure 5A). The correlation coefficient of the 8 snoRNAs between microarray and q-PCR was 0.912. While q-PCR is regarded to be

superior to microarrays for comparative analysis, microarrays offer a high-throughput method that generally captured changes in snoRNA expression.

The microarray results were also validated by northern blot (Figure 5B). Since the expression level of snoRNA is low in the liver tissue, seven abundant snoRNAs were chosen for northern blot analysis to observe the change of expression in the KO mice compared with the WT mice, and the size of the product was investigated. Four H/ACA snoRNAs (SNO1104, 1045, 1023 and 1099) significantly decreased, and three C/D snoRNA (U39/U55, U30, and SNO1020) increased as suggested by microarray results. Ribosome 5.8S and 5S were used as loading controls. Moreover, the intron containing pre-mRNA was not detected in these northern blots (Figure 1S), indicating that the amount of the precursor is very small compared with that of the mature snoRNA.

Decreased levels of H/ACA RNAs but increased levels of C/D RNAs in the absence of dyskerin

Of 50 H/ACA RNAs represented on the microarray 40 significantly decreased in abundance in the KO livers. This is not surprising since dyskerin is a crucial component of H/ACA snoRNPs and is involved in the post transcriptional assembly of the snoRNP as well as providing the pseudouridylation activity. In the absence of dyskerin snoRNP assembly cannot occur and presumably snoRNAs are degraded. The amount of reduction in H/ACA snoRNA abundance is much less than the decrease in dyskerin levels. This could be explained if there was a rapidly turned over component of dyskerin that is in excess of snoRNAs, and a second more stable component in mature snoRNPs. Further experiments will be necessary to determine the exact relationship between dyskerin levels and those of individual snoRNAs.

We did not expect the relative abundance of C/D snoRNAs, which do not associate with dyskerin to increase. However, of 95 C/D snoRNAs represented on the microarray 59 were significantly up-regulated and 2 were down-regulated in the dyskerin KO livers. A possible explanation is that the transcription of the snoRNA host genes, which are almost all involved in ribosome biogenesis, is higher in the KO hepatocytes. This would constitute a mechanism for increasing ribosome biogenesis in response to its attenuation caused by lack of dyskerin. While many of the host genes for C/D snoRNAs encode ribosomal proteins, the host genes for 9 C/D snoRNAs are non-protein-coding multiple snoRNA genes, UHG (U22 host gene) and gas5 (growth arrest-specific transcript 5) [9]. U22 and gas5, as well as most other snoRNA host genes, belong to a class of genes with a specific sequence in the 5' UTR, known as the terminal oligopyrimidine tract (5'TOP) sequence [17]. Though the 5'TOP sequence is known as a translational control element it may be that the increase in C/D snoRNAs is due to increased transcription of host genes, or alterations in splicing [17], mediated by the 5'TOP sequence.

Two C/D snoRNAs, MBII-163 (SNO1003) and MBII-170 (SNO 1004), were exceptional in that they decreased in abundance in the absence of dyskerin. Interestingly these snoRNAs are similar in that that they do not have an identified methylation target and that their host genes are not genes whose products, huntingtin interacting protein 1 and amiloride sensitive cation channel 1 respectively, are predicted to be involved in ribosome biogenesis [18]. In fact both of these genes are expressed predominantly in the brain. Nevertheless they produce detectable amounts of snoRNA, according to the microarray analysis, in the liver.

