

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR α (PPAR α) IS
REGULATOR OF COLORECTAL CANCER CELL
GROWTH AND DIFFERENTIATION

By

Rajnish Anand Gupta

Dissertation

Submitted to the Faculty of the
Graduate School of Vanderbilt University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in

Cell Biology

May, 2004

Approved By:

Professor Raymond N. DuBois

Professor Al Reynolds

Professor Stephen J. Brandt

ACKNOWLEDGEMENTS

I would like to thank my mother and father, my sister Archana, and my brother Shailen for their unconditional support and encouragement. I would like to thank my graduate thesis advisor, Dr. Raymond DuBois, for all his support, guidance, and patience over the last four years. I have learned a great deal from him, including the importance of identifying meaningful research problems and on how one can successfully integrate clinical and basic science as a physician-scientist. I would also like to thank the members of my thesis advisory committee, Drs. Reynolds, Brandt, Granner, Magnuson, and Crawford for their support and guidance and for always willing to meet with me to discuss my work. All members of the DuBois lab (past and present) provided me with a great deal of help during the last four years, including Jeff Brockman, Chris Williams, Rebecca Shattuck-Brandt, Wade Krause, Sharada Katkuri, Hongmiao Sheng, Jinyi Shao, Radhika Aramandala, and Zhounghou Zhang. I would in particular like to thank Howard Crawford, whose bench was down the hall from mine and who was always willing to take the time to teach me and help me with my research the many times I wandered down to talk with him. Finally, Pasha Sarraf (Dana Farber Cancer Institute), S. K. Dey (University of Kansas), and Tim Willson (GlaxoSmithKline) were all collaborators on various projects and their involvement has been extremely beneficial to me.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	vi
LIST OF FIGURES.....	vii
 Chapter	
I. INTRODUCTION.....	1
Introduction to the nuclear hormone receptor superfamily.....	1
NHR binding motifs – the hormone response element.....	2
Classification of NHRs.....	2
Structure of NHRs.....	5
Transcriptional activities of NHRs.....	8
The role of coregulators in NHR function.....	9
NHR coactivators.....	11
NHR corepressors.....	12
The coregulator exchange in NHR function.....	13
Introduction to peroxisome proliferator-activated receptors.....	14
Overview of PPAR α	18
Overview of PPAR β	19
Introduction to PPAR γ	19
PPAR γ ligands.....	20
PPAR γ coregulators.....	23
PPAR γ as a regulator of adipocyte differentiation.....	24
The role of PPAR γ in insulin signaling.....	25
PPAR γ and the control of cholesterol trafficking in macrophages..	27
PPAR γ and the inflammatory response.....	28
PPAR γ and the control of cell cycle.....	28
Cyclooxygenases and colorectal cancer.....	29
Cyclooxygenase and prostaglandin synthesis.....	29
A second cyclooxygenase enzyme.....	31
NSAIDs and colorectal cancer.....	32
Evidence for a role for COX-2 in colorectal carcinogenesis.....	33
How does COX-2 promote tumor development.....	34
Summary.....	36
II. METHODS.....	38
Cell culture.....	38
Nuclear receptor ligands.....	38
Plasmids.....	39
RT-PCR for PPAR subtypes.....	40
Antibodies.....	41
Western blot analysis.....	41

	Detection of PPAR α protein by IP/Western blot.....	42
	Transient transfections.....	42
	Luciferase assays.....	43
	Cell growth measurements.....	43
	Anchorage independent growth assay.....	43
	Tumor growth in athymic mice.....	44
	Flow cytometry.....	44
	cDNA microarray screening.....	44
	Oligonucleotide microarray screening.....	45
	Northern blot hybridization.....	46
	cDNA probes for northern blots.....	48
	Cell aggregation assay.....	48
	PPAR α gene mutation detection.....	49
	Electromobility shift assay.....	49
	Mammalian two-hybrid.....	50
	Generation of stable cell lines using retroviral infection.....	50
	Generation of stable cell lines using plasmid transfection.....	51
	Immunoprecipitations.....	51
	In situ hybridization.....	52
III.	ACTIVATION OF PPAR α INHIBITS COLORECTAL CANCER CELL GROWTH.....	53
	Introduction.....	53
	Results.....	54
	PPAR α expression and transcriptional activity in a panel of human colorectal cancer cell lines.....	54
	Activation of PPAR α inhibits human colorectal cancer cell growth <i>in vivo</i>	58
	Activation of PPAR α delays cell cycle progression.....	61
	Conclusion.....	61
IV.	TARGET GENES OF PPAR α IN COLORECTAL CANCER CELLS.....	62
	Introduction.....	62
	Results.....	64
	Evaluation of a cell culture system to monitor PPAR α target genes.....	64
	Identification of PPAR α target genes using microarrays.....	64
	Characterizing the specificity and selectivity of each target gene induction/repression.....	69
	Activation of PPAR α induces an increase in CEA- dependent homotypic aggregation.....	72
	Conclusion.....	75
V.	A LOSS OF FUNCTION PPAR α ALLELE IN COLORECTAL CANCER CELLS CAUSED BY A MUTATION THAT DISRUPTS BASAL TRANSCRIPTIONAL REPRESSION.....	77
	Introduction.....	77
	Results.....	79
	PPAR α ligand sensitivity and PPAR α gene mutations in a panel of human colorectal cancer cell lines.....	79

