Virginia Commonwealth University
School of Medicine

This is to certify that the dissertation prepared by Qifang Zhang entitled "Role of Jak/Stat pathway in the pathogenesis of breast cancer" has been approved by his or her committee as satisfactory completion of the dissertation requirement for the degree of Doctor of Philosophy

Andrew C. Larner, MD, Ph.D, Director of Dissertation

Paul Dent, Ph.D, School of Medicine

Masoud Manjili, Ph.D, School of Medicine

Jolene J. Windle, Ph.D, School of Medicine

Xianjun Fang, Ph.D, School of Medicine

Sarah Spiegel, Ph.D, Chair, Department of Biochemistry and Molecular Biology

Jerome F. Strauss III, MD, Ph.D, Dean, School of Medicine

Dr. F. Douglas Boudinot, Dean of the Graduate School

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RolE OF JAK/STAT IN THE PATHOGENESIS OF BREAST CANCER
A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of philosophy at Virginia Commonwealth University.

by

QIFANG ZHANG
Master of Science, China University of Geoscience, P.R. China, 1992

Director: Andrew C. Larner, MD, Ph.D
Full professor, Department of Biochemistry and Molecular Biology

Virginia Commonwealth University
Richmond, Virginia
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APRF	acute-phase response factor
BRAC1	Breast cancer antigen 1
CBP/p300	cAMP response element-binding (CREB) binding protein
C/EBP	CCAAT/enhancer binding protein
cDNA	complemenatary DNA
CFSE	carboxyfluorescein diacetate, succinimidyl ester
CLL	chronic lymphocytic leukemia
CNTF	ciliary neurotrophic factor
DC	dendritic cell
DMEM	Dulbecco's modification of Eagle's medium
EDTA	ethylene diamine tetraacetic acid
ETC	electron transport chain
ER	estrogen receptor
FACS	fluorescence activated cell sorting
G-CSF	granulocyte colony-stimulating factor
GFP	green fluorescence protein
GRIM-19	gene associated with retinoid-interferon-induced mortality-19
HBSS	Hank's balanced salt solution
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HPV	human papilloma virus
IFN	interferon
IRES	internal ribosome entry site

IL	interleukin
Jaks	Janus kinases
JH domain	Jak homology domain
LIF	leukemia inhibitory factor
LCMV	lymphocytic choriomeningtis virus
LPS	lipopolysaccharide
MAPK	mitogen activated protein kinase
MSCV	murine stem cell virus
MEF	mouse embryonic fibroblast
MEP	4-mercaptoethylpyridine
MES	2-N-morplolino ethane sulfonic acid
MDSC	myeloid derived suppressor cells
MnSOD	manganese superoxide dismutase
MLS	mitochondrial-localized sequence
NK cell	natural killer cell
NADH	reduced nicotinamide adenine dinucleotide
Nmi	N-Myc interactor
NOD2	nucleotide oligomerization domain 2
OSM	oncostatin M
PBS	phosphate-buffered saline
ROS	reactive oxygen species
RNS	reactive nitrogen Species
TFLC	truncated free light chain
SCID	severe combined immune deficiency
SH2 domain	Src homology domain2
Stat	signal transducer and activator of transcription

Abstract

ROLE OF JAK/STAT IN THE PATHOGENESIS OF BREAST CANCER

By Qifang Zhang, M.Sc.

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

Virginia Commonwealth University, 2010

Major Director: Andrew C. Larner, MD, Ph.D.
Full Professor, Department of Biochemistry and Molecular Biology

The Jak/Stat signaling cascade mediates cell proliferation, differentiation, survival, apoptosis and immune responses. Aberrant activation of this pathway mediates neoplastic transformation and abnormal growth of many malignancies including breast cancer, the most common cancer among women, and the second leading cause of cancer deaths in women in United States. The mechanism by which the Jak/Stat pathway modulates the pathogenesis of breast cancer is unclear. This dissertation elucidates roles of Jak/Stat members that mediate the pathogenesis of breast cancer. For these studies, we used 4T1 mouse mammary tumor cells as a model which mimics human breast cancer. First, we investigated the role of Tyk2 tyrosine kinase in the pathogenesis of breast cancer. Here we show for the first time that compared with wild type mice, Tyk2 $^{-/-}$ mice show increased tumor growth rate as well as metastatic disease and splenomegaly when inoculated with 4T1 breast cancer cells. Such increased tumorigenicity was associated with a significant decrease of IFN production in 4T1 tumor-bearing Tyk2 deficient mice T cells compared with wild type (WT) mice. We demonstrated that NK cells or CD8+ T cells control tumor growth in both Tyk2$^{-/-}$ and WT mice, but neither Tyk2$^{-/-}$ NK cells alone nor Tyk2$^{-/-}$ CD8^{+} T cells alone do not contribute to enhanced tumor growth and metastatic disease of Tyk2$^{-/-}$ mice. Tumor-bearing Tyk2-/- mice have increased level of myeloid-derived

suppression cells than tumor-bearing mice. $Tyk2^{-/-}$ MDSCs have a slight increase in suppression of T cell proliferation**.**

Since elevated phosphorylation of Stat3 has been seen in human and murine breast cancer, and expression of Stat3 in the mitochondria (mitoStat3) appears to have important affects on cell growth, we studied the ability of Stat3 targeted to the mitochondria (MLS Stat3) to influence growth and metastasis of 4T1 cells. We show that a serine mutant of Stat3 expressed in the mitochondria (Stat3 S727A) inhibits the ability of 4T1 tumor cells to grow and metastasize. In contrast, a serine to aspartic acid mutant of Stat3 (S727D) enhances tumorigenesis. We found that expression of mitochondrial-targeted Stat3 does not affect cell growth rate in cell culture under normal conditions, however in low glucose, the serine to alanine mutant shows reduced growth rate and ability to invade. Moreover, we found that expression of mitochondrial-targeted Stat3 protects cells from hypoxia. Our data indicate that serine phosphorylation of mitochondrial-localized Stat3 is required for cell transformation.

In summary, our studies provided new insights into the role of Stat3 in breast cancer and suggest new therapeutic targets for the treatment of this disease.

VITA

March 28, 1966. . . . . Born – Hunan province, P.R. China
1987. . . . . B.S., Geology, Chendu University of Techonolgy
1992 . . . . . M.S., Mineralogy, China University of Geosciences
2004-2007. . . . . Ph.D. Candidate, Cleveland State

University, Biological, Geological and Environmental Science, Department of Immunology, Lerner Research Institute, Cleveland Clinic Foundation
2007-- present . . . . . .Ph.D. Candidate, Biochemistry and Molecular Biology

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