MiR-122 and -200a in Exosomes of ART+ HIV-1 Infected Individuals with Liver Disease

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Aim

- The primary objective was to investigate whether circulating miRNAs were associated with liver disease and/or liver related mortality in a set of ART-treated HIV-1 infected individuals.
- Any miRNAs found to be associated with liver disease were analysed to determine if they were increased in the Ago or the exosome portion of circulating miRNAs.

Introduction

- Circulating microRNAs (miRNA) are promising biomarkers.
- They exist outside the cellular environment either bound to Ago protein, non-specifically released due to cell death, or protected inside exosomes, specifically released and involved in inter-cellular communication.
- We previously analysed the levels of 21 miRNAs in the serum of 126 cases (individuals who died whilst on ART during the SMART and ESPRIT trials) and 247 matched controls as predictors of mortality (All-cause, cardio-vascular and malignancy) (Murray, DD et al. PLOS ONE, 2015).
- While none of these miRNAs showed any associations with mortality (All-cause, cardiovascular and malignancy), we further analysed these individuals to explore associations between circulating miRNAs and liver disease.

Methods

- 21 miRNAs were measured, by RTqPCR, in the serum of cases and controls from the SMART and ESPRIT studies.
- These miRNA were analysed for associations with liver disease
- The liver cases and controls from these two groups (Figure 1) had their exosomes purified using the Exoquick™ reagent .
- After purification the size and number of exosomes were measured using a Malvern NanoSight NS300.
- Total RNA (including miRNA) was extracted from the purified exosomes using Trizol Reagent with cel-miR-39 as a spiked in control.
- Levels of miR-122, miR-200a, Let-7e and Cel-miR-39 were quantified, by RTqPCR, using Taqman® miRNA assays.
- RTqPCR data was normalised to cel-miR-39 and divided by exosome number to give a relative quantity of miRNA per 10^8 exosomes.

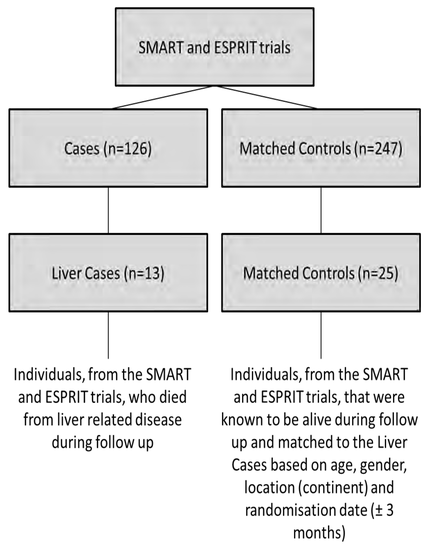

Figure 1. Selection of Liver cases and controls from the SMART and ESPRIT studies. 13 of the original 126 cases died from liver related diseases. These 13 cases had 25 matched controls.

Table 1. Causes of death for the liver cases.

Cause of Death	Number	%
HCV with cirrhosis	4	30
HCV with liver failure	1	8
HCV with liver cancer	1	8
HBV with cirrhosis	2	15
HBV with liver failure	1	8
HBV with liver cancer	1	8
Liver failure (other than HCV and HBV Liver failure)	3	23

Results

- MiR-122 and miR-200a are increased in the serum of HCV/HIV co-infected individuals, from the SMART and ESPRIT studies, compared to HIV-1 mono-infected individuals (HIV-M) (Figure 2).

