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Circulating microRNA profile in a mouse model of Crimean-Congo haemorrhagic fever

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ABSTRACT

Crimean-Congo haemorrhagic fever (CCHF) is a severe disease leading to high mortality in humans. Early diagnosis and evaluation of the severity are necessary to improve patient survival. In a model of CCHF virus-infected interferon-receptor-deficient (IFNAR) KO mice, we found a specific circulating miRNA (c-miRNA) profile when compared to wild-type (wt), resistant mice. Among this response, 20 c-miRNA were shown to be specifically altered, including miR-122-5p, miR-216a-5p, 217-5p, miR-29a-3p and miR-511-5p. Using a logistic regression analysis, a combination of 8 miRNAs allowed a 100% discrimination of mice developing a severe illness (IFNAR-KO) from non-detectable clinical signs (wt).

Crimean-Congo haemorrhagic fever virus (CCHFV) is a negative strand RNA virus (Orthonairovirus genus, Bunyaviridae family), endemic in African, European and Asian areas. CCHFV is transmitted to humans mainly by tick bites. Crimean Congo haemorrhagic fever (CCHF) is severe and often associated to a multiple organ failure (MOF) leading to a high mortality (up to 30%). The infection is associated to clinical findings (leukopenia, thrombocytopenia, disseminated intravascular coagulation, haemorrhagic syndrome, etc), severity and fatality predictors, such as TNF-α, IL-6, viral load and the absence of antibodies (Ergonul et al., 2006). CCHFV induces asymptomatic infections in most host animals and rarely in humans.

MicroRNAs (miRNA) are involved in numerous biologic processes. They can be measured in most biological fluids because they are secreted by the cells or passively leak through altered cell membranes. Circulating miRNA (c-miRNA) had gained attention as biomarkers of various illnesses. Several viral diseases are associated with an altered c-miRNA expression corresponding to host responses to infection. C-miRNA profiling has been described in several acute and chronic viral infections such as Hepatitis c virus (HCV), Ebola virus (EBOV), Hendra virus (HeV), Foot-and-mouth disease virus (FMDV), whereas c-miRNA signature was described as a biomarker to predict the outcome or therapy efficacy (Cowled et al., 2017; Stenfeldt et al., 2017). Little is known about c-miRNA in CCHFV infection. In this study, c-miRNA profile was analysed in an *in-vivo* model of CCHFV infection using the interferon-receptor-deficient (IFNAR-KO) mice model (Bereczky et al., 2010). IFNAR-KO and wild-type (wt) mice were infected with CCHFV. The peripheral blood was collected during the course of the infection to perform c-miRNA detection in sera. We observed c-miRNA expression and profile alteration in infected mice and a characteristic panel of miRNA was identified that could highlight a new class of outcome predictive biomarkers.

Groups of ten wt (129S2/SvPasCrl) and fifteen IFNAR-/- mice were inoculated with 12.5 LD50; corresponding to a 10 FFU challenge dose of CCHFV (IbAr10200, Nigeria). Control mice were mock infected with supernatant from non-infected cells. Each group was monitored until death or up to 14 days post infection (p.i.). Daily, between days (D) 1 to 6 p.i., sera were harvested from sacrificed mice. Liver, spleen, lung and brain were stored at −80 °C until viral titration. CCHFV was quantified

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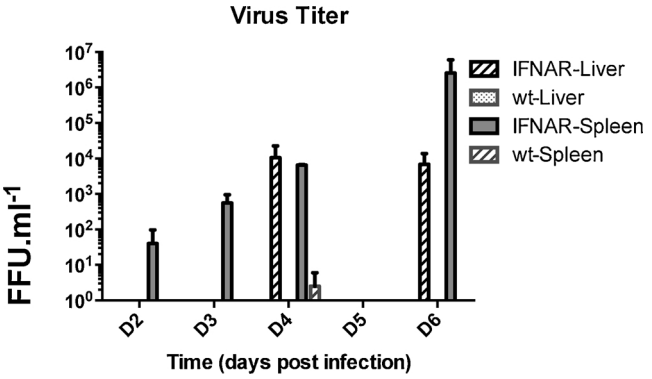

Fig. 1. Virus load in liver and spleen of CCHFV-infected wt or IFNAR-KO mice (FFU ml^{-1}).