	Characterization of K422Q mutant allele.....	82
	Wild type, but not K422Q, PPAR α can rescue PPAR α ligand unresponsiveness in resistant cells.....	85
	The K422Q apo-receptor cannot repress the basal expression of target genes.....	88
	Conclusion.....	92
VI.	PPAR α AND TRANSFORMING GROWTH FACTOR β PATHWAYS INHIBIT COLON EPITHELIAL CELL GROWTH BY REGULATING LEVELS OF TSC-22.....	93
	Introduction.....	93
	Results.....	95
	Cellular response of colorectal cancer cells to PPAR α and TGF- β 1.....	95
	Identification of TSC-22 as a PPAR α and TGF- β target gene in colon epithelial cells.....	95
	Transcriptional regulation of TSC-22 by PPAR α and TGF- β 1.....	100
	The ability of PPAR α to induce TSC-22 is independent of TGF- β 1.....	103
	Overexpression of wild type TSC-22 inhibits colon epithelial cell growth and induces elevated levels of p21.....	106
	Expression of full-length and mutant TSC-22 constructs.....	107
	Wild type TSC-22 inhibits cell growth and leads to increased levels of p21 but not keratin 20.....	108
	Overexpression of dominant negative TSC-22 partially inhibits PPAR α ligand and TGF- β 1 induced growth inhibition and p21 induction.....	109
	Conclusion	109
VII.	DISCUSSION.....	112
	Activation of PPAR α inhibits colorectal cancer cell growth.....	112
	Target genes of PPAR α in colorectal cancer cells.....	113
	A loss of function allele in colorectal cancer cells caused by mutation that disrupts basal transcriptional repression.....	117
	PPAR α and TGF- β 1 pathways inhibit colon epithelial cell growth by regulating levels of TSC-22.....	121
VIII.	FUTURE AIMS.....	125
	Future Aims.....	125
	Selective PPAR α modulators.....	126
	REFERENCES.....	127

LIST OF TABLES

Table		Page
1.	PPAR target genes with identified PPREs.....	3
2.	Summary of genes induced or repressed after exposure of M-S colon carcinoma cells to the PPAR α ligand rosiglitazone.....	68
3.	PPAR α ligand sensitivity and PPAR α receptor mutations in a panel of human colorectal cancer cell lines.....	81

LIST OF FIGURES

Figure	Page
1. The DNA binding motif of NHRs and NHR classification.....	3
2. Anatomy of NHRs.....	7
3. Transcriptional activities of NHRs and the coregulatory exchange.....	11
4. Overview of PPARs.....	15
5. PPAR α ligands.....	20
6. Metabolic consequences of PPAR α activation.....	25
7. The cyclooxygenase signaling cascade.....	29
8. PPAR α is expressed and functionally active in colorectal cancer cells....	54
9. Rosiglitazone inhibits cell growth of colon cancer cells <i>in vitro</i>	55
10. The PPAR α selective agonist rosiglitazone inhibits anchorage independent growth of cells that express functional PPAR α	57
11. Rosiglitazone reduces the volume of tumors grown in vivo from cells expressing functional PPAR α	59
12. PPAR α activation induces G ₁ cell cycle arrest.....	60
13. PPAR α is expressed and transcriptionally active in the M-S colon carcinoma line.....	65
14. PPAR α specifically and selectively inhibits the growth of the M-S colon carcinoma line.....	66
15. The PPAR α target genes adipophilin and L-FABP are also targets of PPAR α and/or PPAR β	70
16. The PPAR α target genes RegIA, Gob-4, NGAL, and keratin 20 are specifically and selectively regulated by PPAR α	71
17. PPAR α induces three different members of the CEA family of proteins.....	73

18.	PPAR α ligands induce an increase in CEA-dependent aggregation of M-S colon carcinoma cells.....	74
19.	Expression of PPAR α in human colorectal cancer cell lines.....	80
20.	DNA binding and transcriptional activity of K422Q PPAR α	83-4
21.	Generation of HCT 15 colorectal cancer cells expressing WT or K422Q PPAR α by retroviral transduction.....	86
22.	Expression of WT, but not K422Q, PPAR α causes the previously resistant HCT 15 cell lines to become sensitive to PPAR α agonist-induced G ₁ delay and growth inhibition <i>in vitro</i>	87
23.	K422Q PPAR α is defective in repressing the basal expression target genes in the absence of exogenous ligand.....	89
24.	There is no difference between WT and K422Q PPAR α in binding affinity to the corepressors N-CoR or SMRT in solution.....	91
25.	The PPAR α ligand rosiglitazone or TGF- β 1 induces growth inhibition and increases in protein levels of p21 in a panel of colon epithelial cell lines.....	96-7
26.	TSC-22 is a downstream target of both PPAR α and TGF- β 1 in colon epithelial cells.....	98
27.	TSC-22 is localized to the post-mitotic epithelial compartment of the normal human colon.....	99
28.	Time and dose dependent induction of TSC-22 by PPAR α and TGF- β 1....	101
29.	TSC-22 is a direct target of PPAR α	102
30.	TSC-22 is specifically and selectively induced by PPAR α	104
31.	The induction of TSC-22 by PPAR α is not dependent on an intact TGF- β 1 signaling pathway.....	105
32.	Dominant negative TSC-22 blocks the ability of PPAR α or TGF- β to induce p21 and inhibit cell growth.....	110