29. Use of miRNA-483 its target genes

(Emory University)

5TH KDDF GLOBAL C&D TECH FAIR

Asset Overview

Product Type	Gene therapy
Disease Area	Cardiovascular Disease, Others
Indication	Aortic Stenosis
Current Stage	Lead Optimization
Target	UBE2C, pVHL, and HIF1-alpha
MoA	• The miR-483 target, UBE2C, regulates the pVHL and HIF1α pathway, leading to endothelial inflammation, EndMT, and subsequent AV calcification. • miR-483 mimic and HIF1α inhibitors may serve as potential therapeutics to reduce CAVD.
Brief Description	• Aortic stenosis limits the amount of blood that can leave the valve and thus causes the heart to pump blood through the valve at an increased force, this is mainly caused by narrowing due to aortic valve calcification. Aortic valve calcification calcium deposits form on the aortic valve in the heart. Said deposits can cause narrowing at the opening of the aortic valve which can require valve repair surgery. • A differentially expressed, flow sensitive, microRNA (miRNAs) in the aorta and heat takes part in controlling inflammation and progressive calcification of the aortic valve. Using human endothelial cell lines, Inventors demonstrated that expression of miR-483 decreased under conditions of oscillatory flow. • Ubiquitin E2 ligase expression is regulated by miR-483 which in regulates inflammation via degrading von-Hippel-Lindau tumor suppressor protein and then increasing expression of HIF1α thus leading to inflammation and progressive calcification.
Intellectual Property	US20220090079A1
Publication	Disturbed flow increases UBE2C via loss of miR-483-3p, inducing aortic valve calcification by the HIF1α pathway in endothelial cells. Arterioscler Thromb Vasc Biol. (2019)
Inventors	Hanjoong Jo, Joan FERNANDEZ ESMERATS, Nicolas Villa-Roel

Highlights

- miR-483 expression is shear-sensitive and side-specific, and it inhibits shear-induced EndMT and endothelial inflammation
- UBE2C regulates OS- and miR-483-dependent inflammation and EndMT in HAVECs.
- UBE2C mediates flow-sensitive expression of pVHL and HIF1α.
- UBE2C binds and ubiquitinates pVHL, leading to its degradation.
- pVHL and HIF1α mediate UBE2C-dependent inflammation and EndMT in HAVECs.
- MiR-483 mimic and HIF1α chemical inhibitor PX478 inhibit calcification in porcine aortic valves.

