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### **Publications in this thesis**

1. Cai J, Pardali E, Sánchez-Duffhues G, Ten Dijke P. BMP signaling in vascular diseases. *FEBS Letters*. 2012.
2. Shi S\*, Cai J\*, de Gorter DJ, Sanchez-Duffhues G, Kemaladewi DU, Hoogaars WM, Aartsma-Rus A, 't Hoen PA, ten Dijke P. Antisense-oligonucleotide mediated exon skipping in activin-receptor-like kinase 2: inhibiting the receptor that is overactive in fibrodysplasia ossificans progressiva. *PLoS One*. 2013 (co-first author)
3. Spiekerkoetter E, Tian X, Cai J, Hopper RK, Sudheendra D, Li CG, El-Bizri N, Sawada H, Haghghat R, Chan R, Haghghat L, de Jesus Perez V, Wang L, Reddy S, Zhao M, Bernstein D, Solow-Cordero DE, Beachy PA, Wandless TJ, Ten Dijke P, Rabinovitch M. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *Journal of Clinical Investigation*. 2013
4. Cai J, Orlova V V, Cai X, Eekhoff E M W, Zhang K, Pei D, Pan G, Mummery C L, ten Dijke P. Induced Pluripotent Stem Cells to Model Human Fibrodysplasia Ossificans Progressiva. *Stem Cell Reports*. 2015

### **Other publications**

5. Esteban MA, Peng M, Deli Z, Cai J, Yang J, Xu J, Lai L, Pei D. Porcine Induced Pluripotent Stem Cells May Bridge the Gap between Mouse and Human iPS, *IUBMB Life*, 2010
6. Yang J\*, Cai J\*, Zhang Y, Wang X, Li W, Xu J, Li F, Guo X, Deng K, Zhong M, Chen Y, Lai L, Pei D, Esteban MA. Induced pluripotent stem cells can be used to model the genomic imprinting disorder Prader-Willi syndrome. *The Journal of biological chemistry*. 2010 (co-first author)
7. Huang L, Fan N, Cai J, Yang D, Zhao B, Ouyang Z, Gu W, Lai L, Establishment of a Porcine Oct-4 Promoter-Driven EGFP Reporter System for Monitoring Pluripotency of Porcine Stem Cells. *Cellular Reprogramming*. 2011
8. Liao B, Bao X, Liu L, Feng S, Zovoilis A, Liu W, Xue Y, Cai J, Guo X, Qin B, Zhang R, Wu J, Lai L, Teng M, Niu L, Zhang B, Esteban MA, Pei D. MicroRNA cluster 302-367 enhances somatic cell reprogramming by accelerating a mesenchymal-to-epithelial transition. *The Journal of biological chemistry*. 2011

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## **Curriculum Vitae**

Jie Cai was born on the 10<sup>th</sup> of May 1987 in Xishui, Hubei, China. She was admitted to Xishui No.1 middle school in 2001 and graduated in 2004. From 2004 to 2008, she did her bachelor in the department of Life Science in Hubei University. In 2008, she started her master study in Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science. From August 2009 to June 2011, she did her master training in the lab of Prof. Miguel Esteban. During that period of time, she was trained on how to establish human disease models by using induced pluripotent stem cells (iPSCs). On July 2011, she earned her master degree on the topic of modeling human Prader Willi symptom by using iPSCs. Besides the study of model disease phenotypes, she was also interested on the study of interpreting disease mechanisms. Therefore, she joined Prof. Peter ten Dijke's lab in 2011 to study BMP signal pathway in disease contexts. From July 2011 to July 2015, her work was mainly about clarifying how disturbed BMP signaling contributes to the development of human vascular disease pulmonary hypertension and bone disease fibrodysplasia ossificans progressive, and to develop novel therapeutic strategies for these diseases. These results in this thesis were performed during her PhD period.

