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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

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February, 2010

Acknowledgement

I would like to express my greatest gratitude to those whom I am so fortunate to meet and who have contributed to this effort for a doctoral degree.

First of all, it is hard for me to find words to thank my advisor, Dr. Andrew C. Lerner, for giving me the opportunity to work and finish my Ph. D. in his lab, for his able guidance, constant support and encouragement. I am so amazed about his insights to almost everything across many different fields. I have learned a great deal from him on how to be a good scientist.

I would also like to extend my sincere appreciation and gratitude to all my advisory

committee members – Dr. Paul Dent, Dr. Masoud Manjili, Dr. Jolene J. Windle and Dr. Xianjun Fang for their invaluable suggestions and input in my dissertation work. I would like to thank the Department of Biochemistry and Molecular Biology for supporting my study. Especially, I feel deeply thankful to Dr. Suzanne Barbour for her instruction in the Ph.D program.

I would like to also express my gratitude to Dr. Daniel Conrad for his great suggestions and teaching in immunology. I am also grateful to Dr. V. Ramakrishnan (Ramesh) for his help in statistical analysis of my data.

I also would like to express my gratitude to professors when I studied in Cleveland: Dr. Jeffrey Dean, Dr. Crystal Weyman, Dr. Xiaoxia Li and Dr. Anton Komar for their excellent teaching.

My special thanks go to Dr. Maciej Kmiecik and Jamie Sturgill for teaching me many assays in immunology and providing great suggestions and invaluable knowledge in tumor immunology.

I would like to thank Dr. Ross B. Mikkelsen and Dr. Vasily A. Yakovlev for their help in oxidative stress studies. I am thankful to Dr. Yun Dai for his instruction in my cancer study. I am also grateful to thank Dr. Edward Lesnefsky and his lab members, Dr. Qun Chen and Dr. Lodovic Camelle Jules, for teaching me a lot about mitochondrial respiration.

My life at VCU would not have been so fruitful without many talented colleagues and

friends I have met here. Dr. Shuang Chen, Laura J. Graham, Dr. Zaozhong Su, Dr. Swadesh K. Das and Dr. Rupeth Dash taught me many assays used in my studies. Hossein Hamed instructed me on preparation of tissue sections. Dr. Tomasz Kordula helped me to design primers for mutagenesis. Dr. David Stratus showed me how to look for the lymphatic system of mouse. Frances White and Julie Farnsworth provided me a lot of help in sorting cells and doing flow cytometry analysis.

I want to thank my lab colleagues for making the lab a family: Ramesh, Joanna, Magda, Yong Joon, Marta, Karol, Jenny, Vidisha and Catherine. In this family, we help each other in both work and life. I have gotten huge help and support from them. I am lucky to have them in the most important phase of my life.

I can not fully express my gratitude to my parents for their unconditional love and unlimited support to take care of my daughter. I can never say enough to thank my mom whom without her support and trust in me I could have never achieved this goal and I pray for her well being in her battle with stroke.

Table of Contents

	Page
Acknowledgements	
iii	
List of Tables	
ix	
List of Figures	
ix	
List of Abbreviations	
xii	

Abstract.....

.....xiv

Vitaxvii

Chapter

1 Introduction

1

1.1 Cytokine signal transduction and the Jak/Stat pathway

1

1.1.1 Jak family of protein tyrosine kinase

1

1.1.2 Signal transducers of activators of transcription

6

1.2 Tyk2

9

1.2.1 Biology of Tyk2

9

1.2.2 Tyk2 and cancer

10

1.3 Stat3

11

1.3.1 Biology of Stat3

11

1.3.2 Relationship between Grim19 and Stat3

14

1.3.3 Stat3 and cancer

15

1.4 Immune system and anti-tumor responses

16

1.4.1 Innate immunity

17

1.4.2 Adaptive immunity

19

1.4.3 Antitumor immune response

20

1.4.4 The effects of immune cells on tumor development

25

1.5 Breast cancer and 4T1 mouse mammary carcinoma model

25

1.6 Reactive oxygen species and their effects on tumor growth

26

2 Tyk2 deficiency promotes breast tumor growth

30

2.1 Abstract

30

2.2 Introduction

31

2.3 Materials and Methods

34

2.4 Results

44

2.5 Discussion

64

2.6 References for Chapter 2

69

3 Mitochondrial-localized Stat3 mediates breast tumor growth

73

3.1 Abstract

73

3.2 Introduction

74

3.3 Materials and Methods

77

3.4 Results

85

3.5 Discussion

100

3.6 References for Chapter 2

104

4 Discussion

107

General references (for chapters 1 and 4)

List of Tables

Page

Table 1.1: Phenotypes of Jak-deficient mice.

