APPENDICES

APPENDIX A

List of chemical and materials used in this study

Name of chemicals	Company
40% Polyacrylamide	AMRESCO, USA
Absolute ethanol	E.Merck, Germany
Ammonium persulfate	Sigma-Aldrich, USA
Anti-Bax primary Ab	Cell Signaling Technology, USA
Anti-Bcl-xL primary Ab	Cell Signaling Technology, USA
Anti-CyclinD1 primary Ab	Cell Signaling Technology, USA
Anti-CyclinE primary Ab	Cell Signaling Technology, USA
Anti-CYP2A13 primary Ab	Santa Cruz Biotechnology, USA
Anti-CYP2A6 primary Ab	Santa Cruz Biotechnology, USA
Anti-DNMT1 primary Ab	Santa Cruz Biotechnology, USA
Anti-iNOS primary Ab	Abcam PLC, UK
Anti-K-ras primary Ab	Santa Cruz Biotechnology, USA
Anti-mouse secondary Ab	Abcam PLC, UK
Anti-NF-κB primary Ab	Santa Cruz Biotechnology, USA
Anti-PARP primary Ab	Santa Cruz Biotechnology, USA
Anti-PCNA primary Ab	Cell Signaling Technology, USA

Anti-p-MEK1/2 primary Ab	Santa Cruz Biotechnology, USA
Anti-Pro-caspase-3 primary Ab	Cell Signaling Technology, USA
Anti-Pro-caspase-8 primary Ab	Cell Signaling Technology, USA
Anti-Pro-caspase-9 primary Ab	Cell Signaling Technology, USA
Anti-rabbit secondary Ab	GE Healthcare, UK
Anti-β-actin primary Ab	Santa Cruz Biotechnology, USA
ApopNexin FITC apoptosis detection kit	Merck Millipore, Germany
Aprotinin	Sigma-Aldrich, USA
Benzamidine	AMRESCO, USA
BrdU Cell Proliferation Assay Kit	Merck Millipore, Germany
Coomassie Brilliant Blue G-250	Thermo scientific, USA
Cox2 primer	TAKARA BIO INC, Japan
CYP2A13 primer	Bio Basic Inc, Canada
Cyp2a5 primer	TAKARA BIO INC, Japan
CYP2A6 primer	Bio Basic Inc, Canada
DAB	Dako Corp, Denmark
Dextran sodium sulfate	MP Biomedicals, USA
Dithiothreitol	Sigma-Aldrich, USA
DMSO	RCI Labscan Ltd, Thailand
Dulbeco's modified eagel's medium	GIBCO, USA
EDTA	Sigma-Aldrich, USA
EGTA	Sigma-Aldrich, USA
Ethanol	RCI Labscan Ltd, Thailand
Fetal bovine serum	Hyclone, USA
GAPDH primer	Bio Basic Inc, Canada
Glycerol	Sigma-Aldrich, USA
Glycine	Research Organics Inc, USA
HEPES	VWR International Ltd. England
iNos primer	TAKARA BIO INC, Japan
KCl	Sigma-Aldrich, USA
KH_2PO_4	Sigma-Aldrich, USA

Ki67 primary Ab	Dako Corp, Denmark
Leupeptin	Sigma-Aldrich, USA
Methanol	RCI Labscan Ltd, Thailand
Methylene blue	Sigma-Aldrich, USA
MTT dye	Invitrogen, USA
Na_2HPO_4	Sigma-Aldrich, USA
NaCl	E.Merck, Germany
$NaHCO_3$	Sigma-Aldrich, USA
NNK	Toronto, Canada
NucleoSpin® RNA II kit	Macherey Nagel, Germany
OxiSelect™ Comet assay kit	Cell Biolabs Inc, USA
Penicillin/Streptomycin	GIBCO, USA
PMSF	Sigma-Aldrich, USA
Precision Plus Protein™ Standard	Bio-Rad Laboratories, USA
Propidium iodide solution	Invitrogen, USA
Restore™ PLUS Western Blot Stripping Buffer	Thermo scientific, USA
ReverTra Ace® qPCR RT Kit	Toyobo, Japan
Ribonuclease A	United States Biological, USA
RNeasy Mini Kit	Qiagen sciences, USA
RPMI 1640	GIBCO, USA
Sodium dodecyl sulfate	Merck Millipore, Germany
Sodium vanadate	Sigma-Aldrich, USA
Sodiun fluoride	Sigma-Aldrich, USA
SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR kit	Invitrogen, USA
SuperSignal® West Pico Chemiluminescent Substrate	Thermo scientific, USA
TaKaRa SYBR Premix Ex Taq	TAKARA BIO INC, Japan
TEMED	Sigma-Aldrich, USA
THUNDER-BIRD™ SYBR® qPCR Mix	Toyobo, Japan
Tnf-α primer	TAKARA BIO INC, Japan