**List of Abbreviations**

A	ActR2A	Activin receptor type II A	
	ALK	Activin receptor-like kinase	
	ALP	Alkaline phosphatase	
	AMH	Anti-müllerian hormone	
	AONs	Antisense oligonucleotides	
	Apo	Apolipoprotein	
	AV	Aortic valve	
	AVMs	Arteriovenous malformation	
	B	BAECs	Bovine aortic endothelial cells
		BAMBI	BMP and activin membrane-bound inhibitor
bFGF		Basic fibroblast growth factor	
BMPs		Bone morphogenetic proteins	
BMPER		BMP endothelial cell precursor derived regulator	
BMPR2		BMP Type II receptor	
BRE		BMP-responsive element	
BS3		bis(sulfosuccinimidyl)suberate	
BSP		Bone sialoprotein	
C		COL 1	COLLAGEN type I
	cox-2	Cyclo-oxygenase-2	
	CV2	Cross-veinless 2 or BMPER	
D	DAN	Differential screening-selected gene aberrative in neuroblastoma	
	Dll4	Delta-like 4	
	DMD	Duchenne muscular dystrophy	
	DSS	Disuccinimidyl suberate	
	DTT	dithiothreitol	
	E	ECs	Endothelial cells
		EC-20	20% of maximal response
		ECD	extracellular domain
EndoMT		Endothelial to mesenchymal transition	
eNOS		Endothelial nitric-oxide synthase	
EPCs		Endothelial progenitor cells	
ER		Endoplasmic reticulum	
ESCs		Embryonic stem cells	
ETV2		Early endothelial transcription factor	

List of abbreviations

F	FACS	Flow cytometry
	FBS	Fetal bovine serum
	FDA	US food and drug administration
	FGF	Fibroblast growth factor
	FKBP12	FK-binding protein-12
	FOP	Fibrodysplasia ossificans progressiva
	FPAH	Hereditary or familial PAH
G	GAGs	Glycosaminoglycans
	GDFs	Growth and differentiation factors
	GS	Glycine-serine-rich
H	HEK293	human embryonic kidney 293 cells
	hESC	Human embryonic stem cell
	HHT	Hereditary hemorrhagic telangiectasia
	His-tag	histidine-tag
	HIV	Human immunodeficiency virus
	HLH	Helix-loop-helix
	HMEC-1	Human microvascular endothelial cells
	HO	Heterotopic ossification
	HUVECs	Human umbilical vein endothelial cells
I	IL	Interleukin
	IPAH	Sporadic or idiopathic PAH
	iPSCs	Induced pluripotent stem cells
	I-Smads	Inhibitory Smads
K	KD	Kinase domain
	KCNK3	Potassium channel subfamily K member 3
L	LAP	Latency associated peptide
	LBD	Ligand binding domain
	LDL	Low-density lipoprotein
	LDN	LDN-193189
	LTBP	Latent TGF- $\beta$ binding protein
M	MCs	Mesenchymal cells
	MGP	Matrix GLA protein
	miR	Micro RNAs
	MMP-14	Matrix metallo proteinase 14
	mvPAECs	Microvessel PAECs
N	NFATs	Nuclear factor of activated T cells

	NMD	nonsense-mediated decay
	NSAIDs	Non-steroidal anti-inflammatory drugs
O	OSC	Osteocalcin
P	PAEC	Pulmonary artery endothelial cells
	PAH	Pulmonary arterial hypertension
	PASMCs	Pulmonary artery SMCs
	PDGF-BB	Platelet-derived growth factor subunit BB
	PDGFR	Platelet-derived growth factor receptor
	PEI	Polyethyleneimine
	PKC	Protein kinase C
	PRDC	Protein related to DAN and Cerberus
	pSMAD1/5	Phospho-SMAD1/5
	PTPN14	Tyrosine-protein phosphatase non-receptor type 14
Q	qPCR	Quantitative real-time PCR
R	RGM	Repulsive guidance molecule
	ROS	Reactive oxygen species
	R-Smads	Receptor-regulated Smads
	RVH	Right ventricular hypertrophy
	RVSP	Right ventricular systolic pressure
S	SBE	Smad-binding elements
	SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
	sEng	Soluble endoglin
	SMCs	Smooth muscle cells
T	TGF- $\beta$	Transforming growth factor- $\beta$
	TGF $\beta$ R2	TGF- $\beta$ receptor 2
	TM	Transmembrane domain
U	USAG-1	Uterine sensitization-associated gene-1
V	VEGF	Vascular endothelial growth factor
	VEGFR2	Vascular endothelial growth factor receptor 2
	vWF	Von Willebrand factor