Nuclear Protein Kinase C- theta (PKC-θ) Directly Regulates Inducible Genes of Epithelial to Mesenchymal Transition and Breast Cancer Stem Cells

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Achievements

Journal Publications

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Kristine Hardy, **Fan Wu**, Anjum Zafar, Wenjuan Tu, Tara Boulding, A Theodoratos, Sudha Rao. (**2015**) Identification of chromatin accessibility domains in human breast cancer stem cells. *Nucleus*, submitted, review in progress.

Tara Boulding, **Fan Wu**, Anjum Zafar and Sudha Rao. (**2015**) Multi-Layered Epigenetic Regulatory Mechanisms Mediate Epithelial to Mesenchymal Transition in Cancer. *Journal of Integrative Oncology*, 4:127

Anjum Zafar, Kristine Hardy, **Fan Wu**, Jasmine Li, Sudha Rao. (**2015**) The role of protein kinase-C theta in control of epithelial to mesenchymal transition and cancer stem cell formation. *Genomics Data*, Volume 3, March 2015, Pages 28–32

Fan Wu, Sudha Rao. (**2015**) Development of a novel nuclear PKC-theta inhibitor that abrogates breast cancer stem cell formation *in vivo*. *Nature Medicine*, manuscript in preparation.

Fan Wu, Robert McCuaig, Sudha Rao. (**2015**) Novel epigenetic mechanism of H3K27Ac regulation by p300 in epithelial to mesenchymal transition and cancer stem cells formation in breast cancer. Molecular Cell, manuscript in preparation.

Anjum Zafar, **Fan Wu**, Kristine Hardy, Jasmine Li, Wen Juan Tu, Robert McCuaig, Janelle Harris, Kum Kum Khanna, Joanne Attema, Philip A Gregory, Gregory J Goodall, Kirsti Harrington, Jane E Dahlstrom, Tara Boulding, Rebecca Madden, Abel Tan, Peter J Milburn and Sudha Rao. (**2014**) Chromatinized PKC-θ directly regulates inducible genes in epithelial to mesenchymal transition and breast cancer stem cells. *Molecular Cell Biology*, 2014 Aug; 34(16): 2961–2980

Hilary S. Warren, **Fan Wu**, Peggy L. Horn, David B. Pyne, Nic West, Allan Cripps. (**2013**) Peripheral blood natural killer (NK) cell function in healthy adults assessed using the target-induced NK loss (TINKL) assay. *Journal of Immunological Methods*, 2013 Jun 28; 392 (1-2): 68-70

Ben Duell, CK Keong Tan, Alison Carey, **Fan Wu**, Allan Cripps, Glen Ulett. (**2012**) Recent insights into microbial triggers of interleukin-10 production in the host and the

impact on infectious disease pathogenesis. *FEMS Immunology and Medical Microbiology*, 2012 Apr; 64(3): 295-313

Conference Presentation

Fan Wu, Anjum Zafar, Sudha Rao. (**2015**) Direct regulation of nuclear PKC-θ in epithelial to mesenchymal transition and breast cancer stem cells. The EMT International Association (TEMTIA) Early Career Research (ECR) Forum, Melbourne, VIC, Australia. (Oral presentation)

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Fellowships and Awards

Recipient of University of Canberra CResTS Travel Award, **2015**.

Recipient of The EMT International Association (TEMTIA) Travel Bursary, **2015**

Recipient of University of Canberra CResTS Annual Symposium Best Presentation, **2013**.

Recipient of International Postgraduate Research Scholarship (IPRS), **2012**.

Abstract

Epithelial to mesenchymal transition (EMT) is a key event in cancer progression and the process of metastasis that creates a reservoir for cancer stem cells (CSCs) and is associated with highly aggressive traits. CSCs play a vital role in metastasis, therapeutic resistance and relapse in breast cancer patients. Protein Kinase C theta (PKC-θ) is signal transduction kinase that has been implicated in inflammatory disorders, tumor progression, and metastasis has been recently linked to aggressive breast cancer. Rao lab has previously shown that PKC-θ can directly translocate to nucleus to regulate inducible immune responsive gene transcription and micro-RNAs that essential for effective immune response in T cells. Hence, it will be a crucial step to unravel the molecular role of PKC-θ in EMT and CSCs formation process.

Using cancer biological and epigenetics analysis, we have shown that PKC-θ promotes EMT by directly tethering chromatin for mediating inducible genes via transforming growth factor beta (TGF-β) and the key inflammatory regulatory protein NF-kappa B. Chromatinized PKC-θ acts as an essential active transcription complex for establishing permissive chromatin state at signature EMT genes. Genome-wide analysis identifies a unique cohort of EMT inducible genes that are directly tethered to PKC-θ. Overall, PKC-θ plays an irreplaceable role in regulating inducible transcription programs that drive EMT and CSCs formation via cross-talking with chromatin, which provide a novel mechanism to target breast cancer using epigenetic therapy.

