TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS	iii
ABSTRACT (English)	iv
ABSTRACT (Thai)	viii
LIST OF TABLES	xix
LIST OF FIGURES	xxii
ABBREVIATIONS	xxvi
CHAPTER I INTRODUCTION	
1.1 Statement of problem	1
1.2 Literature review	7
1.2.1 Leukemia	7
1.2.1.1 Etiology and risk factor	7
1.2.1.2 Host factor	7
1.2.1.2.1 Hereditary defect in leukemia	7
1.2.1.2.2 Congenital chromosomal abnormalities	8
1.2.1.2.3 Immunodeficiency	8
1.2.1.2.4 Chronic marrow dysfunction	8
1.2.1.3 Environmental factor	9
1.2.1.3.1 Ionizing radiation	9
1.2.1.3.2 Chemicals and drugs	9
1.2.1.3.3 Viruses	10

1.2.1.4 Incidence 10

1.2.1.5 Leukemia Classification 11

1.2.1.5.1 Acute myelogenous leukemia (AML) 15

1.2.1.5.2 Acute lymphoblastic leukemia (ALL) 28

1.2.1.5.3 Chronic myelogenous leukemia (CML) 29

1.2.1.5.4 B-cell chronic lymphocytic leukemia (B-CLL) 32

1.2.2 *Wilms'tumor 1* gene (*WT1*) 36

1.2.2.1 Structure and function of WT1 37

1.2.2.2 Normal expression of WT1 41

1.2.2.3 WT1 in normal hematopoiesis 41

1.2.2.4 WT1 in leukemia 43

1.2.3 Curcumin 45

1.2.3.1 Curcumin structure 45

1.2.3.2 Solubility and stability 46

1.2.3.3 Metal-binding chemistry 49

1.2.3.4 History and traditional uses of curcumin 52

1.2.3.5 Curcumin and tumor signaling 53

1.2.3.5.1 Curcumin suppress NF-κB activation 56

1.2.3.5.2 Curcumin suppress AP-1 57

1.2.3.5.3 Curcumin suppress mitrogen-activated protein kinases 58

1.2.3.5.4 Curcumin suppress protein kinases 59

1.2.3.5.5 Curcumin suppress Egr-1 60

1.2.3.5.6 Curcumin inhibits growth factor receptor protein Tyrosine kinases 61

1.2.3.6 Curcumin and leukemia 63

1.2.3.7 Curucmin in human clinical trials 66

1.2.3.8 Safety and pharmacology 75

1.2.3.8.1 Pharmacokinetic of curcumin 75

1.2.3.8.2 Safety 76

1.3 Objectives 78

CHAPTER II MATERIALS AND METHODS

2.1 Chemicals and reagents 79

2.2 Cells and cell culture condition 79

2.3 Vector construction 79

2.4 Transfection procedure 80

2.4.1 Stable clone 80

2.4.2 Transient transfection 80

2.5 Extraction and isolation of pure curcumin 81

2.6 Proliferation and viability assay 82

2.6.1 MTT assay 82

2.6.2 Trypan blue exclusion assay 83

2.7 Total RNA extraction and quantitative reverse transcriptase PCR (qRT-PCR) 83

2.8 Protein extraction 85

2.8.1 Whole protein lysate (crude protein) 85

2.8.2 Nuclear, cytosolic and membrane fractions 86

2.8.3 Protein concentration measurement 87

2.9 Protein detection using SDS-PAGE, Western blot analysis and ECL analysis 89
2.9.1 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) 89
2.9.2 Western blot analysis and ECL detection 90
2.9.3 Stripping method 91
2.10 Cytotoxicity of pure curcumin on K562 and stable clone transfected U937 cells 92
2.11 Effect of pure curcumin on WT1 mRNA levels in K562 and stable clone transfected U937 cells in a dose dependent manner 92
2.12 Effect of pure curcumin on WT1 mRNA levels in K562 and stable clone of transfected U937 cells in a time dependent manner 93
2.13 Effect of pure curcumin on WT1 protein levels in K562 and stable clone of transfected U937 cells in a dose dependent manner 93
2.14 Effect of pure curcumin on WT1 protein expression in K562 and stable clone transfected U937 cells in a time dependent manner 93
2.15 Effect of pure curcumin on phosphorylated kinase protein 93
2.16 Effect of pure curcumin on activated PKC isoform 94
2.17 Protein half life assay 95
2.18 Proteasome assay 96
2.19 mRNA stability assay 97
2.20 Chromatin immunoprecipitation (ChIP) assay 97
2.21 Primer design for ChIP experiment 100
2.22 Reporter gene assay 100
2.23 Mutation promoter target assay 101

