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Apabetalone Reduces Cardiac Events in CKD Patients, while Downregulating Fibrotic and Inflammatory Processes in Renal Cells and Patient Plasma

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INTRODUCTION

Major adverse cardiac events (MACE) are a leading cause of mortality in patients with chronic kidney disease (CKD).

Apabetalone is an inhibitor of bromodomain & extraterminal (BET) proteins – epigenetic readers modulating gene expression involved in fibrosis & inflammation.

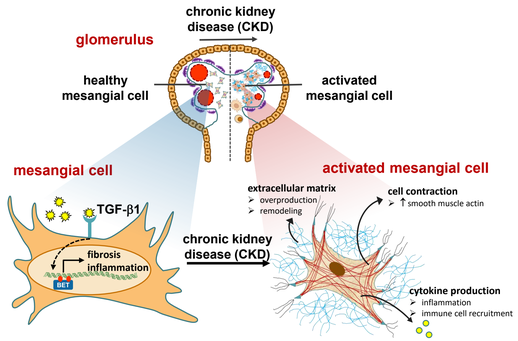
In the phase 3 BETonMACE trial in patients with CVD & diabetes mellitus, **apabetalone reduced risk of MACE by 50%** in the subpopulation with CKD, indicating favorable response along the kidney-heart axis (eGFR < 60; HR 0.50 95% CI 0.26,0.96 p = 0.04) .

AIMS

1: To examine effects of apabetalone in human renal mesangial cells (HRMCs) on fibrotic & inflammatory pathways.

2: To assess plasma levels of fibrotic & inflammatory proteins in CKD patients & matched controls after receiving apabetalone.

CELL SYSTEM



HRMC activation to fibroblast-like cells frequently accompanies and drives CKD regardless of how CKD is initiated

TGF-β1, a pro-fibrotic cytokine, activates HRMCs *in vitro* and *in vivo*

METHODS

HRMCs were stimulated with TGF-β1, a pro-fibrotic cytokine, or lipopolysaccharide (LPS) ± apabetalone, JQ1 or MZ1 - BET inhibitors (BETi) with chemical scaffolds different than apabetalone.

Gene expression was measured by RNA-seq & real-time PCR. RNA-seq was evaluated by Reactome Enrichment Analysis & Ingenuity Pathway Analysis (IPA).

Proteins were measured by immunofluorescence or ELISA.

Collagen gel contraction was assessed in 3D cultures.

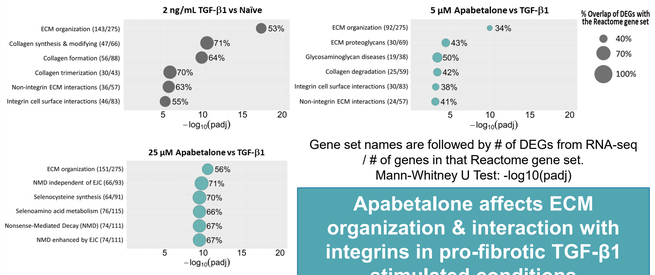
In human subjects, plasma proteomics was performed via SOMAscan 1.3k before and 12 hours after 100mg of apabetalone in CKD patients (n=8, eGFR < 30 stage 4 or 5) and matched controls (n=8, eGFR > 60).

RESULTS

Bioinformatics of RNA-seq from TGF-β1 Stimulated HRMC

1: Reactome Enrichment Analysis

Reactome gene sets associated with ECM production & remodeling are in the top 6 affected by TGF-β1 and by apabetalone



Gene set names are followed by # of DEGs from RNA-seq / # of genes in that Reactome gene set.
Mann-Whitney U Test: -log10(padj)

Apabetalone affects ECM organization & interaction with integrins in pro-fibrotic TGF-β1 stimulated conditions

2: Ingenuity Pathway Analysis: Upstream Regulators

Upstream Regulator	IPA z-score					
	5μM Apa		25μM Apa		0.15μM JQ1	
	Predicted Activation State	z-score	Predicted Activation State	z-score	Predicted Activation State	z-score
NF-kB RelA	No prediction	**-1.18**	Inhibited	**-2.53**	Inhibited	**-2.00**
NF-kB Complex	No prediction	**-0.443**	Inhibited	**-3.14**	Inhibited	**-2.00**

Blue – inhibition; **Bold p < 0.05** Fisher's Exact Test

IPA predicts apabetalone inhibits NF-κB, a master regulator of inflammation

Bioinformatics of RNA-seq (cont.)