List of Acronyms and Abbreviations

× g	Relative centrifugal force
°C	Degrees Celsius
µ	Micro
µL	Micro-liter
µM	Micro Molar
3' UTR	3' untranslated region
5' UTR	5' untranslated region
ADP	Adenosine diphosphate
Akt	Alpha serine/ threonin-protein kinase
ALDH	aldehyde dehydrogenase
ANK	ankyrin
ANU	The Australian National University
APC	Allophycocyanin
aPKCs	atypical PKCs
Arg	Arginine
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
bHLH	Basic helix-loop-helix
BIM	Bisindolymaleimide 1
BMI1	B lymphoma Mo-MLV insertion region 1 homologue
bp	Base pair
BSA	Bovine serum albumin

CD24	Cluster of differentiation 24
CD44	Cluster of differentiation 44
cDNA	Complementary DNA
ChIP	Chromatin Immuno-precipitation
ChIP-seq	Chromatin Immuno-precipitation sequencing
Chr.	Chromosome
CpG	CpG dinucleotide
cPKCs	classic PKCs
CResTS	Centre for Research in Therapeutic Solutions
CSCs	Cancer stem-like cells
Ct	Threshold cycle
CTBP	C-terminal-binding protein
CTRL	Control
DAG	Domains bind diacylglycerol
DAPI	4',6-diamidino-2-phenylindole
DEPC	Diethylyprocarbonate
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DNMTs	DNA methyltransferase enzymes
DPBS	Dulbecco's Phosphate-Buffered Saline
DTT	Dithiothreitol
DUSP	Dual Specificity Phosphatase
E-box	Enhancer box
E-cadherin	Epithelial cadherin

EDTA	Ethylenediamine tetraacetic acid
EGF	Epidermal growth factor
EMT	Epithelial-to-mesenchymal transition
EMT-TFs	EMT-inducing transcription factors
EpCAM	Epithelial cell adhesion molecule
ER	Estrogen receptor
ESA	Epithelial-specific antigen
EZH2	Enhancer of zeste homolog 2
FACS	Fluorescence-Activated Cell Sorting
FAK	Focal adhesion kinase
FAM	6-Aminofluorescein
FBS	Foetal Bovine Serum
FGF	Fibroblast growth factor
FITC	Fuorescein isothiocyanate
FOXOs	Fork head transcription factors
GFP	Green fluorecent protein
H2A	Histone 2A
H2B	Histone 2B
H3	Histone 3
H3	Histone 4
H3K4me3	Tri-methylated lysine 4, histone 3
H3K9ac	Acetylated lysine 9, histone 3
HATs	Histone acetyltransferases
HDAC	Histone deacetylases
HGF	Hepatocyte growth factor

HIF-α	Hypoxia-inducible factor 1-α
HM LE	Human mammary luminal epithlial
hr	Hour (s)
ID	Inhibitor of differentiation
IKK	Inhibitor of NF-κB kinase complex
IL6	Interleukin-6
IκB	Inhibitor of NF-κB
JCSMR	John Curtin School of Medical Research
JmjC	Jumanji C
k	Lysine
kb	Kilobase (pairs)
kDa	Kilo Dalton (s)
L	Liter
LiCl	Lithium chloride
LOCKs	Large organised heterochromatin K9-modifications
LSD1	Lysine-specific demethylase 1
LSD2	Lysine-specific demethylase 2
Lys	Lysine
m	Milli
M	Molar
MAPK	Mitogen activated protein kinase
MCF-IM	MCF7 inducible model
MET	Mesenchymal-to-epithelial transition
MgCl2	Magnesium chloride
min	Minute

miRNA	microRNA
mL	Milli-liter
MMP	Matrix metalloproteinase
mRNA	messageRNA
MUC1	Mucin1
MW	Mplecular weight
n	Nano
N-	Amino terminal
NCSC	Non-cancer stem cell
NEMO	NF-κB essential modulator
NFKB1	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B Cells 1
NF-κB	Nuclear factor-κB
NID	Notch intracellular domain
NLS	Nuclear localization signals
nM	Nano Molar
No.	Number
nPKCs	novel PKCs
NS	Non-stimulate
P	Phosphorylation
PAK1	p21-activated kinase-1
PAR6	Partitioning-defective proten-6
PcG	Polycomb group
PE	Phycoerythrin
PFA	Paraformaldehyde

PI	Propidium Iodide
PI3K	Phosphatidylinositide 3-kinases
PKC	protein kinase C
PKC-θ	Protein Kinase C-theta
PMA	Phorbol 12-myristate-13-acetate
Pol II	Ribonucleic acid polymerase II
PRCs	Polycomb repressive complexes
pre-miRNA	precursor miRNA
PSN	Penicillin-Streptomycin-Neomycin
PTMs	Post-translational modifications
qPCR	Quantitative real-time polymerase chain reaction
Ras	Renin-angiotensin system
Rev	Reverse
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
RT-PCR	Real-Time Polymerase Chain Reaction
sec	Second
Ser	Serine
siRNA	Silencing RNA
SP1	SMAD-interacting protein 1
SSC	Side scatter
ST	Stimulated
SUZ12	Suppressor of zeste 12 homolog

TACE	Tumour necrosis factor-α-converting enzyme
TGF-β	Transforming growth factor β
TI	Total input
TNF-α	Tumor necrosis factor-α
Tris	Trisaminomethane
UC	University of Canberra
uPAR	Urokinase-type plasminogen activator receptor
UV	Ultra violet
Wnt	Wingless-type
ZEB	Zinc Finger E-Box Binding Homeobox

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