Transcription of host genes

To gain insight into the mechanism whereby expression of H/ACA snoRNAs decrease and that of C/D snoRNAs increase in dyskerin deficient cells we measured the mRNA

expression level of 3 host genes of each type by q-RT PCR (Table 2). The mRNA of host genes of the 3 C/D snoRNA increased 20~75%. Surprisingly in the light of decreased H/ACA snoRNAs, the mRNA of the host genes of the 3 H/ACA snoRNAs also increased 15~25%. This might be expected if there was a global increase in ribosome related gene transcription and mRNA is more stable than free snoRNA. Since unspliced mRNA precursors give rise to both mRNA and snoRNA we next investigated levels of mRNA precursors for 2 H/ACA RNA host genes and 2 C/D RNA host genes. The results (Table 3) show that precursor levels in 3 cases were not altered while the RPS8 precursor was twice as abundant in the liver samples after dyskerin ablation. These results indicate that while dyskerin ablation may affect the transcription of host genes, this is not consistently reflected in increased levels of rapidly turned over premRNA. Alternatively the increase in C/D snoRNA abundance may be due to post-transcriptional events. mRNAs for proteins involved in ribosome biogenesis can be preferentially recruited to polysomes via the 5'TOP sequences in their 5'UTR. This can result in increased stability of the mRNAs[19] but does not obviously account for the increase in C/D snoRNA level.

Because we observed differences between KO1 and KO2 in PCA analysis we examined the correlation of the expression of dyskerin and snoRNA. We compared the expression level of snoRNAs in KO1 and KO2 mice as the dyskerin protein level in the KO1 liver was much lower (90% reduction) than that in KO2 (60%) (Figure 2). While the dyskerin level of KO1 was 4× lower as that of KO2 mice, the expression of most H/ACA snoRNAs in KO1 was ~10%–20% lower compared with those in KO2 mice. However, the increased expression of C/D snoRNAs in KO1 and KO2 did not show a similar correlation (Figure 6). This may be related to the fact that the level of H/ACA snoRNAs is directly affected by the absence of dyskerin whereas the increase in C/D abundance may be an indirect consequence of dyskerin ablation, though the precise mechanism remains obscure.

We conclude that the absence of dyskerin in mouse liver decreases the level of H/ACA snoRNAs and increases the level of C/D snoRNAs. The snoRNA microarray is an excellent tool to study the expression of snoRNA, and this innovative technical approach will provide valuable insights into the molecular and biological consequences of pathogenic dyskerin mutations. It may also prove useful in exploring the unknown regulation of snoRNA levels in development and disease.

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Abbreviations used

snoRNA	small nucleolar RNA
KO	knock out
WT	wild type
Cy3	cyanine dye 3
PMT	photomultipliers
ULS	universal linkage system
ANOVA	analysis of variance

UTR	untranslated region
PolyI:C	polyinosinic: polycytidylic acid
q RT-PCR	quantitative real time PCR
TOP	terminal oligopyrimidine tract sequence

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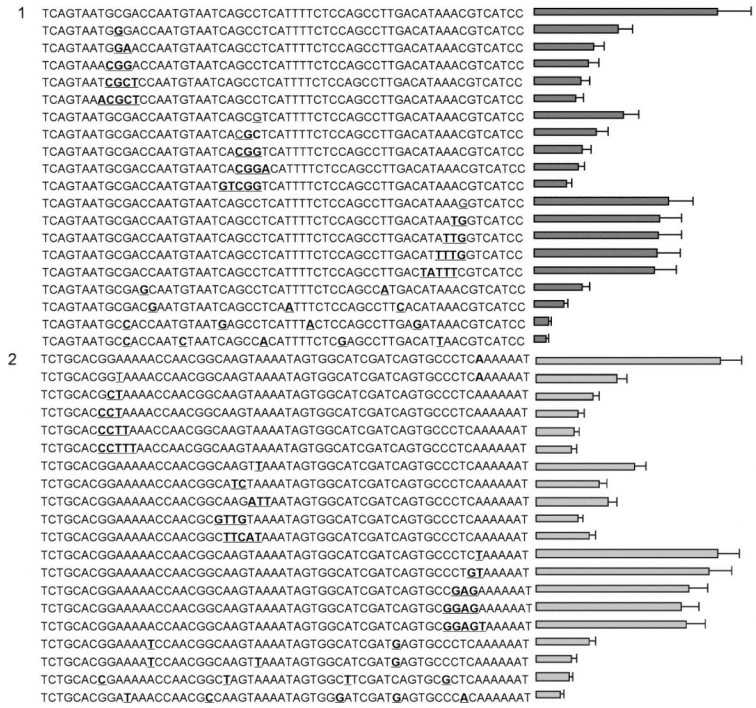

Figure 1.