using plaque assay and qRT-PCR as previously described (Peyrefitte et al., 2010). All procedures involving infectious CCHFV were performed in a Biosafety Level 4 facility (INSERM P4 Jean Merieux, Lyon, France) according to standard operating procedures approved by the institutional biosafety committee. Animal experiments were approved by the regional ethical committee CECCAPP (C2EA15) and French Animal regulation (committee number B69-387-05-02). After intraperitoneal inoculation, mice were monitored twice a day and euthanized by using endpoint-scoring criteria. All animals were handled in accordance to European laws on ethics in animal experiment; all efforts were made to minimize suffering. All wt and IFNAR control mice survived, as well as the wt mice challenged with 12.5 LD50 while none of the challenged IFNAR-mice survived day 6 p.i. For the wt CCHFV-challenged mice, CCHFV was undetectable in tissues from day 1 to 6 p.i. (except for a very low titer in the spleen of one mouse at D3). In IFNAR CCHFV-challenged mice, CCHFV was observed in the spleen (D2) and the liver (D4) (Fig. 1), but not in lung or brain.

miRNA measurement was performed after total RNA isolation from 100 µL of serum with Trizol LS (Invitrogen). Reverse transcription was performed using Universal cDNA Synthesis Kit (Exiqon). First, a pool of cDNA was prepared for each KO groups (non-infected; D1-D2; D3-D4; D6). C-miRNA profiling was performed with miRnome Mouse&Rat panel I&II (Exiqon). The serum profiling identified 84 reliably detectable miRNAs in CCHFV-infected KO mice compared to non-infected KO mice (Fig. 2A).

Then, 44 miRNA were selected based on their detectability and response to infection. They were measured on each individual sample with Pick&Mix plates (Exiqon). The PCR results were normalized using 3 endogenous reference miRNA (miR-103-3p, let-7f-5p, let-7g-5p). KO and wt groups for D1-D2, D3-D4 and D6 were compared with a non-parametric ANOVA (Kruskal-Wallis).

Among selected miRNAs measured in individual samples, 20 were significantly ($p \leq 0.05$) altered in KO groups (Fig. 2B). A significant increase of several miRNA at D3-D4 or D6 was observed in response to infection. The miR-28a-5p and miR-29c-3p had a similar profile but did not reach statistical significance (Fig. 3A). The logistic regression analysis identified a combination of 8 miRNAs that allowed a 100% discrimination of mice developing a severe illness (IFNAR-KO) from non-detectable clinical signs (wt), as shown by a resulting area under the curve (AUC) = 1 of the Receiver Operating Characteristics ROC curve (Fig. 3B).

This study showed that a specific c-miRNA profile could be observed in our *in vivo* model of CCHFV infection. Moreover, a panel of miRNA is likely predictive of the mice outcome. Whether a similar profile can be found in human is not known. However this approach deserves further study to determine if circulating miRNAs could be new, clinically relevant prognostic tools.

As previously described for viral infections, we observed an altered c-miRNA profile in CCHFV-infected IFNAR-KO mice, different from miRNAs altered in peripheral blood mononuclear cells from CCHF patients (Demir et al., 2017). The origin of c-miRNA modulations are not fully understood, however two hypotheses can be made: i) high levels of circulating c-miRNA could reflect a passive and non-specific release from injured cells and organs. Interestingly, miR-122-5p is highly expressed in hepatocytes and is a relevant biomarker of liver damage (Bala et al., 2012; Schueller et al., 2018). Similarly, miR-216a-5p and 217-5p are biomarkers of pancreatic tissue damage (Goodwin et al., 2014). Part of the c-miRNA modulation observed in CCHFV-infected mice could result from the tissue damage and possibly the MOF; ii) c-miRNA response reflects the host response to infection. We described here a substantial increase in miR-146a-5p. This miRNA was increased in blood and sera of EBOV, HeV and FMDV-infected organisms (Cowled et al., 2017; Duy et al., 2016; Stenfeldt et al., 2017). This specific miRNA likely plays a role in HeV replication and is up regulated in HIV-infected T-cells (Goodwin et al., 2014; Reynoso et al., 2014). Therefore the miR-146a up regulation in our model could reflect the host response to CCHFV infection. Likewise, the increase in miR-29a-3p and miR-511-5p highlighted in our model had also been reported during an EBOV infection (Duy et al., 2016).

The logistic regression analysis identified an 8-miRNA set that allowed the discrimination of seriously ill from non-sick mice. This observation deserves further work to strengthen this result. However, if confirmed, it could provide a new clinical severity predictive tool for the CCHFV infection.

The main limitation is this study is the low number of samples in each group, mainly due to the experimental constraints of CCHFV handling and animal use rule. However, because of the very substantial differences we observed and the consistency during the course of time, the data suggest that these results are reliable enough to propose a proof of concept.

In this study, infected IFNAR-KO mice developed the classical disease, associated with weight loss and 100% mortality, and a different c-miRNA profile compared to the non-infected mice, suggesting that a characteristic c-miRNA signature resulted upon CCHFV infection. Further studies are needed to confirm that this specific miRNA profile could be used as predictive biomarkers of severity and outcome of the disease.