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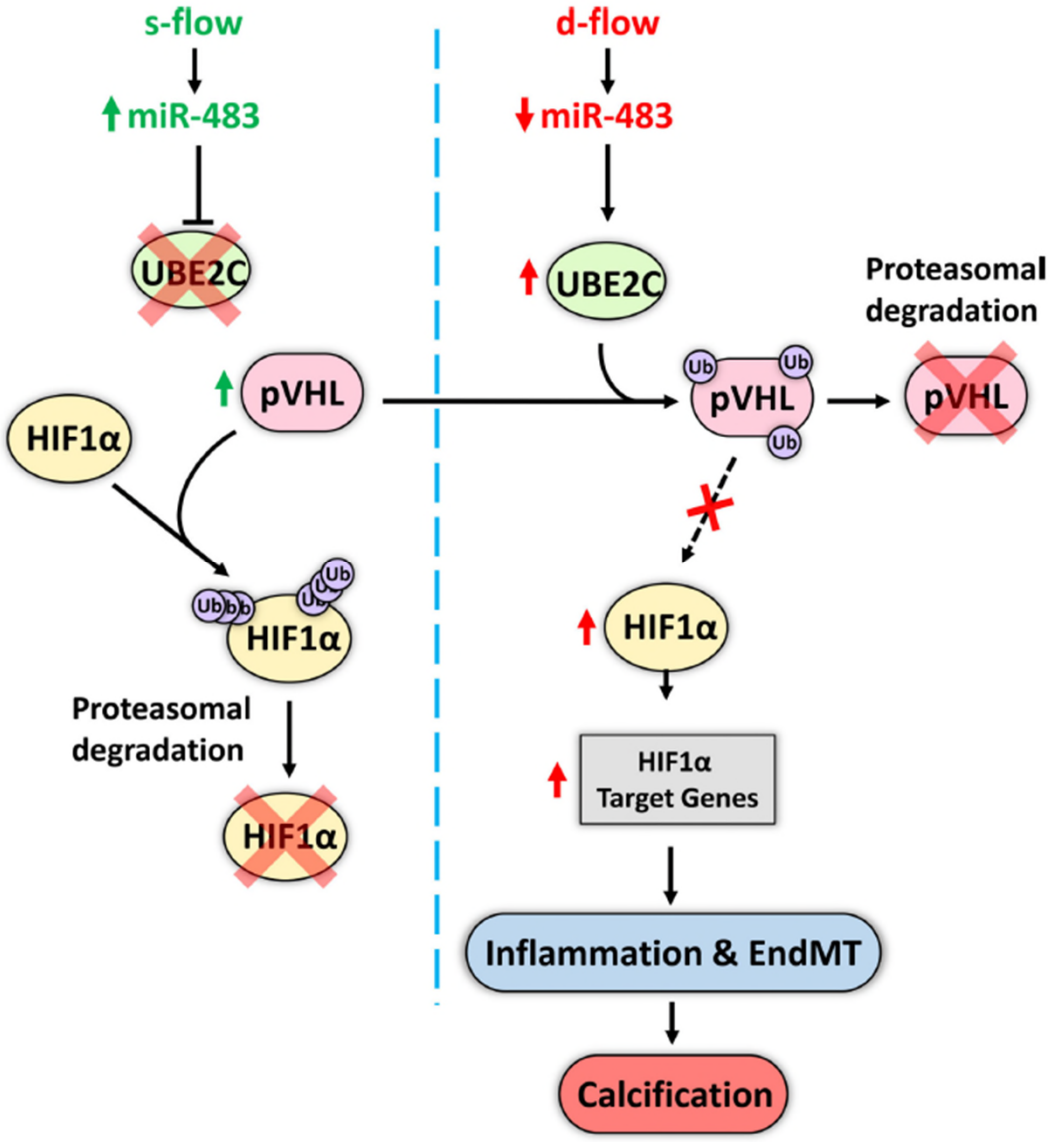
Key Data

Overall working hypothesis



UBE2C targets pVHL by binding and mediating its degradation in a ubiquitination-dependent manner. pVHL ubiquitination site mutants indicates that binding of UBE2C to pVHL is independent of the ubiquitination sites on pVHL;
the UBE2C-dependent degradation of pVHL requires at least one of its ubiquitination sites.
The degradation of pVHL further led to increased stabilization of HIF1α, which in turn induced endothelial inflammation, EndMT and AV calcification.

