

Stability of microRNA in partly degraded RNA extracted from porcine lung tissue



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Conclusion

Initial results indicate that miR-23a, miR-26a and miR-34a are comparatively stable at room temperature in porcine lung tissue within the first 24 h after retrieval.

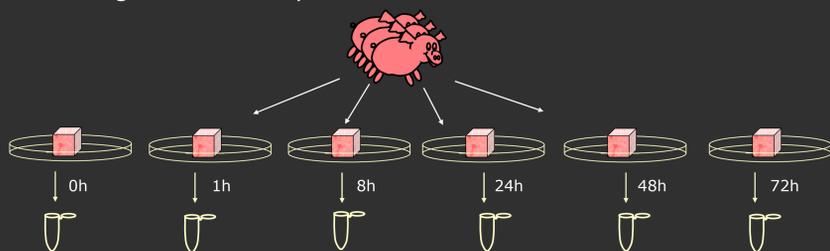
Introduction

MicroRNAs have gained considerable interest as small non-coding RNA playing a prominent role in the post-transcriptional epigenetic regulation of gene expression. In the diagnostic field it is being investigated if expression profiles of these small non-coding RNAs can be used as a host signature or fingerprint to yield information on the nature of ongoing infections. In principle this involves the investigation of tissue to reveal correlations between the known infection status of an animal and the presence and composition of microRNAs in its tissue.

We investigated the usability of microRNAs for quantitative RT-PCR in partly degraded RNA isolated from lung tissue. The stability of microRNA was previously found to be relatively high in formalin-fixed paraffin-embedded tissue (Li et al., 2007). The small size of microRNA could account for the greater tolerance to degradation as compared to normal-sized mRNA (Fleige et al., 2006).

Materials and Methods

1. Lung tissue obtained from three healthy pigs by necropsy was cut into 1cm x 1cm pieces and stored at room temperature in Petri dishes for 0 h, 1 h, 4h, 8h, 24 h, 48 h, and 72 h, respectively.
2. At the stated time points RNA Later (Invitrogen) was added to stabilize the RNA
3. Total RNA (including small RNA) was extracted using TRI Regent (Sigma)
4. The integrity of the RNA was determined using two different chips (RNA Nano and Small RNA) on the Agilent Bioanalyser.
5. cDNA synthesis and real time PCR for reference genes and microRNA using SYBR green chemistry.



Results and Discussion

RNA degradation:

A correlation coefficient of 0.87 was found between RNA degradation (RNA integrity number (RIN)) and time (h), confirming a linear increase with time in the degradation of RNA in the lung tissue at room temperature. During the time span of this study RIN decreased from 9.0 (± 0.12) at time 0 h to 4.7 (± 1.57) at time 72 h (Table 1).

Electropherograms of small RNA confirmed these results, ranging from high quality RNA samples, with a clear tRNA peak at time 0 h to highly degraded samples after 72 h (Figure 1).

Table 1: RIN over time

PIG	0h	1h	4h	8h	24h	48h	72h
101	9.1	8.6	8.9	6.4	8.4	6.3	6.1
102	8.9	9.1	8.6	8.5	7.4	6.7	5.0
104	8.9	9.0	8.5	8.9	7.5	6.2	3.0
Mean	9.0	8.9	8.7	7.9	7.8	6.4	4.7
SD	0.1	0.3	0.2	1.3	0.6	0.3	1.6

Relative concentration of mRNA and microRNA:

The relative concentration of three well described reference genes (B2M, β -actin and GAPDH) was compared with the relative concentration of three putative microRNA reference genes: miR-23a, miR-26a and miR-34a as well as SNORD48 at five time points, using quantitative RT-PCR after total RNA extraction and quantification (Nanodrop spectrophotometry). The concentration of miR-23a, miR-26a and miR-34a was found to be relatively stable within the first 24 h, whereas the concentration of the three mRNA reference genes started to decrease within less than 8 h (see Figure 2)

Previous studies have established that RNA stability is dependent on variation in the type and quantity of active ribonucleases as well as differences in tissue structure (Schoor et al., 2003; Seear and Sweeney, 2007). In this study we tested the stability of microRNA and mRNA in partly degraded RNA isolated from porcine lung tissue. Initial results indicate that mRNA coding for B2M, GAPDH and β -actin are degraded consecutively during the 72 h period, whereas miR-23a, miR-26a miR-34a, and SNORD48 are comparatively stable at room temperature in lung tissue within the first 24 h after retrieval.