CHAPTER III RESULTS

3.1 Effect of pure curcumin on cell viability in K562 cells 102

3.2 WT1 overexpression reverses the effect of pure curcumin activity 102

3.3 Effect of pure curcumin on *WT1* gene expression in K562 cells 106

3.3.1 Effect of pure curcumin on WT1 mRNA expression in K562 cells 106

3.3.1.1 Effect of various concentrations of pure curcumin on WT1 mRNA expression at 24 h of incubation time 106

3.3.1.2 Effect of 15 μM of pure curcumin on WT1 mRNA expression at various periods of time 106

3.3.2 Effect of pure curcumin on WT1 protein expression in K562 cells 111

3.3.2.1 Effect of various concentration of pure curcumin on WT1 protein expression in K562 cells at 24 h of incubation time 111

3.3.2.2 Effect of 15 μM pure curcumin on WT1 protein expression in K562 cells in different time points 111

3.4 Effect of pure curcumin on *WT1* gene stability in K562 cells 116

3.4.1. Effect of pure curcumin on WT1 mRNA stability in K562 cells 116

3.4.2. Effect of pure curcumin on WT1 protein stability in K562 cells 116

3.4.3 Effect of proteasome inhibitor on WT1 protein half-life 121

3.5. Effect of pure curcumin on phosphorylated protein kinase in K562 cells 121

3.5.1 Effect of 15 μM pure curcumin on phosphorylated protein kinase in K562 cells using human phosphor-kinase array 121

3.5.2 Effect of 15 μM pure curcumin on protein kinase C signaling cascade in K562 cells 130

3.5.3 Effect of 15 μM pure curcumin on protein kinase A cell signaling in K562 cells 130

3.5.4 Effect of various concentrations of pure curcumin on protein kinase Cα in K562 cells 133

3.6 Effect of specific protein kinase inhibitor on *WT1* gene expression in K562 cells 135

3.6.1 Effect of PKC inhibitor (GF109203x)on WT1 mRNA expression in K562 cells 135

3.6.2 Effect of PKC inhibitor (GF109203x) on WT1 protein expression in K562 cells 135

3.7 PKCα activation up-regulates WT1 expression and reverses pure curcumin inhibition of WT1 expression in K562 cells 140

3.8 Investigation upstream and downstream of PKCα signaling cascade in K562 cells 145

3.8.1 Investigation upstream of PKCα signaling cascard in K562 cells 145

3.8.2 Investigation downstream of PKCα signaling cascard in K562 cells 145

3.9 Effect of pure curcumin on WT1 proximal promoter in K562 cells 155

3.10 Identification of the effect of pure curcumin on Sp1 transcription factor binding *WT1* gene promoter in K562 cells 159

3.10.1 Effect of various concentrations of pure curcumin on Sp1 protein in K562 cells 159

3.10.2 Effect of pure curcumin on binding of Sp1 protein and *WT1* gene promoter in K562 cells 159

3.11 Effect of pure curcumin on WT1 proximal promoter activity 164

3.12 Establishment and characterization of U937 clones constitutively expressing WT1 168

3.13 Cytotoxic effects of pure curcumin on four WT1 isoforms stably transfected U937 cells 172

3.14 WT1 overexpression reverses the effect of pure curcumin activity 172

3.15 Effect of pure curcumin on mRNA levels in four WT1 isoform stably transfected cells 176

3.16 Effect of pure curcumin on exogenous WT1 protein in WT1 +/+ isoform transfected U937 cells 176

3.16.1 Effect of various concentrations of pure curcumin on exogenous WT1 protein in stably transfected U937 cells 176

3.16.2 Effect of 15 μM pure curcumin on exogenous WT1 protein in stably transfected U937 cells at various time points 181