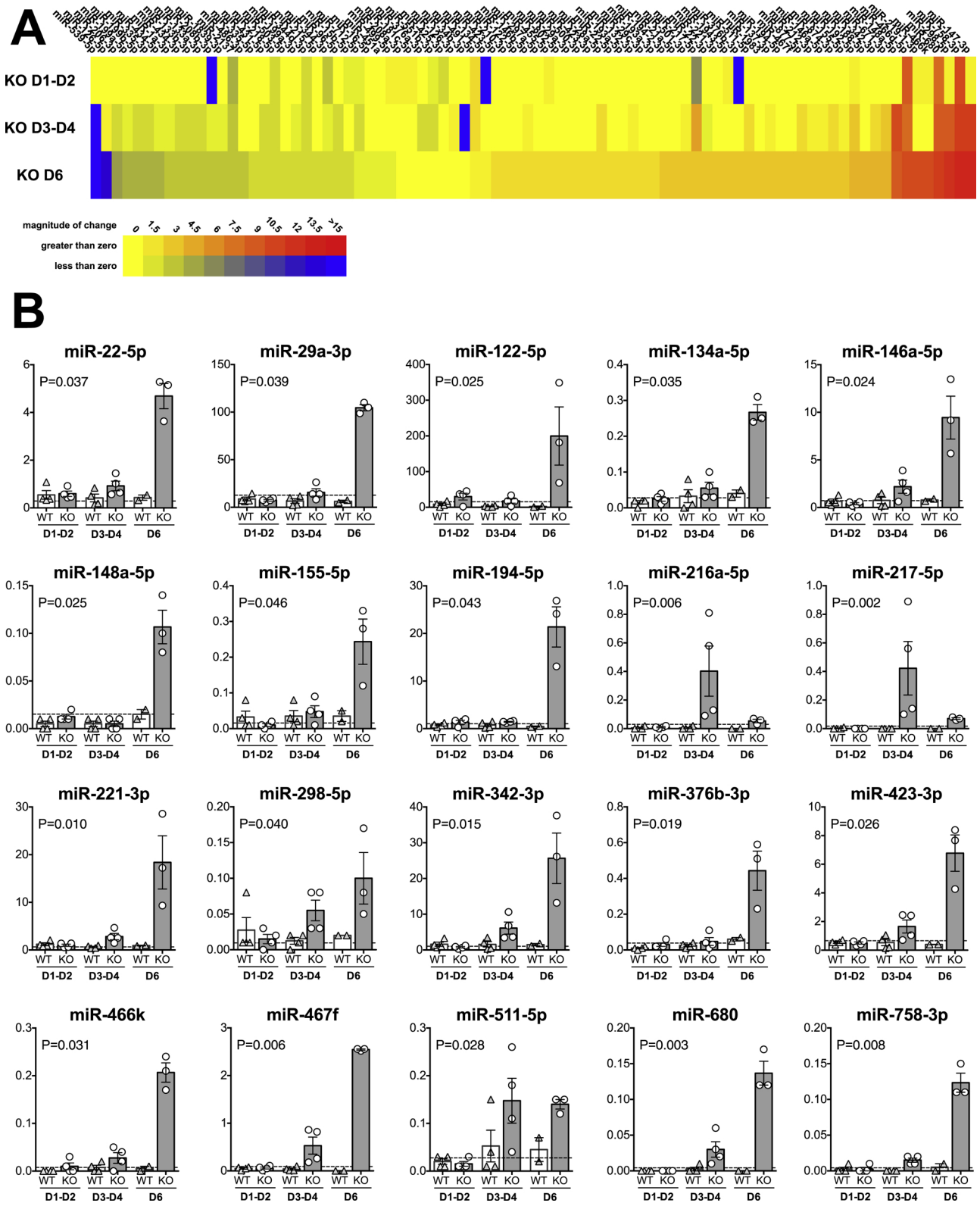

Fig. 2. A) Circulating miRNA PCR profiling: heatmap of miRNA expression in pools of sera from CCHFV-infected IFNAR-KO mice at day 1–2, 3–4 or 6 post-infection compared to non-infected IFNAR-KO mice (Arbitrary units); B) Circulating levels of 20 miRNAs significantly altered by CCHFV infection. MiRNA were measured in sera of CCHFV-infected wt or IFNAR-KO mice at day 1–2, 3–4 and 6 post-infection (Arbitrary units). The bars represent the mean ± SEM, the dashed line represents the mean value for uninfected IFNAR-KO mice, the p value of the overall effect is presented (Kruskall-Wallis).