Tris	AMRESCO, USA
Triton X-100	Sigma-Aldrich, USA
Trizol reagent	Invitrogen, USA
Tween 20	Sigma-Aldrich, USA
VECTASTAIN® Elite ABC Kit	Vector Laboratories Inc, USA
β-actin primer	TAKARA BIO INC, Japan

APPENDIX B

List of instrument used in this study

Instrument	Company
8μm Membrane filters	Merck Millipore, Germany
Analytical balance AC 100	Sartorius AG, Germany
Autoclave	Tomy Tech, USA
Automatic pipette	Sartorius Biohit, Finland
Culture disk	Corning Incorporated, USA
Culture flask	Nunc, Denmark
Deionized water machine	ELGA Labwater, UK
ELISA plate reader	BioTek Instruments Inc, USA
Flow cytometer	BD Biosciences, USA
Fluorescence microscope	Nikon Instruments, USA
Freezer (-20 °C)	Whirlpool, USA
Freezer (-80 °C)	NuAire Laboratory, USA
Glassware	DURAN® Laboratory, Germany
Hot air oven	Heraeus Holding, Germany
HPLC machine	Agilent Technologies, USA
Laminar flow biological cabinet	NuAire Laboratory, USA

Light microscope	Nikon Instruments, USA
Light-Cycler	Roche Diagnostics, Germany
Microcentrifuge	Hettich Instruments, USA
Microplate	Nunc, Denmark
Microtube and Tip	Axygen Inc, USA
Molecular Imager ChemiDoc XRS System	Bio-Rad Laboratories, USA
NanoDrop® ND-1000 UV-Vis Spectrophotometer	NanoDrop Technologies, USA
Nitrocellulose membrane	GE Healthcare, UK
Pascal pressure cooker	Dako Corp, Denmark
Pasture pipette	Corning Incorporated, USA
PCR machine	Eppendorf Scientific Inc, USA
pH meter	Hanna Instruments, USA
Polystyrene FACS tube (12x75 mm)	BD Biosciences, USA
Power supply	E-C Apparatus Corporation, USA
Real-time PCR machine	Applied Biosystems, USA
Refrigerator	Toshiba, Japan
Scanning electron microscope	JEOL Inc, USA
Serological pipette	HBG, Germany
Sonicator	Crest Ultrasonics, USA
Spectrophotometer microplate reader	BioTek Instruments Inc, USA
Vortex	Scientific industries, USA
Water bath	Julabo Inc, USA
YMC C-30 reverse-phase (RP) column	YMC CO., Ltd, Japan

APPENDIX C

Preparation of some reagents and buffers

Cell culture

1. Incomplete DMEM medium with phenol red

DMEM	1	package (13.5 g)
HEPES	2.603	g
$NaHCO_3$	3.7	g
Deionize distilled water	800	mL

Adjust pH to 7.2-7.4 then top up volume with deionized water to 1,000 mL and sterile by suction filter (membrane pore size 0.2 µm).

2. Incomplete RPMI 1640 medium

RPMI 1640	1	package (13.5 g)
HEPES	2.603	g
$NaHCO_3$	3.7	g
Deionize distilled water	800	mL

Adjust pH to 7.2-7.4 then top up volume with deionized water to 1,000 mL and sterile by suction filter (membrane pore size 0.2 µm).

3. Completed culture media (DMEM and RPMI)

Incomplete medium	89	mL
Fetal bovine serum	10	mL
Penicillin/Streptomycin	0.5	mL

Stored at 4°C.