Specificity of microarray probes. The sequences of the 2 groups of maize control probes and the variants with 1–5 mutations are on the left. Mutations are underlined. The maize control RNA oligonucleotides (1μg each) were chemically labeled with the Cy3 dye, and hybridized with the microarray. The mean intensity of signals and standard deviations are shown as horizontal bars on the right, and the length of the bar is proportional to the hybridization signal intensity. Error bars represent the standard deviation of the mean.

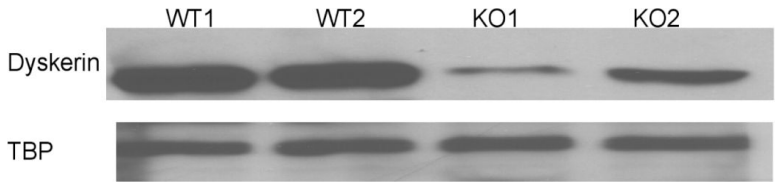

Figure 2.

Confirmation of dyskerin attenuation in the *Dkc1*^{lox}*Tg:Mx Cre* transgenic mice. The transgenic mouse was constructed as described before [13]. Nuclear liver proteins were purified and 20 μg proteins were employed for SDS/PAGE followed by immunoblotting for dyskerin and TATA box binding protein (TBP) used as a loading control. Western blot shows a 90% reduction and a 60% reduction in the amount of dyskerin in the liver of 2 mice, KO1 and KO2 respectively.

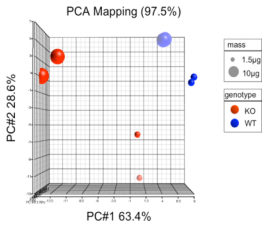

Figure 3.

Principle Component Analysis (PCA) plot of snoRNA expression from the dyskerin WT and KO liver samples. SnoRNA expression data generated by the customized Agilent microarray and derived from 2 KO and 2 WT samples at two RNA mass (1.5 μg and 10 μg) were analyzed by PCA. A three-dimensional model was developed, and there is an obvious separation of the WT and KO samples.

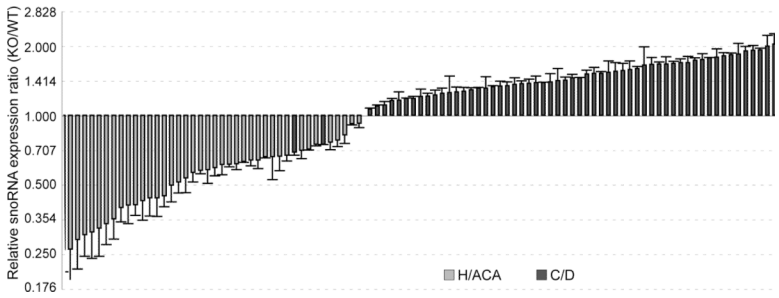

Figure 4.

Regulation of H/ACA and C/D snoRNAs in the dyskerin knock out liver by snoRNA microarray analysis. Four total RNAs, (two from KO mice and two from WT mice) from liver tissue, were chemically labeled at two input masses (10 μg and 1.5 μg). Total RNA were chemically labeled with the Cy3 dye, and hybridized with the microarray. Each column represents the average of the relative snoRNA expression ratio (KO/WT) of a different snoRNA. The y-axis is a logarithmic scale of the level of snoRNA ratio. The level of the101 snoRNAs which were significantly changed (multiple testing corrected $p<0.05$) in the KO liver, the 59 increasing C/D snoRNAs, 2 decreasing C/D snoRNAs, and 40 decreasing H/ACA snoRNAs are shown. Error bars represent the standard deviation of the mean.