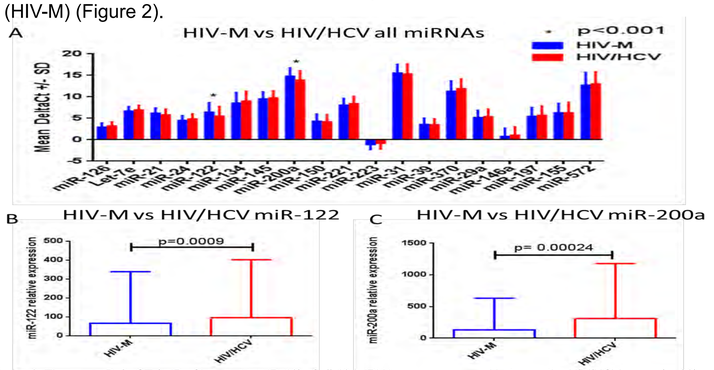

Figure 2. The mean DeltaCT (miR of interest – miR-16) of all 19 miRNAs were compared between the HIV/HCV group (n=82) and the HIV-M group (n=272) using a multiple-t test with sidak-bonferroni correction (A). The relative expression of the two significantly different miRNAs between the two groups is also plotted (B and C). 18 individuals from the SMART and ESPRIT cohorts did not have their HCV status recorded and were excluded from the analysis while 1 individual failed PCR and was also excluded.

- Serum levels of miR-122 and miR-200a correlate with the liver transaminases ALT and AST in the SMART and ESPRIT individuals (Figure 3).

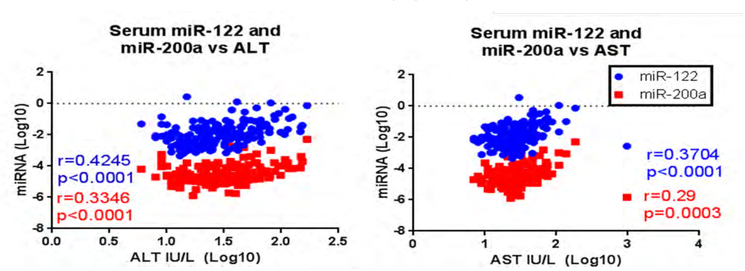

Figure 3. Relative expression (normalised to miR-16) of miR-122 and miR-200a, measured in the serum of the 126 cases and controls from the SMART and ESPRIT trials, and AST/ALT were log normalised and plotted. Correlations were analysed using Spearman's non-parametric correlation coefficient with a relationship deemed significant with p<0.05. Matched XY pairs were available for 178 individuals in the miR-122 vs AST analysis, 154 in the miR-200a vs AST, 180 in the miR-122 vs ALT and 160 for miR-200a vs ALT.

- MiR-122 (2 fold) and miR-200a (1.22 fold) levels were significantly higher in the serum of the liver cases (Figure 4a and Figure 4b).
- There was no difference in let-7e (p=0.75), included as a control (Figure 4c).

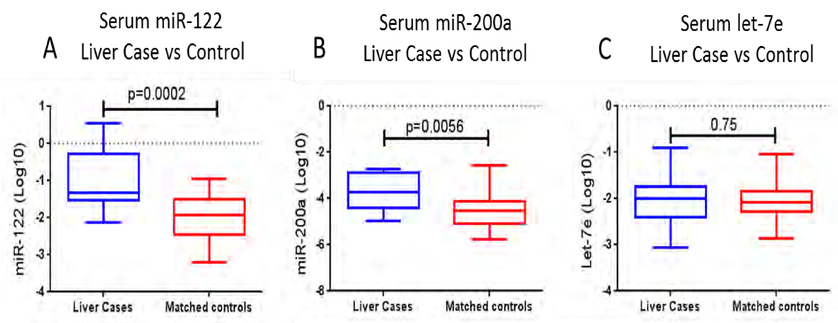

Figure 4. Relative expression (normalised to miR-16) of A) miR-122, B) miR-200a and C) Let-7e were log10 normalised and plotted for the Liver Cases (n=13) and their matched controls (n=25). Differences were measured using a Mann-Whitney T test with differences deemed significant with p<0.05.

- In the purified exosomes of the liver cases expression of miR-122 was significantly up-regulated (3.3 fold increase in mean of liver cases compared to controls) (Figure 5a).
- miR-200a expression, while not significant, was also increased in the liver cases (4.5 fold increase) (Figure 5b).
- miR-200a (n=31) was amplified in less individuals than miR-122 (n=38) and if sample size is increased significance may emerge.
- Let-7e was unchanged (Figure 5c).