3.17 Effect of pure curcumin on exogenous WT1 protein expression via pCMV promoter 181

3.18 Effect of pure curcumin on exogenous WT1 protein half-life in WT1 +/+ isoform stably transfected U937 cells 186

3.19 Effect of PKC inhibitor on exogenous WT1 +/+ protein expression in transfected U937 cells 191

CHAPTER IV DISCUSSION 194

CHAPTER V CONCLUSION 207

REFERENCES 209

APPENDICES 249

Appendix A 250

Appendix B 261

Appendix C 265

CURRICULUM VITAE 280

LIST OF TABLES

Table		Page
1	Classification of leukemia	13
2	Clinical feature of acute myelogenous leukemia	17
3	FAB classification of acute myeloblastic leukemia	18
4	The MIC classification of AML	20
5	FAB classification of acute lymphoblastic leukemia	30
6	Effect of curcumin on different cell signaling pathways	53
7	Clinical trial of curcumin	66
8	Preparation of stock BSA standard solution	88
9	Inhibitory concentration at 20% and 50% values of pure curcumin on K562 cells	103
10	Percentage of relative WT1 mRNA expression in K562 after treatment with various concentration of pure curcumin	107
11	Percentage of relative WT1 mRNA levels in K562 cells after treatment with 15 μM pure curcumin treatment for 0, 3, 6, 12, and 24 h	109
12	Percentage of relative WT1 protein levels in K562 cells after treatment with various concentrations of pure curcumin	112
13	Percentage of relative WT1 protein levels in K562 cells after treatment with 15 μM pure curcumin in different time points	114

14 Percentage of relative WT1 mRNA level and half-life in K562 cells after treatment with actinomycin D in the presence or absence of 15 μM pure curcumin at different time points 117

15 Percentage of relative WT1 protein levels and half-lives in K562 cells after treatment with cycloheximide in the presence or absence of 15 μM pure curcumin at different time points 119

16 Percentage of relative WT1 protein levels in K562 cells after treatment with or without MG132 in presence or absence of 15 μM pure curcumin in difference time points 122

17 Percentage of relative WT1 protein levels in K562 cells after treatment with or without EGCG or lactacystin in presence or absence of 15 μM pure curcumin 124

18 Relative ratio of phosphor-kinease protein after pure curcumin treatment in K562 cells 126

19 Percentage of relative of protein kinase C isoenzyme and protein kinease A levels in K562 cells after 15 μM pure curcumin treatment 131

20 Percentage of relative activated PKCα protein levels in treated K562 cells with various concentrations of pure curcumin 133

21 Percentage of relative WT1 mRNA level in treated K562 cells with 0.0084 μM (IC_{50} value) GF109203x (PKCα inhibitor) 136

22 Percentage of relative WT1 protein level in treated K562 cells with 0.0084 μM (IC_{50} value) GF109203x (PKCα inhibitor) 138

23 Percentage of relative WT1 protein level is high levels after transfection with activated PKCα 141

24 Activated PKCα stimulated endogenous WT1 protein expression and reversed pure curcumin activity in K562 cells 143

25 Percentage of relative WT1 mRNA level in treated K562 cells with 1.4 μM (IC_{50} value) LY294002 (PI3K inhibitor) 147

26 Percentage of relative WT1 mRNA level in treated K562 cells with 0.53 μM (IC_{50} value) U0126 (MEK inhibitor) 150

27 Percentage of relative WT1 mRNA level in treated K562 cells with 40 nM (IC_{50} value) SP600125 (JNK inhibitor) 152

28 Percentage of relative WT1 and Sp1 protein levels after pure curcumin treatment with various concentration for 24 h 160

29 Relative enrichment ratio of SYBR green qPCR amplification of promoter DNA containing WT1 binding site in K562 cell which were treated with 15 μM pure curcumin 162

30 Firefly luciferase activity of 301 bp consensus WT1 promoter constructed pGL3 vector was treated with 15 μM pure curcumin for 24 h 165

31 IC_{20} and IC_{50} value of pure curcumin on cytotoxicity of transfected U937 cells 173

32 Percentage of relative levels of WT1 isoforms mRNA in transfected U937 cells after treatment with pure curcumin or DMSO 177

33 Percentage of relative exogenous WT1 +/+ protein level in transfected U937 cells after treatment with 10, 15, 17 μM pure curcumin or 0.03% DMSO for 24 h 179

34 Percentage of relative levels of exogenous WT1 +/+ protein in transfected U937 cells after treatment with 15 uM pure curcumin in different time points 182