4. Freezing solution

Fetal calf serum	9.2	mL
DMSO	0.8	mL

Stored at 4°C.

H&E staining

1. Eosin Y Stock Solution (1%)

Eosin Y	10	g
Distilled water	200	mL
95% Ethanol	800	mL

Mix to dissolve and store at room temperature.

2. Eosin Y Working Solution (0.25%)

Eosin Y stock solution	250	mL
80% Ethanol	750	mL
Glacial acetic acid (concentrated)	5	mL

Mix well and store at room temperature.

3. Hematoxylin Solution

Potassium or ammonium (alum)	100	g
Distilled water	1,000	mL

Heat to dissolve. Add 50 mL of 10% alcoholic hematoxylin solution and heat to boil for 1 minute. Remove from heat and slowly add 2.5 g of mercuric oxide (red). Heat to the solution and until it becomes dark purple color. Cool the solution in cold water bath and add 20 mL of glacial acetic acid (concentrated). Filter before use.

IHC staining

Citrate Buffer (10mmol/L Citric Acid, 0.05% Tween 20, pH 6.0)

Citric acid (anhydrous)	1.92	g
Distilled water	1,000	mL

Mix to dissolve. Adjust pH to 6.0 with 1N NaOH and then add 0.5 mL of Tween 20 and mix well. Store this solution at room temperature for 3 months or at 4^{o}C for longer storage.

Measurement of cell survival

1. MTT stock dye solution (5mg/mL)

MTT	0.2	g
PBS pH 7.4	40	mL

Filtrate with membrane filter pore size 0.2 µm, collect in dark container.

2.Phosphate buffer saline (PBS) pH 7.4

KH_2PO_4	0.24	g
Na_2HPO_4	1.44	g
NaCl	8.0	g
KCl	0.2	g

Dissolve in 800 mL of deionized distilled water, adjust pH to 7.4, and then top up to 1,000mL. Sterile by autoclave.

Western blot analysis

1. Whole cell lysis buffer without proteinase inhibitor

5M NaCl	75	µL
1M HEPES pH7.9	30	µL
10% NP-40	150	µL
Deionized water	1,180	µL
10mg/mL Aprotinin	2	µL
10mg/mL Leupeptin	2	µL
100mM PMSF	5	µL
1M NaF	10	µL
0.2M Na_3VO_4	10	µL
0.5M EDTA pH8.0	7.5	µL
0.1M EGTA pH 7.0	6.0	µL

3. Cytoplasmic extraction buffer without protease inhibitor

1.0 M HEPES, pH 7.9	1	mL
2.0 M KCl	0.5	mL
0.5 M EDTA, pH 8.0	0.02	mL
0.1 M EGTA, pH 7.0	0.1	mL
Sterilized deionized water	96.28	mL

4. Cytoplasmic extraction buffer with protease inhibitors

Lysis buffer without proteinase inhibitor		
0.1 M DTT	0.1	mL
100 mM PMSF	0.05	mL
1 mg/mL Leupeptin	0.02	mL
1 mg/mL Aprotinin	0.02	mL
250 mg Benzamidine	0.02	mL

5. Nuclear extraction buffer without protease inhibitor

1.0 M HEPES, pH 7.9	1	mL
2.0 M NaCl	4	mL
0.5 M EDTA, pH 8.0	0.1	mL
0.1 M EGTA, pH 7.0	0.5	mL
Sterilized deionized water	43.1	mL

6. Nuclear extraction buffer with protease inhibitors

Lysis buffer without proteinase inhibitor	974	mL
0.1 M DTT	10	mL
100 mM PMSF	10	mL
1 mg/mL Leupeptin	2	mL
1 mg/mL Aprotinin	2	mL
250 mg Benzamidine	2	mL

7. Phosphate buffered saline (PBS)

NaCl	8.18	g
KCl	0.201	g

Na_2HPO_4	1.42	g
KH_2PO_4	0.244	g

Adjust volume to 1000 mL with DI water.

8. PBS-T (0.1% v/v Tween)

PBS	500	mL
Tween 20	500	µL

Mix to dissolve and store at 4°C.