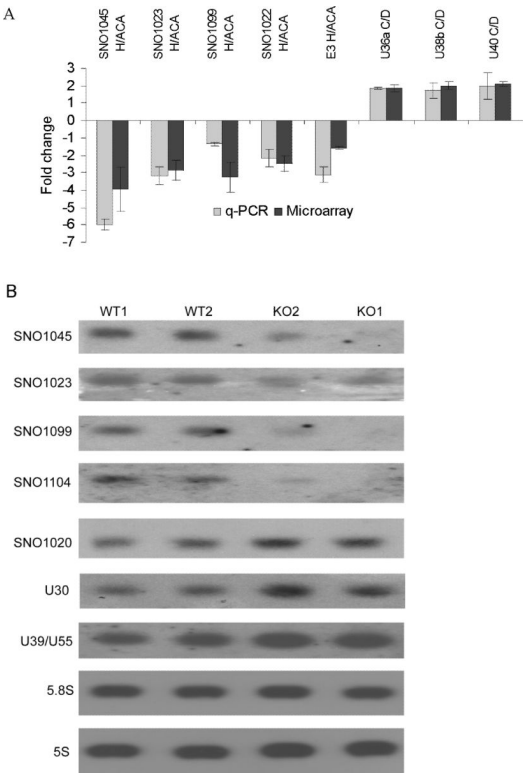

Figure 5.

Confirmation of the microarray data with q-RT PCR and northern blot. Total liver RNA was utilized for q-RT PCR analysis and northern blot A: The RT PCR results were quantified and normalized relative to β-actin mRNA levels. The KO/WT ratio of 8 randomly selected snoRNAs, including 5 H/ACA (SNO1045, 1023, 1099, 1022) and 3 C/D (U36, U38b, U40) snoRNAs, was measured by q-PCR and was compared to the level measured by the microarray. The relative change of snoRNA expression assayed using methods was consistent, and the correlation coefficients of 8 snoRNAs between microarray and q-PCR were 0.912. B: Northern blot, RNA (50 μg) was electrophoresed in 1.5% (w/v) agarose–formaldehyde gels. Nine oligonucleotides (10 pmol) were end-labeled with [γ-^{32}P]ATP and hybridized with the membrane. The four H/ACA snoRNA (SNO1104, 1045, 1023 and 1099) significantly decreased in the KO samples, and three C/D snoRNA (U39/U55, U30, and SNO1020) increased as suggested by the microarray results. Ribosome 5.8S and 5S were used as loading controls.

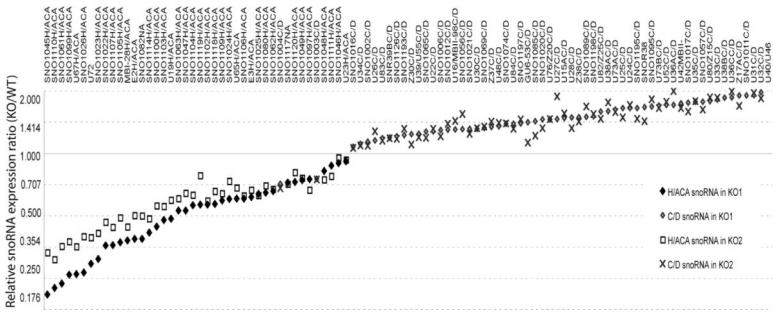

Figure 6.

Varied expression ratio of the H/ACA snoRNA in the KO mice. The dyskerin protein level is 10% (KO1) and 40% (KO2) compared to the protein level in the WT liver tissue. The relative expression ratio of snoRNA (KO/WT) from microarray data is presented in a logarithmic scale. The level of the101 snoRNAs which were significantly changed (multiple testing corrected $p<0.05$) in the KO liver are presented. The expression level of H/ACA is lower in the KO1 mice, while the expression level of C/D shows no difference.

