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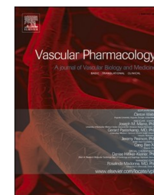

miR-10b promotes aortic aneurysm formation and aortic rupture in angiotensin II-induced ApoE-deficient mice

A R T I C L E I N F O

Keywords
Aorta
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A B S T R A C T

Abdominal aortic aneurysm (AAA) is associated with increased plasma levels of microRNA (miR) -10b. 5 nmols of miR-10b or miR control was administrated to Apolipoprotein *E*-deficient mice three days prior implantation of osmotic mini-pumps containing angiotensin II, and for three additional times once a week, which increased expression of miR-10b in plasma. Animals receiving miR-10b had a mortality rate due to aortic rupture of 61% compared to 11% in the miR controls ($p < 0.05$). Further, miR- 10b resulted in an increased aneurysm formation and growth ($p < 0.05$), which was accompanied by increased elastin degradation, neutrophil and mast cell markers ($p < 0.05$). In conclusion, miR-10b is functionally affecting aneurysm development and rupture and not only a marker of AAA. More mechanistic studies are required to better understand miR-10b's role in AAA formation.

Subject terms

Animal models of human disease
Basic science research
Aneurysm

1. Introduction

Abdominal aortic aneurysm (AAA) is characterized by aortic dilatation and weakening of the vascular wall [1]. Remodeling of the vascular wall is associated with inflammatory processes [2] leading to extra cellular matrix (ECM) destruction. Lately, microRNAs (miR) have emerged as biomarkers. Several miRs are altered in plasma from humans with AAA [3,4] of which miR-10b was strongly upregulated. The mechanism behind such strong association is unknown. MiR-10b has been explored in different types of cancer [5,6] and cholesterol efflux in leukocytes [7]. Its role in the vascular wall is scarce, however miR-10b is known to induce SMC proliferation from atherosclerotic plaque [8]. We hypothesize that miR-10b affect aneurysm development and is not only a marker of such disease.

The aim of this study was to explore if miR-10b influence aneurysm development in a mouse model of AAA and explore possible mechanisms.

2. Methodology

2.1. Mouse model of abdominal aortic aneurysm

Experiments were performed in accordance with the local ethical committee. 10 weeks old male Apolipoprotein *E*-deficient ($ApoE^{-/-}$) mice were implanted with osmotic pumps (Aztec Mod #1004) containing 100 μl of either saline solution (vehicle, $n = 5$) or angiotensin II (Ang II, 1 μg/kg/min, Sigma # A9525). Mice receiving Ang II were given i.p. injections of 5 nmol miR-10b mimic ($n = 18$) or miR mimic control ($n = 9$) three days before surgery, during surgery, and every 7 days for another two weeks. miRs were obtained from Horizon™. After 4 weeks ultrasound was performed, and supra renal aortic size was measured. The supra renal aorta was isolated and divided into two parts, the superior part was collected for mRNA analysis, whereas the inferior part for histological analysis. AAA was defined as aortic diameter ≥ 50% increase from control mice.

2.2. Semi-quantitative PCR measuring alterations in gene expression

Total RNA was extracted from aortas of mice that did not die due to rupture, complementary DNA (cDNA) synthesized and standard semi-quantitative PCR (qPCR) was performed. Relative levels of gene expression were normalized using a housekeeping gene (TATA-binding protein, TBP) and fold changes were normalized against the specific control groups.

2.3. Histology and quantification of elastin degradation

OCT-fixed blocks were cut into 10 μm sections. Hematoxylin-eosin staining was performed, and aortic size was measured by the perimeter of external elastic layer from where diameter of vessels was obtained. ImageJ (Wayne Rasband) was used for analysis. Neutrophil elastase (antibody Cat # PA5–29659, Thermo Fisher) was used following protocol and scored based on their positivity from 0 to 3. Elastin degradation was observed in aortic sections, scored blinded and graded from 0 (elastin fibers intact) to 3 (destruction of elastin fibers). Mast cells were stained using Toluidine blue (Sigma).

2.4. Statistical analysis

All data are expressed as means ± standard errors of the mean (SEM).

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Table 1
Mortality and AAA incidence of angiotensin II induced and miR-10b treated mice and controls.