35 Percentage of relative exogeousWT1 protein level and half-life of WT1 +/+ transfected U937 cells after treatment with cycloheximide in the presence or absence of 15 μM pure curcumin at different time points 187

36 Percentage of relative exogenous WT1 +/+ protein level in stably transfected U937 cells after treatment with or without MG132 in presence or absence 15 μM pure curcumin 189

37 Percentage of relative exogenous WT1 protein level in transfected U937 cells were treated with 2.5 μM GF109203x (PKCα, β1, δ, and ε inhibitor) 192

38 The percentage of SDS-PAGE used for preparing gel 268

LIST OF FIGURES

Figure		Page
1	Structure of *WT1* gene and WT1 protein	38
2	Diagrammatic representation of major and minor WT1 protein isoforms	40
3	A stemless rhizomatous of *Curcuma longa*	46
4	Nature yellow dye	48
5	Tautomerism of curcumin under physiological conditions	49
6	Nomenclature of regions of curcumin I	51
7	Stages in tumor progression inhibited by curcumin	63
8	Structure of curcumin and its metabolite	77
9	Standard curve of BSA	88
10	The chromatin immunoprecipitation (ChIP) assay and various methods of analysis	99
11	Cytotoxicity of pure curcumin on K562 cells	103
12	Effect of WT1 isoform overexpression on pure curcumin activity	105
13	Effect of various concentration of pure curcumin on WT1 mRNA expression in K562 cells	108
14	Effect of pure curcumin on WT1 mRNA expression of K562 cells in a time-dependent manner	110
15	Effect of various concentration of pure curcumin on WT1 protein expression in K562 cells by Western blot analysis	113

16 Effect of pure curcumin on WT1 protein expression in K562 cells by Western blot analysis 115

17 Effect of pure curcumin on WT1 mRNA stability using actinomycin D 118

18 Effect of pure curcumin on WT1 protein stability using cycloheximide 120

19 Effect of pure curcumin on WT1 protein stability by using MG132 123

20 Effect of pure curcumin and proteosome inhibitors on proteasomal degradation 125

21 Effect of pure curcumin on phosphorylated protein kinase in K562 cells 128

22 Effect of pure curcumin on the level of protein kinase C isoenzymes and protein kinase A expression in K562 cells 132

23 Effect of various pure curcumin on the level of activated protein kinase Cα expression at cell membrane in K562 cells 134

24 Effect of protein kinase C inhibitor (GF109203x) on the level of WT1 mRNA expression in K562 cells 137

25 Effect of PKC inhibitor on the level of WT1 protein expression in K562 cells 139

26 Activated PKCα stimulated endogenous WT1 protein expression in K562 cells 142

27 PKCα activation stimulated endogenous WT1 protein expression in K562 cells 144

28 Overview of signaling transduction pathway 146

29 Effect of PI3K inhibitor (LY294002) on the level of WT1 mRNA expression in K562 cells 148

30 Effect of MEK inhibitor (U0126) on the level of WT1 mRNA expression in K562 cells 151

31 Effect of SP600125 (JNK inhibitor) on the level of WT1 mRNA expression in K562 cells 153

32 Effect of 0.014% DMSO on the level of WT1 mRNA expression in K562 cells 154

33 Sequence of the WTl proximal promoter region 156

34 Pure curcumin treatment attenuated WT1 protein transcription factor binding to the proximal *WT1* gene promoter 157

35 Time-dependent comparison of WT1 protein levels versus promoter occupancy by qPCR 158

36 Effect of various concentration of pure curcumin on Sp1 protein expression in K562 cells 161

37 Pure curcumin treatment attenuated WT1 transcription factor protein binding to the WT1 proximal promoter 163

38 Effect of pure curcumin on WT1 gene activity *via* Firefly luciferase reporter 166

39 Site directed mutagenesis of the WT1 consensus sequence (-50 to -39) abolished the WT1 promoter activity compared to the wild type WT1 promoter construct (301 bp WT1) 167