Table 1

Primer sequences for q-PCR.

ID	Target	Primer sequence
SNO1045 1F	H/ACA snoRNA	GCTAGCAGAGTACACACAAGAAGAAAAGTA
SNO1045 1R		GAAAGAGGTCCACCCCAGTCT
SNO1023 1F	H/ACA snoRNA	GCACTCAGTGAGCCTTTTGCTA
SNO1023 1R		TGTTTTTCACTCTGCCCCTTCT
SNO1099 F	H/ACA snoRNA	CTC AGG TAT TGC TTG TTG CCT TAG T
SNO1099 R		AGC CAG TGA ATA AGG TCA GCA GTT
SNO1022 1F	H/ACA snoRNA	TCTGTCTGCGTTTTGCATTCA
SNO1022 1R		AAAAAAACACAACACTAGGCCAGTACTA
E3 F	H/ACA snoRNA	ACA ATA TAG CTG CTC AGT GCT GTG T
E3 R		TGG CAG ACA GGT ATA GTT AAG AAA CAA
U36 1F	C/D snoRNA	TGTGAGGTTCTGTGTGGCTTCT
U36 1R		TCTTTTCCCCAGCTCTTCAAAC
U38b F	C/D snoRNA	CAG GCC AGC GTG GAC TTC
U38b R		AGG CAG TCT TCA GTA AAG GAC AAG A
U40 1F	C/D snoRNA	GTAACTAGGGTGATGAAAACGTATCCT
U40 1R		TGTGTGTACAGGTGGCCAAGAC
RPS3 F	mRNA	GTTGTCCAGAAGCGCTTTGG
RPS3 R		GTGGCCACTTTCTCTGCATAAA
RPS3a 3F	mRNA	GGAACCAAAATTGCCTCTGATG
RPS3a 3R		CTGTAGATCAGCAAGGCTCACTTC
RPS8 1F	mRNA	GCTCTGAGTGTTGTACTCGCAAAA
RPS8 1R		CAAGCTCGTTGTTGGATGCAT
RPL27a F	mRNA	CACCACCACAGGATCAACTTTG
RPL27a R		TAATGCCGCATACCAACTTTCC
RPSa 1F	mRNA	CTGCGCTATGTGGACATTGC
RPSa 1R		CCACATCAGACCCACTGAGTGA
EIF4a1 1F	mRNA	GCGTCATCGAGAGTAACTGGAA
EIF4a1 1R		GAGGGACTCTGAGAGATTCATGTCA
RPS8 2F	mRNA precusor	GGCGGGGCTGAGTTTTAGAAACCG
RPS8 3R		GTCGGGAGGCAGGATGGAGAC
RPS3 2F	mRNA precusor	AGAGTGGGGCCAAGGGCTGC
RPS3 3R		CAGCTACTTACCCTGTCTGA
EIF4a1 3F	mRNA precusor	GCCAGGCTCAGATGACACGCA
EIF4a1 3R		ATCGTGGGTACCCCTGGCCG
RPSa 4F	mRNA precusor	GCTGGCCCGCAGTTAGGTGG
RPSa 4R		CCGTCCCATCTCCATGCGCC

Table 2

Function and host genes of the significantly changed snoRNAs ($p<0.05$)