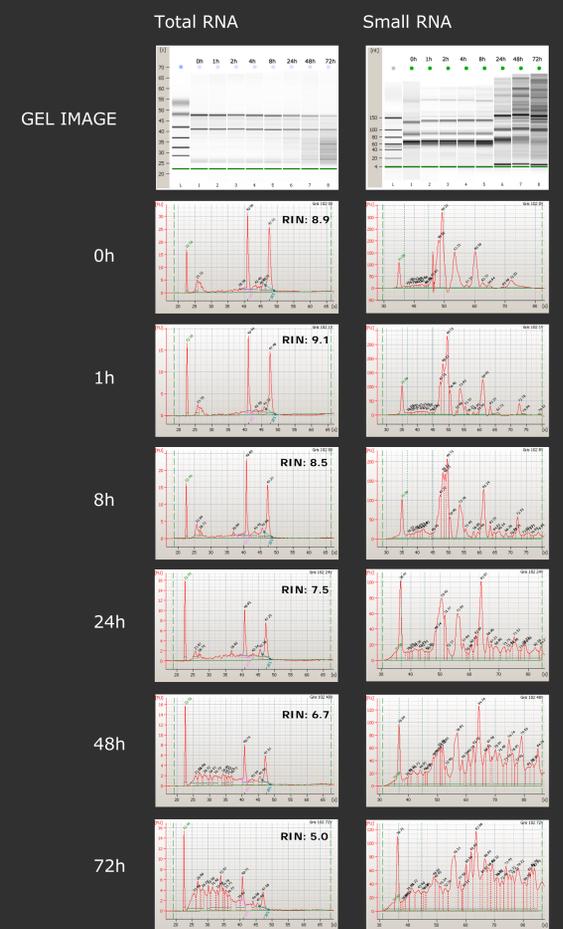


Figure 1: Gel images and elektropherograms at different timepoints

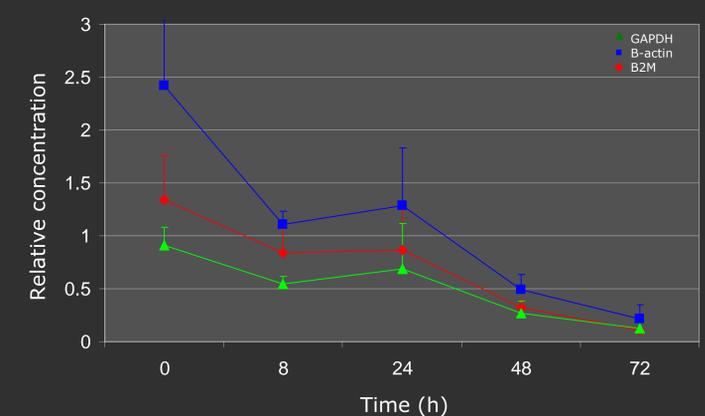
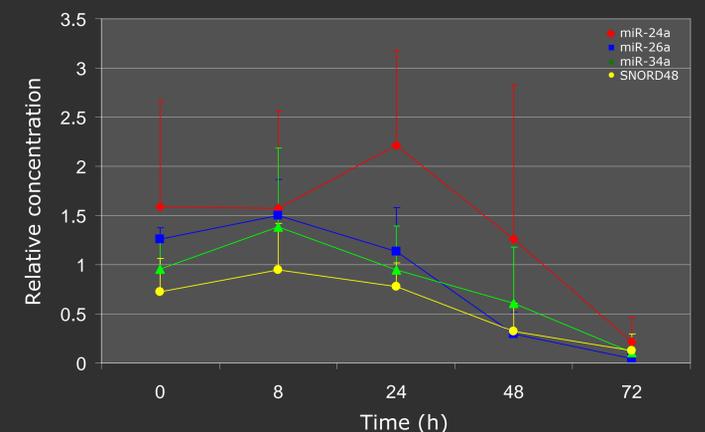


Figure 2: Relative concentration of small RNAs above and mRNA below over time