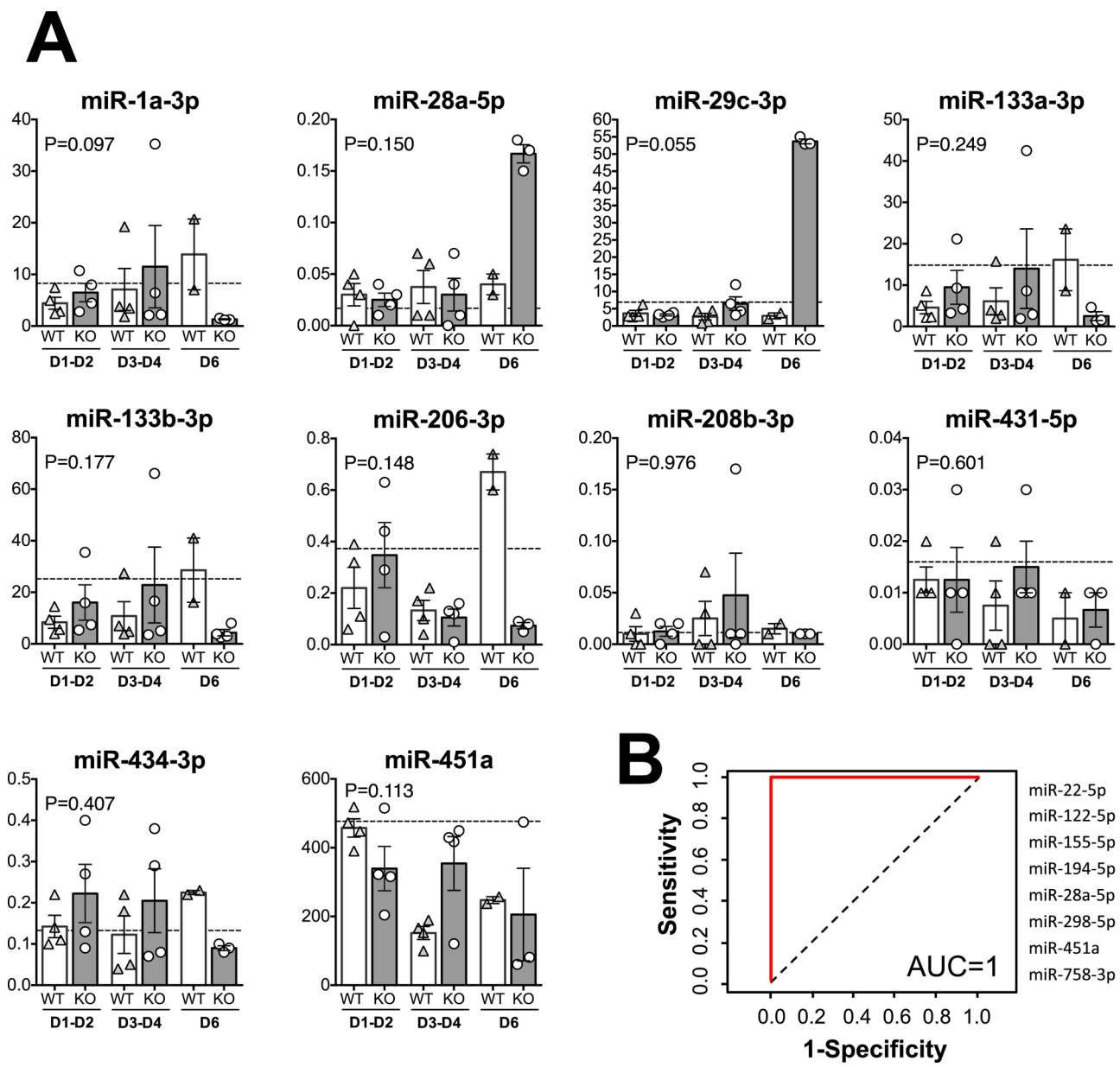

Fig. 3. B) Circulating levels of 10 miRNAs not significantly altered by CCHFV infection. MiRNA were measured in sera of CCHFV-infected wt or IFNAR-KO mice at day 1–2, 3–4 and 6 post-infection (Arbitrary units). The bars represent the mean ± SEM, the dashed line represents the mean value for uninfected IFNAR-KO mice, the p value of the overall effect is presented (Kruskall-Wallis). B) Diagnostic performance of a combination of 8 miRNAs identified with a logistic regression analysis. The Receiver Operating Characteristics (ROC) curve and the resulting area under the curve (AUC) are presented.

Conflict of interest

The authors declare to have no conflict of interest.

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