Column ID	Ratio	Target RNA	Host gene
SNO1045H/ACA	0.27±0.09	? 28S	EIF4a
SNO1110H/ACA	0.27±0.06	U2 snRNA	T-complex protein 1
SNO1061H/ACA	0.29±0.09	18S	NA
U67H/ACA	0.31±0.07	18S	EIF4a1
SNO1099H/ACA	0.32±0.08	28S	RPL27a
SNO1026H/ACA	0.33±0.09	18S	EIF5
U72	0.34±0.07	5.8S	RPL30
SNO1023H/ACA	0.36±0.07	28S	RPL23
SNO1107H/ACA	0.40±0.06	18S; 28S	Tumor protein, translationally-control led1
SNO1022H/ACA	0.41±0.08	28S	importin
MBI-28H/ACA	0.41±0.04	28S	RPL27a
SNO1105H/ACA	0.43±0.08	28S	RPL32
SNO1082NA	0.44±0.08	28S	RPSa
E2 H/ACA	0.44±0.08	28S	RPSa
SNO1114H/ACA	0.45±0.05	? 28S	RPL18a
SNO1100H/ACA	0.50±0.08	28S	Glycogen synthase kinase B
SNO1103H/ACA	0.51±0.06	18S	Tbrg4
U19H/ACA	0.54±0.08	28S; U3	U19H noncoding gene
SNO1063H/ACA	0.56±0.05	? 5.8S; 28S	NA
SNO1102H/ACA	0.58±0.02	28S	RPS12
SNO1047H/ACA	0.58±0.08	? 18S	EEF1b2
SNO1104H/ACA	0.59±0.05	18S; 28S ? 18S;	NA
SNO1118H/ACA	0.61±0.06	5.8S	RPL39
SNO1109H/ACA	0.61±0.03	U6	Constitutive photomorphogenic protein 1
SNO1106H/ACA	0.61±0.02	28S	RPS16
SNO1025H/ACA	0.63±0.01	28S; 18S	NA
U65H/ACA	0.64±0.05	28S	RPL12
E3H/ACA	0.64±0.04	28S	EIF4a
SNO1024H/ACA	0.67±0.09	28S	RPL23a
SNO1119H/ACA	0.67±0.15	?18S	Ribosomal protein 10
SNO1062H/ACA	0.66±0.01	28S	NA
SNO1080H/ACA	0.67±0.04	U6 ? rRNA	WD repeat domain 22
SNO1004C/D	0.69±0.02	folding	Amiloride-sensitive cation channel 1
SNO1097H/ACA	0.71±0.06	28S	Ribosomal protein, large P2
SNO1117NA	0.71±0.01	28S	EIF4a
SNO1003C/D	0.75±0.01	NA	Huntingtin-interacting protein-1 protein interactor
SNO1049H/ACA	0.75±0.01	NA	NA
SNO1048H/ACA	0.78±0.05	NA	dyskerin

Column ID	Ratio	Target RNA	Host gene
SNO1120H/ACA	0.77±0.06	? 28S	RPSa
SNO1111H/ACA	0.82±0.07	18S	nucleolin
U23H/ACA	0.92±0.01	18S	nucleolin
SNO1046H/ACA	0.92±0.04	? 18S	chapronin subunit 6a
SNO1016C/D	1.06±0.01	NA	WD Repeat domain 43 isoform 5
U34C/D	1.09±0.01	NA	RPL13a
SNO1002C/D	1.11±0.04	NA	Glioma tumor suppressor candidate region gene 2
Z30C/D	1.16±0.10	U6 snRNA	NA
U83C/D	1.16±0.02	NA	BAT1
SNR39BC/D	1.18±0.01	28S	EIF4a2
SNO1126C/D	1.18±0.02	28S	EIF4a2
U39/U55C/D	1.21±0.03	28S	RPS8
U26C/D	1.21±0.09	NA	UHG
SNO1065C/D	1.22±0.06	NA	NA
SNO1006C/D	1.25±0.06	NA	NA
GU6-53C/D	1.26±0.20	NA	Chromodomain helicase DNA binding protein 8
SNO1193C/D	1.26±0.06	18S	RPS12
SNO1021C/D	1.27±0.05	NA	Chromodomain helicase DNA binding protein 8
U22C/D	1.29±0.03	NA	UHG
SNO1085C/D	1.32±0.15	NA	NA
U30C/D	1.31±0.01	NA	UHG
SNO1012C/D	1.34±0.06	NA	NA
SNO1069C/D	1.33±0.01	NA	Adaptor protein complex AP-1
U84C/D	1.34±0.05	NA	BAT1
U16/MBII-98C/D	1.36±0.09	18S	RPL4
U48C/D	1.37±0.04	28S	C6orf48
SNO1064C/D	1.40±0.16	NA	NA
Z37C/D	1.37±0.06	5.8S	guanine nucleotide binding protein (G protein)
SNO1074C/D	1.38±0.01	NA	WD Repeat domain 43 isoform 5
SNO1020C/D	1.38±0.09	NA	RPL3
SNO1197C/D	1.42±0.03	28S	NA
U28C/D	1.40±0.12	NA	UHG
SNO1056C/D	1.42±0.17	NA	Nucleolar protein 5
U76/Z20C/D	1.45±0.01	28S	GAS5
Z38C/D	1.45±0.05	28S	guanine nucleotide binding protein (G protein)
SNO1198C/D	1.52±0.02	28S	unigene Hs281434
U15AC/D	1.51±0.05	28S	RPS3
U82/Z25C/D	1.52±0.09	18S	Nucleolin
SNOR38	1.54±0.17	28S	unigene Hs281434
SNO1195C/D	1.55±0.13	28S	unigene Hs281434
U25C/D	1.56±0.12	NA	UHG
SNO1089C/D	1.58±0.13	NA	RPL4