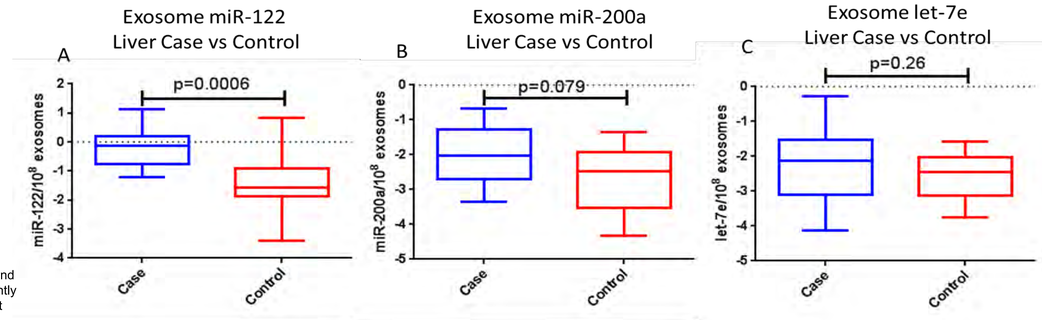

Figure 5. The levels of A) miR-122, B) miR-200a and C) let-7e were measured in the purified exosomes of the liver cases and controls. miRNA levels were normalised to cel-miR-39 and divided by exosome concentration to give an estimation of relative amount of miRNA per 10^8 exosomes. miR-122 was clearly increased in liver cases (n=13) compared to controls (n=25) and miR-200a also approached significance showing a clear trend for increase in liver cases (n=11) compared to controls (n=20). Let-7e was no different between liver cases (n=11) and controls (n=20). Not all PCRs were successful at amplifying the miRNAs so the full complement of cases and controls were not always available for the analyses. Differences were measured using a Mann-Whitney T test with differences deemed significant with p<0.05.

- Exosomal miR-122 and miR-200a correlated with ALT but not AST (Figure 6).

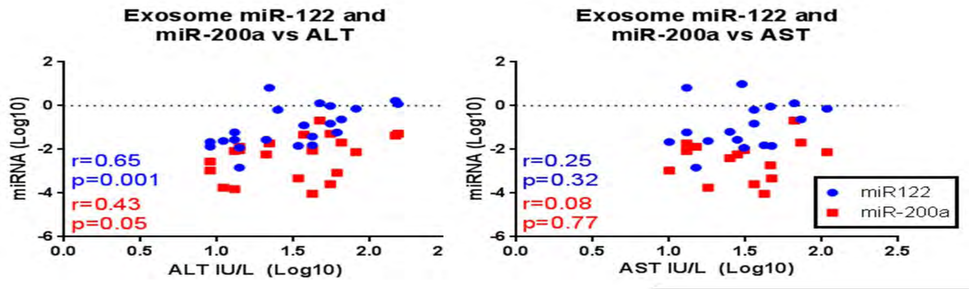

Figure 6. Relative expression (normalised to cel-miR-39 and exosome number) of miR-122 and miR-200a, measured in the exosomes of the Liver cases and controls and controls from the SMART and ESPRIT studies, and AST/ALT were log normalised and plotted. Correlations were analysed using Spearman's non-parametric correlation coefficient with a relationship deemed significant with p<0.05. XY pairs were available for 22 individuals in the miR-122 vs ALT analysis, 21 for miR-200a vs ALT, 17 in the miR-122 vs AST analysis, 15 for miR-200a vs AST.

Conclusion

- In HIV-1 positive, ART-treated individuals with liver disease, circulating levels of miR-122 and miR-200a are elevated.
- Both miR-122 and miR-200a are increased, at baseline, in the serum and exosomes of HIV-1 infected individuals that went on to develop fatal liver disease.
- These miRNAs may provide novel biomarkers for liver disease in both the HIV-1 infected and non-HIV-1 infected populations.

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