Source: Arterioscler Thromb Vasc Biol. (2019). Fig.7

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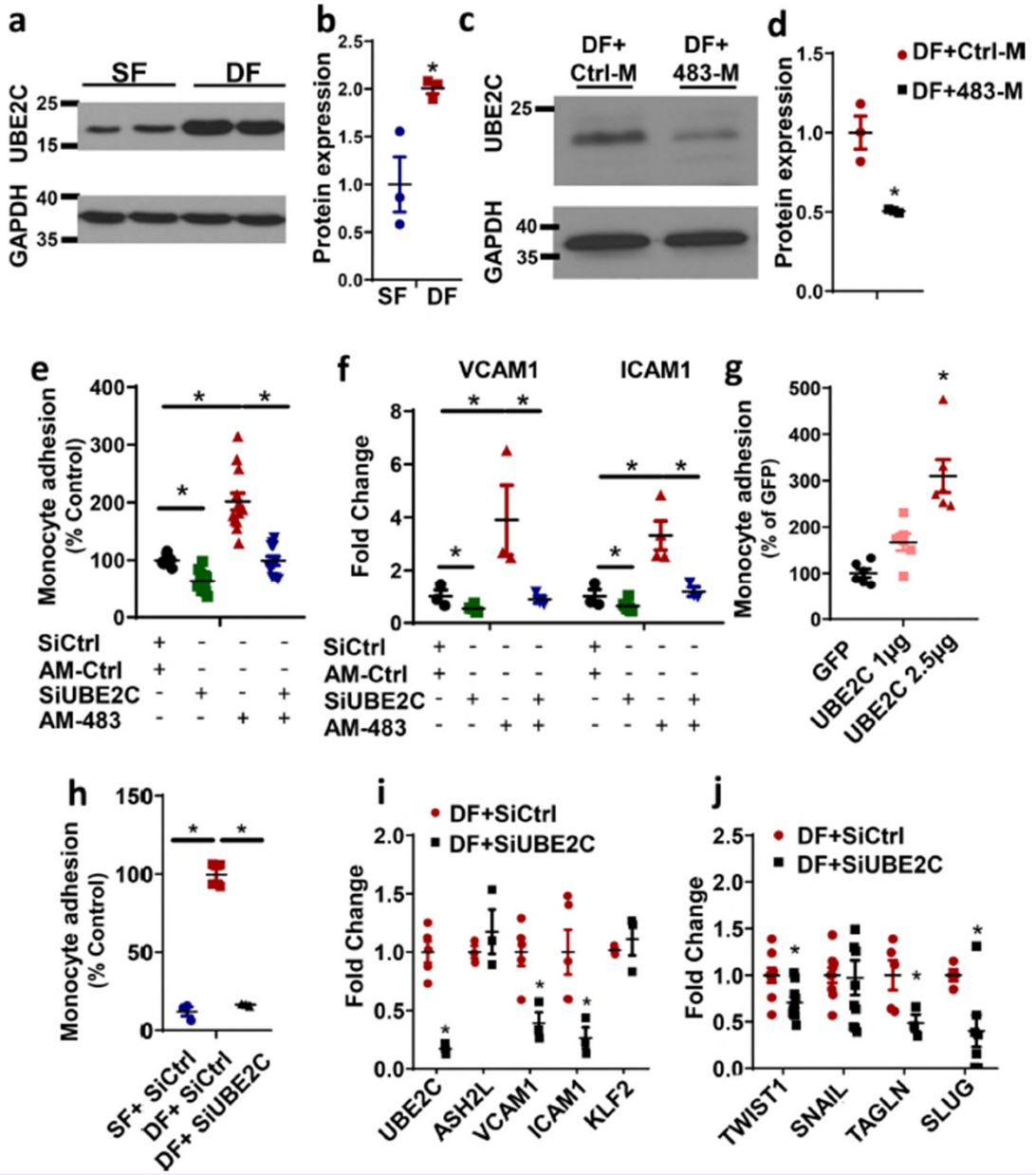
UBE2c is a shear-dependent target of miR-483 and regulates DF- and miR-483-induced inflammation EndMT in HAVECs



a,b) UBE2C expression was increased in HAVECs exposed to d-flow (DF) compared to s-flow (SF) for 24 hours, as determined by Western blot and its quantification by ImageJ (n=3). **c,d**) HAVECs treated with miR-483-mimic (483-M) or control mimic (Ctrl-M) followed by DF were analyzed by UBE2C Western blot and its quantification (n=3). **e-g**) HAVECs were co-transfected with siUBE2C or siCtrl and anti-miR-483 (AM-483) or anti-miR-control (AM-Ctrl), followed by monocyte adhesion (n=11) (**e**) and qPCR analysis for inflammatory markers (n=3) (**f**). **g**) HAVECs transfected with UBE2C overexpression plasmid or GFP plasmid were used for monocyte adhesion assay (n=6). **h-i**) HAVECs treated with siUBE2C or siRNA control (SiCtrl) for 24 hours, followed by DF or SF conditions for another 24 hours. Then, monocyte adhesion (**h**) and qPCR analyses for markers of inflammation (**i**) and EndMT (**j**) were carried out, normalized to 18S (n=4–6). Mean±sem, *p<0.05.