Treatment	Mortality	P value	AAA incidence	P value
	N (%)		N (%)	
miR-10b Control	1/9 (11%)		3/9 (33%)	
miR-10b	11/18 (61%)	**0.039**	16/18 (88%)	**0.011**

Student's *t*-tests or Mann-Whitney *U* tests were performed when two groups were compared. Chi square with Yate's correction was used for mortality and incidence rates. Statistical analyses of experimental data were performed using SigmaPlot version 12.5 (Systat Software Inc). All graphics were made using GraphPad Prism 5.0. A p value <0.05 was considered significant.

3. Results and discussion

3.1. Administration of miR-10b is associated with AAA growth and aortic rupture

All mice infused with Ang II presented larger aortic diameter compared to saline (data not shown). Administration of miR-10b resulted in increased levels in plasma (data not shown). The mortality rate in the miR-10b-treated mice was 61% compared to 11% in the Ang II miR control animals (Table 1, $p < 0.05$). Additionally, aortic diameter were incresed by miR-10b treatement, leading to a 2.5-fold increase in AAA formation, with an incidence of 88% compared with 33% in miR controls (Table 1, $p < 0.05$). Aortic diameters both from ultrasound and histology examinations are expressed in Fig. 1A and B, which correlated ($R = 0.67, p < 0.001$). Such result align with human data that miR-10b was associated with bigger aneurysms in AAA patients [3]. No changes in media area were observed in miR-10b-treated mice (data not shown). Blood pressure was not measured in the treated animals, which is a potential limitation of the study.

3.2. miR-10b promotes elastin degradation and neutrophil recruitment

In order to explore the mechanism of which miR-10b could impact aneurysm formation, histology sections were analyzed for degradation of elastic fibers. The elastin in aortas of miR-10b-treated mice were significantly more disrupted compared with that of miR-10b control mice (Fig. 1C). To confirm this, staining of neutrophil elastase showed that miR-10b-treated mice were more positive to neutrophils compared to controls (Fig. 1D with representative pictures). mRNA levels of mast cell chymase 1 (*CMA1)* and neutrophil gelatinase-associated lipocalin 2 (*LCN2)* were up-regulated in miR-10b-treated mice compared to controls (Fig. 1E and F), suggesting that mast cells and neutrophils are responsible for the elastin degradation. Staining for mast cells did not reveal any difference in numbers but mRNA induction indicates an increased mast cell activity. Chymases are serine proteases produced by granulocytes that can promote ECM destruction and are associated with AAA formation [1]. Additionally, pharmacological inhibition of chymases reduces AAA formation in multiple mouse models [9]. These findings are in line with previous data that propose mast cells and neutrophils are key cells in the pathogenesis of AAA [10]. In order to explore other possible mechanism that miR-10b could be involved in AAA formation, we also screened for markers of leukocytes such as CD3, CD68 or markers of SMC (α-actin, tagln, desmosin or PTen), collagens (Col1A1, Col3A1), lysyl oxidase, cathepsins (CTS G, CTS K and CTS S), MMPs (MMP2, MMP9 and MMP14) and IL-6 but no differences were found between the groups (data not shown).