9. 6X DNA loading dye

Sodium dodecyl sulfate	1.2	g
Bromophenol blue	6.0	mg
Glycerol	4.7	mL
0.5M Tris pH6.8	1.2	mL
Sterilized deionized water	2.1	mL

Dissolve by warming, and then add 0.93 g of DTT. Aliquote and keep frozen at-20ºC.

10. Separating gel for SDS-PAGE

	6%	**8%**	**10%**	**12%**	
Deionized water	4,785	4,365	3,952	3,540	µL
1.5 M Tris (pH8.8)	2,062	2,062	2,062	2,062	µL
10% SDS	82.5	82.5	82.5	82.5	µL
40% Polyacrylamide	1,238	1,650	2,062	2,475	µL
10% APS	82.5	82.5	82.5	82.5	µL
TEMED	8.5	8.5	8.5	8.5	µL
Total Volume	8.25	8.25	8.25	8.25	mL

11. Stacking gel for SDS-PAGE

	3%	**4%**	
Deionized water	1,967	1,892	µL
0.5 M Tris (pH6.8)	750	750	µL
10% SDS	30	30	µL
40% Polyacrylamide	225	300	µL
10% APS	25	25	µL
TEMED	2.5	2.5	µL
Total Volume	3	3	mL

12. Blotting buffer

Tris-base	3.03	g
Glycine	14.4	g
Methanol	200	mL

Deionized distilled water top up to 1000 mL.

Curriculum vitae

Name	Mr.Suphot Phutthaphadoong
Date of Birth	November 19th, 1982
Instituted attended	
1995 - 2001	Certificated Yupparaj Wittayalai School, Chiang Mai, Thailand.
2001 - 2005	2nd class honor, Bachelor Degree in Medical Technology Chiang Mai University, Chiang Mai, Thailand.

Publications

1. **Phutthaphadoong S**, Yamada Y, Hirata A, Tomita H, Taguchi A, Hara A, Limtrakul PN, Iwasaki T, Kobayashi H, Mori H, Chemopreventive effects of fermented brown rice and rice bran against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in female A/J mice, *Oncol Rep*. 2009; 21(2): 321-7.
2. **Phutthaphadoong S**, Yamada Y, Hirata A, Tomita H, Hara A, Limtrakul P, Iwasaki T, Kobayashi H, Mori H, Chemopreventive effect of fermented brown rice and rice bran (FBRA) on the inflammation-related colorectal carcinogenesis in *Apc*$^{Min/+}$ mice, *Oncol Rep*. 2010; 23(1): 53-9.
3. Hatano Y, Yamada Y, Hata K, **Phutthaphadoong S**, Aoki H, Hara A, Genetic ablation of a candidate tumor suppressor gene, Rest, does not promote mouse colon carcinogenesis, *Cancer Sci*. 2011; 102(9): 1659-64.
4. **Phutthaphadoong S**, Yodkeeree S, Chaiyasut C, Limtrakul P, Anti-cancer activities of α- and γ-Tocotrienol against the human lung cancer, *African Journal of Pharmacy and Pharmacology*. 2012; 6(9): 620-9.

Oral presentation

1. **Phutthaphadoong S**, Yamada Y, Hirata A, Tomita H, Taguchi A, Hara A, Limtrakul PN, Iwasaki T, and Mori H, Chemopreventive effects of fermented brown rice and rice bran (FBRA) against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumors in female A/J mouse, The 10th RGJ-Ph.D. Congress, April 3rd-5th 2009, Pattaya, Thailand**.**
2. **Phutthaphadoong S**, Yamada Y, Hirata A, Tomita H, Hara A, Limtrakul PN, and Mori H, Chemopreventive effect of fermented brown rice and rice bran (FBRA) on the inflammation-related colorectal carcinogenesis in *Apc* $^{Min/+}$ mice. RGJ Seminar Series LXXII, July 2nd 2010, Chiang Mai, Thailand.
3. **Phutthaphadoong S**, Yamada Y, Hirata A, Tomita H, Taguchi A, Hara A, Limtrakul PN, Iwasaki T, and Mori H, Chemopreventive effects of fermented brown rice and rice bran (FBRA) against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumors in female A/J mouse, The 3rd Biochemistry and Molecular Biology Conference, April 6th-8th 2011, Chiang Mai, Thailand**.**