3: Ingenuity Pathway Analysis: Canonical Pathways

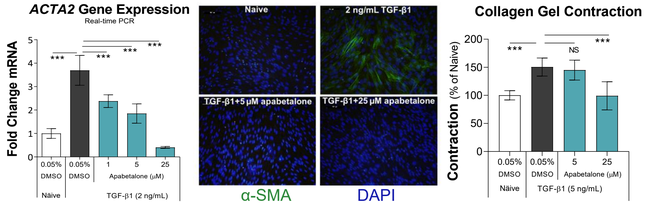
Canonical Pathway	IPA z-score		
	5μM Apa	25μM Apa	0.15μM JQ1
Glycolysis I	2.12	2.31	2.33
Oxidative Phosphorylation	3.50	**5.73**	**5.81**
NRF2-mediated oxidative stress response	**1.62**	**2.29**	**1.24**

Yellow – activation; **Bold p < 0.05** Fisher's Exact Test

IPA predicts apabetalone increases glucose utilization and tolerance of ROS production, which may allow adaptation to elevated glucose in the diabetic kidney

HRMC Activation to Fibroblast-like Cells

α-Smooth muscle actin (gene *ACTA2*) is a marker of HRMC activation to fibroblast-like cells that forms stress fibers to induce cell contraction



ANOVA followed by Dunnett's ***p<0.001, NS not significant

Apabetalone suppresses TGF-β1-induced markers of HRMC activation

Key Drivers of Fibrosis and Inflammation

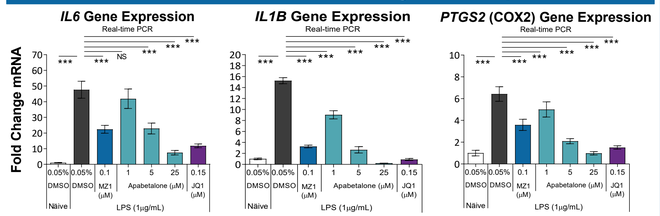
Thrombospondin: activator of latent TGF-β1 **Fibronectin:** key ECM component
Periostin: promotes ECM production **Osteonectin:** regulator of TGF-β1 expression
IL6: pro-inflammatory cytokine

TGF-β1 stimulation 24h, unless specified	Protein	Gene	mRNA			Secreted Protein		
			Fold Change with TGF-β1	% Reduction with Apa		Fold Change with TGF-β1	% Reduction with Apa	
				5μM	25μM		5μM	25μM
Pro-fibrotic	Thrombospondin	*THBS1*	**1.74**	**29.5**	**50.4**	**2.2**	**31.6**	**52**
	Fibronectin	*FN1*	**7.89**	**19.3**	**44.2**	**4.29**	**43.1**	**58**
	Periostin	*POSTN*	**5.52**	**81.2**	**96.5**	**22.3**	**96.6**	**99.2**
	Osteonectin	*SPARC*	**2.49**	**25.6**	**57.1**	**1.53**	**30.8**	**49.7**
Pro-inflammatory	Interleukin-6 (48h)	*IL6*	**2.93**	**48.6**	**67.7**	**3.85**	**75.7**	**89.9**

Yellow – upregulation; Blue – downregulation; **Bold p < 0.05** ANOVA followed by Dunnett's

Apabetalone suppresses TGF-β1 induction of key drivers of fibrosis and inflammation (all validated therapeutic targets)

LPS Stimulated Pro-inflammatory Gene Expression



ANOVA followed by Dunnett's ***p<0.001, NS not significant

Apabetalone suppresses LPS-stimulated expression of inflammatory genes linked to CKD and its progression.

Plasma Proteomics from CKD Patients & Matched Controls

CKD
n = 8, eGFR < 30
not on dialysis

Matched controls
n = 8, eGFR > 60

Single dose of **apabetalone**

Pre-dose 12 hours post-dose
Plasma proteomics

	Protein	% change from pre-dose	
		CKD	Controls
Pro-fibrotic	Thrombospondin	**-36**	17.4
	Fibronectin	**-25**	3.6
	Periostin	-12	**-4.2**
	Osteonectin	**-31**	16.0
Pro-inflammatory	Interleukin-6	**-29**	42.0

Yellow – upregulation; Blue – downregulation;
Bold p<0.05 Wilcoxon Rank Sum

Apabetalone reduced plasma levels of TGF-β1 targets in CKD patients

CONCLUSIONS

Through epigenetic regulation of transcription in HRMCs, apabetalone reduces fibrotic & inflammatory factors known to signal along the kidney-heart axis & exacerbate kidney dysfunction.

Predicted changes in energy metabolism suggest apabetalone facilitates adaptation to high glucose in kidneys.

Cell-based results are consistent with a human clinical trial where plasma levels of fibrotic & inflammatory factors were reduced specifically in CKD patients receiving apabetalone.

Our study provides mechanistic insights into reduced MACE in CKD patients receiving apabetalone in the phase 3 BETonMACE trial & will be further evaluated in an upcoming Phase 3 trial.