40 Expression of *WT1* gene in transfected cells by RT-PCR 169

41 Expression of *WT1* gene in transfected cells by qRT-PCR 170

42 Expression of WT1 protein in transfected U937 cells by immunoprecipitation and Western blot analysis 171

43 Cytotoxicity of pure curcumin on transfected U937 cells 174

44 Cytotoxic effect of pure curcumin on WT1 isoform-transfected U937 cells 175

45 Effect of pure curcumin on WT1 isoforms mRNA level in transfected U937 cells 178

46 Effect of different concentrations of pure curcumin on exogenous WT1 +/+ protein in stably transfected U937 cells 180

47 Effect of pure curcumin at 15 μM on exogenous WT1 +/+ protein expression in WT1 +/+ transfected U937 cells 183

48 Effect of pure curcumin on other proteins driven by the pCMV promoter 184

49 Effect of pure curcumin on exogenous WT1 +/+ protein stability using Cycloheximide 188

50 Effect of pure curcumin on exogenous WT1 +/+ protein stability using MG132 190

51 Effect of GF109203x on exogenous WT1 +/+ protein expression in transfected U937 cells 193

52 Diagram signaling cascade propose relation of WT1 transcription factor activity 201

53 Schematic model of inhibitory mechanism of pure curcumin on endogenous *WT1* gene expression in K562 cells and exogenous WT1 protein in transfected U937 cells 206

ABBREVIATIONS

%	Percent
β	Beta
α	Alpha
γ	Gamma
ε	Epsilon
δ	Delta
°C	Degree Celsius
aa	Amino acid
ALL	Acute lymphocytic leukemia
AML	Acute myelogenous leukemia
AP-1	Activator protein-1
APL	Acute promyelocytic leukemia
ATM	Ataxia telangiectasia mutated
ATPase	Adenosine triphosphatase
BM	Bone marrow
bp	Base pair
BSA	Bovine serum albumin
CD	Cluster of Differentiation
CLL	Chronic lymphocytic leukemia

CML	Chronic myelogenous leukemia
CO_2	Carbon dioxide
CHX	Cycloheximide
COX	Cyclooxygenase
DEPC	Diethyl pyrocarbonate
DMSO	Dimetyl sulfoxide
DNA	Deoxyribonucleic acid
DTT	Dithiotreitol
K_2EDTA	Ethylenediaminetetraacetic acid dipotassium dihydrate
ECL	Enhanced chemiluminescence
EGF	Epidermal growth factor
FAB	French-American-British
FBS	Fetal bovine serum
FGF	Fibroblast growth factor
gm	Gram
GAPDH	Glyceraldehyde-3-phosphate dehydrogenease
GST	Glutathione S-transferase
h	Hour
HEPES	*N*-2-hydroxyethylpiperazine-*N*-2-ethanesufonic acid
HPLC	High performance liquid chromatography
IC_{20}	Inhibitory concentration at 20% growth
IC_{50}	Inhibitory concentration at 50% growth
IL	Interleukin
IU	International unit

JNK	c-Jun N-terminal kinase
kb	Kilobase
kDa	Kilodalton
KTS	Lysine-threonine-serine
L	Liter
LAP	Leukocyte alkaline phosphatatse
LPS	Lipopolysaccharide
LOX	Lipoxygenase
K562	Human erythroid leukemia cell line
µg	Microgram
MIC	Morphologic-immunologic-cytogenetic
µL	Microliter
µM	Micromolar
M	Molar
mM	Millimolar
mL	Milliliter
MMP	Matrix metalloprotease
MPO	Myeloperoxidase
mRNA	Messenger ribonucleic acid
MTT	3-(4, 5 dimethylthiazole-2yl)-2, 5 Diphenyltetrazolium Bromide
NADH	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor kappa B
nM	Nanomolar

nm	Nanometer
NOS	Nitric oxide synthase
OD	Optical density
PAS	Periodic acid shift
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PKC	Protein kinase C
PKCα	Protein kinase C alpha
PTK	Protein tyrosine kinase
RNA	Ribonucleic acid
RPMI 1640	Roswell Park Memorial Institute 1640 medium
RT-PCR	Reverse transcription polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
rpm	Revolution per minute
SBB	Sudan Black B
SD	Standard deviation
SEM	Standard error of sample mean
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Sp1	Stimulating protein 1
TAE	Tris acetate EDTA
TdT	Terminal deoxynucleotidyl transferase
TEMED	*N, N, N, N*,-tetrametyl ethylene diamine

TNF	Tumor necrosis factor
TPA	12-*O*-tetradecanoylphorbol-13-acetate
U937	Human monocytic leukemia cell line
w/v	Weight per volume
WHO	World Health Oranization
WT1	Wilms' tumor 1
v/v	Volume per volume