5

Table 1.2: Phenotypes of tissue-specific conditional Stat3-null mice.

13

Table 2.1: Anti-mouse antibodies used in this study.

36

List of Figures

Page

Figure 1.1: Jak/Stat pathway

2

Figure 1.2: Structure of Jak kinases

4

Figure 1.3: Structure of Stat3

7

Figure 1.4: Three phases of cancer cells against host immune system

22

Figure 1.5: Model of innate recognition and initiation of adaptive antitumor immune

response

23

Figure 1.6: Superoxide production in complex I of mitochondria

28

Figure 2.1: Purification of anti-CD4 and anti-CD8 monoclonal antibodies.

41

Figure 2.2: The deletion of Tyk2 gene promotes tumor growth

46

Figure 2.3: Deletion of Tyk2 promotes splenomegaly induced by 4T1 tumor cells

47

Figure 2.4 : Tyk2^{-/-} mice have increased metastatic disease

49

Figure 2.5: Tyk2 deficiency in the host promotes metastasis

50

Figure 2.6: Tyk2^{-/-} splenocytes produce less IFN than Tyk2^{+/+} splenocytes

51

Figure 2.7: The schedule of NK cell depletion and the efficiency of the depletion

53

Figure 2.8: Tyk2^{-/-} NK cells do not contribute to enhanced susceptibility of Tyk2^{-/-} mice

55

Figure 2.9: The schedule of CD8⁺ and CD4⁺ T cell depletion

57

Figure 2.10: Depleting CD4⁺ T cells do not alter tumor growth rate

58

Figure 2.11 CD8⁺ T cells do not contribute to increased primary tumor growth in Tyk2^{-/-} mice

59

Figure 2.12 Tumor-bearing $Tyk2^{-/-}$ mice have higher levels of MDSCs than tumor-bearing $Tyk2^{-/-}$ mice

61

Figure 2.13 Tumor-bearing $Tyk2^{-/-}$ MDSCs have a slight increase in suppressing T cell proliferation

63

Figure 3.1 Domain structure of MLS Stat3

86

Figure 3.2 MLS Stat3 and its variants were expressed only in mitochondria

87

Figure 3.3 MLS Stat3 and its variant do not alter Stat3-dependent transcription

89

Figure 3.4 MLS Stat3 and its variant do not alter cell growth rates under normal conditions of cell culture

91

Figure 3.5 MLS Stat3 S727A and Y705F/S727A mutants have reduced cell survival and growth in low-glucose media

92

Figure 3.6 MLS Stat3 YFSA shows reduced anchorage-independent growth

.....93

Figure 3.7 Serine to alanine mutation of MLS stat3 impairs the invasion

95

Figure 3.8 MLS Stat3 Y705FS727A mutant slows decreased tumor growth, whereas MLS Stat3 Y705FS727D mutant promotes tumor growth

96

Figure 3.8 MLS Stat3 protects tumor cells from hypoxia induced tyrosine nitration

List of Abbreviations

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	complementary 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
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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

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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 effects 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.

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Wegrzyn, J., R. Potla, Y.-J. Chwae, N. B. V. Sepuri, **Q. Zhang**, T. Koeck, M. Derecka,
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Dulak, D. P. Bake, A. Wolfman, D. Stueh, M. O. Hassan, X.-Y. Fu, N. Avadhan, J. I.
Drake, P. Fawcett1, E. J. Lesnefsky, and A. C. Lerner. 2009. Function of Stat3 in
Cellular Respiration. *Science* **323**:793-797