Column ID	Ratio	Target RNA	Host gene
U73AC/D	1.59±0.03	NA	RPS3a
U27C/D	1.67±0.30	NA	UHG
U73BC/D	1.67±0.03	NA	RPS3a
U24C/D	1.67±0.04	NA	RPL7a
SNO1017C/D	1.66±0.11	NA	Nucleolar protein 5A
U38AC/D	1.67±0.12	28S	RPS8
U42MBII-287C/D	1.69±0.06	18S	RPL23a
SNO1057C/D	1.69±0.11	NA	NA
U52C/D	1.73±0.04	28S	C6orf48
SNO1095C/D	1.75±0.10	NA	NA
U35C/D	1.77±0.01	18S	RPL13a
Z17AC/D	1.79±0.14	18S	RPS11
U80/Z15C/D	1.81±0.05	28S	GAS5
U36AC/D	1.84±0.20	18S	RPL7a
U33C/D	1.83±0.03	18S	RPL13a
U32C/D	1.90±0.09	18S	RPL13a
U36CC/D	1.91±0.09	18S	RPL7a
U31C/D	1.92±0.03	NA	UHG
U38BC/D	2.00±0.21	28S	RPS8
SNO1011C/D	2.04±0.21	NA	NA
U40/U46	2.11±0.13	28S	RPS8

Table 3

Up-regulation of the host genes of the snoRNAs after dyskerin deletion by q- PCR. The mRNA of three host genes for C/D snoRNA and three for H/ACA snoRNA were measured, and the levels of snoRNA related to each host genes was measured by microarray. The last column shows the change in abundance of the 4 pre-mRNAs that we could measure.

SnoRNA	Ratio KO/WT	Host gene	Host gene mRNA KO/WT	Host gene pre-mRNA KO/WT
C/D U73a	1.59±0.03	RPS3	1.74±0.43	1.09±0.03
C/D U73b	1.67±0.03			
C/D U15a	1.51±0.05	RPS3a	1.45±0.60	NA
C/D U38	1.67±0.12	RPS8	1.20±0.22	2.05±0.59
C/D U39	1.21±0.03			
C/D U40	2.11±0.12			
H/ACA MBI-28	0.41 ±0.04	RPL27a	1.15±0.01	NA
H/ACA E2	0.44±0.08	RPSa	1.16±0.03	1.13±0.41
H/ACA SNO1120	0.76±0.06			
H/ACA U67	0.31±0.06	EIF4a1	1.28±0.35	1.02±0.02