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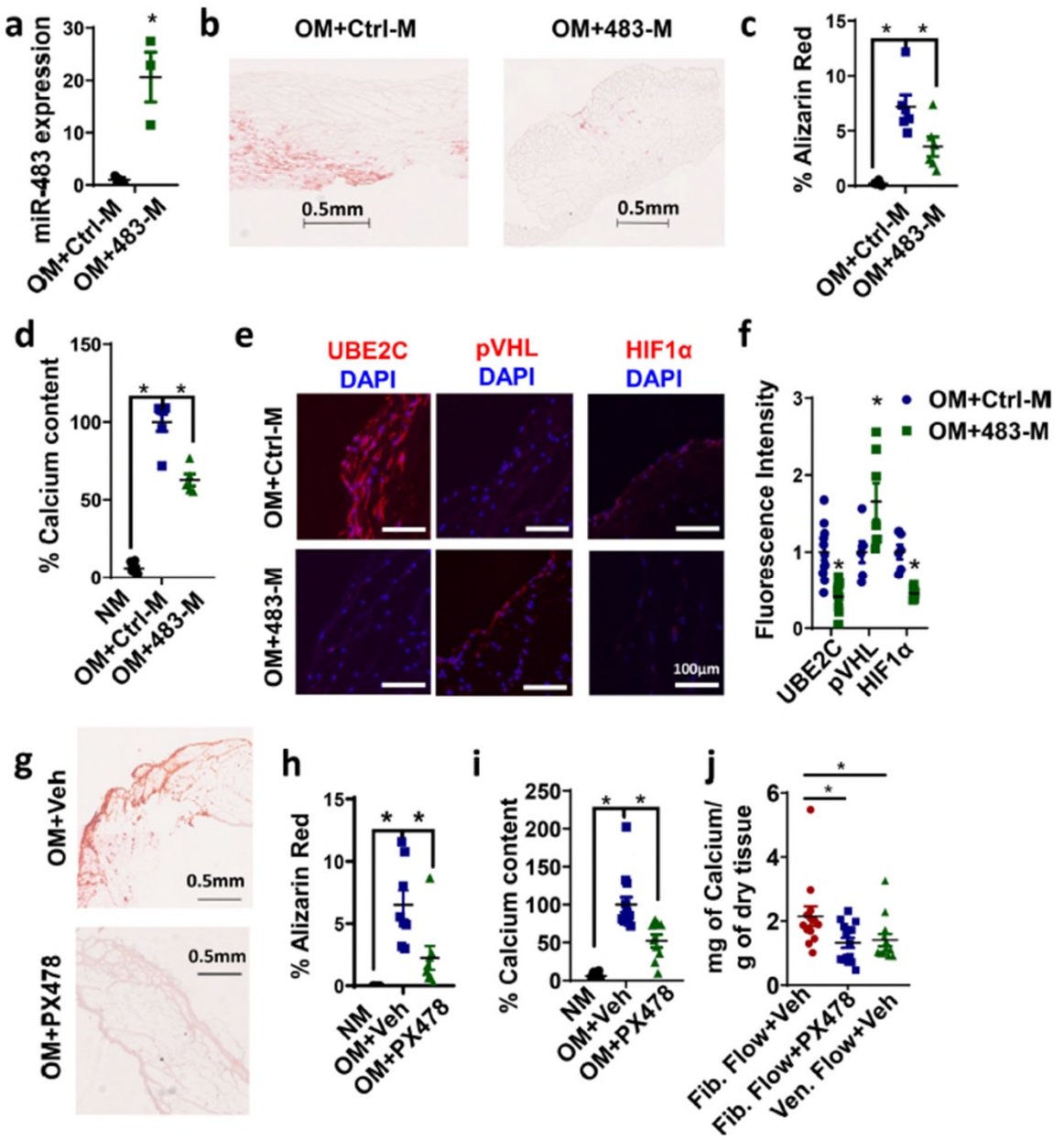
The miR-483-mimic or HIF1a inhibitor inhibits AV calcification



a) Freshly-harvested porcine AV leaflets were transfected with either miR-483 mimic or Ctrl mimic (**a-f**) or PX478 (20 μM) or HBSS vehicle (**g-i**) every 3 days for 2 weeks in osteogenic media (OM). AV leaflets were then divided for total RNA isolation and qPCR assay for miR-483 (**a**) (n=3), immunohistochemical assay using Alizarin Red (**b,c**) (n=6) and antibodies for UBE2C, pVHL and HIF1α (**e,f**) (n=6–12) and Arsenazo calcium assay (**d**) (n=5–6). Alizarin images were quantified using MATLAB (**c,h**) while the fluorescence images (**e**) were quantified by ImageJ (n=3–12). **j)** Porcine AV leaflets were exposed to fibrosa-(Fib.) or ventricularis-(Ven.) flow profiles in osteogenic media for 7 days with PX478 (20μM) or vehicle. Total calcium was quantified via Arsenazo assay and normalized to dry tissue weight (n=13–15). Mean±sem, *p<0.05.