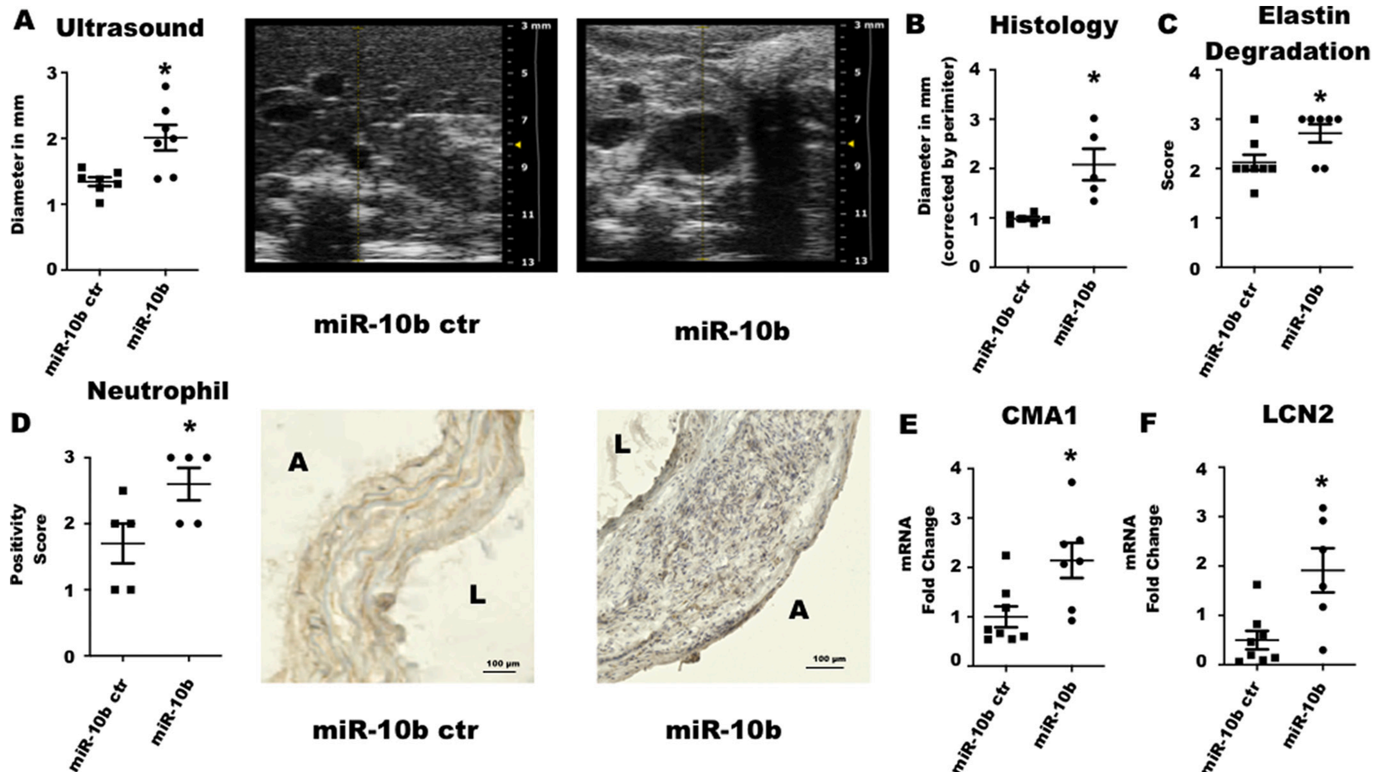

Fig. 1. Ultrasound (A), with representative pictures and histology (B) measurements after 4 weeks' treatment with miR-10b control (miR-10b ctr) or miR-10b. Diameters are presented in millimeters (mm). Elastin degradation is presented by grade, from 0 (intact) to 3 (destroyed), based on aortic sections (C). Score of neutrophil positivity from 0 (negative) to 3 (strong positivity) (D) between miR-10b and miR-10b miR-10b ctr; representative figures are showed with enhanced important areas. mRNA measurements ($2^{\Delta ct}$) of fold changes in *CMA1* (E) and *LCN2* (F) gene expression between miR-10b and miR-10b ctr. * indicates a p value <0.05 vs. control.

4. Conclusion

Taking all together, miR-10b, previously associated with AAA in human, induce aortic rupture, diameter and elastin degradation, in the vascular wall of a mouse model, most likely by activation and recruitment of mast cells and neutrophils. More mechanistic studies are required to better understand miR-10b's role in AAA formation.

Author contributions

Conceptualization: AK, AW, KM, DW, MP
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Formal analysis: AK, AB, MN, MBA, DW, MP
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Investigation: AK, AB, MN, MBA, DW, MP
Methodology: AK, AB, MBA, MP
Project administration: AW, KM, DW, MP
Resources: AW, KM, AK, AB, MBA, MP
Software: DW, MP
Supervision: DW, MP
Validation: AW, KM, AK, AB, MN, MBA, DW, MP
Visualization: AK, AB, MN, MBA, AW, KM, DW, MP
Writing - original draft: DW, AB, MN, AW, KM, MP
Writing - review & editing. AK, AB, MN, MBA, AW, KM, DW, MP

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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