

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

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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	Diethylprocarbonate
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 fluorescent protein
H2A	Histone 2A
H2B	Histone 2B
H3	Histone 3
H4	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 epithelial
hr	Hour (s)
ID	Inhibitor of differentiation
IKK	Inhibitor of NF- κ B kinase complex
IL6	Interleukin-6
I κ B	Inhibitor of NF- κ B
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
MgCl ₂	Magnesium chloride
min	Minute

miRNA	microRNA
mL	Milli-liter
MMP	Matrix metalloproteinase
mRNA	messageRNA
MUC1	Mucin1
MW	Molecular weight
n	Nano
N-	Amino terminal
NCSC	Non-cancer stem cell
NEMO	NF- κ B essential modulator
NFKB1	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B Cells 1
NF- κ B	Nuclear factor- κ B
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 protein-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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