REFERENCES

Adami, F. and others. (1994) Serum levels of tumor necrosis factor-alpha in patients with B-cell chronic lymphocytic leukaemia. Eru. J. Cancer. 30A: 1259-1263.

Aganna E, Aksentijevich I, Hitman GA, Kastner DL, Hoepelman AI, and Posma FD. (2001) Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) in a Dutch family: evidence for a TNFRSF1A mutation with reduced penetrance. Eur. J. Hum. Genet. 9(1) : 63-66.

Alessandra, F. and others. (2002) The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia. Blood 100(4) : 1215-1219.

Ashkenazi A DV. (1998) Death receptors: signaling and modulation. Science (Washington DC). 281: 1305-1308.

Balkwill, F.R. and others. (1986) Human tumor xenografts treated with recombinant human tumor necrosis factor alone or in combination with interferons Cancer Res. 46: 3990-3993.

Baud V. (1999) Signaling by proinflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev. 13: 1297-1308.

Bazzoni F BB. (1996) The tumor necrosis factor ligand and receptor families. N. Engl. J. Med. 334: 1717-1725.

Boryana, K. P. and others. (2008) Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J. Clin. Invest. 118: 560-570.

Carwell EA, Okd Lj, Kassel RL, Green S, Fiore N, and Williamson B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. U S A, 72: 3666-3670.

Chang, H.Y.a.Y., X. (2000) Proteases for cell suicide: functions and regulation of caspases. Microbiol. Mol. Biol. Rev. 64: 821-846.

Creasey AA, Reynolds MT, and Laird W. (1986) Cures and partial regression of murine and human tumors by recombinant human tumor necrosis factor. Cancer Res. 46: 5687-5690.

Cross, M. and Dexter TM. (1991) Growth factors in development, transformation and tumorigenesis. Cell. 64: 271-280.

De Groen P.C., Gores G.J., La Russo N.F., Gunderson L.L. and Nagorney D.M. (1999) Biliary Tract Cancers. N. Engl. J. Med. 341: 1368-1378.

Degterev, A. BM. And Yuan J. (2003) A decade of caspases. Oncogene 22: 8543-8567.

Deng Y, Lin Y, and Wu X. (2002) TRAIL-induced apoptosis requires Bax-dependent mitochondrial release of Smac/DIABLO. Genes Dev 16: 33–45.

Devin A CA, Lin Y. (2000) The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. Immunity 12: 419-429.

Dolcet, X. LD. And Pallares J. (2005) NF-kB in development and progression of human cancer. Virchows Arch. 446: 475-482.

Dudek, H. and others. (1997) Regulation of Neuronal Survival by the Serine-Threonine Protein Kinase Akt.

Duygu, D. and others. (2008) Serum levels of apoptosis biomarkers, surviving and TNF-alpha in nonsmall cell lung cancer. Lung Cancer 59: 240-245.

Fava G, Marzioni M., and Benedetti A. (2007) Molecular pathology of biliary tract cancers. Cancer Letter 250: 155-167.

Ferry S., Laifa H., Yu N., Norifumi N., and Seiji N. (2006) Inhibition of Akt and MAPK pathways elevated potential of TNF-alpha in inducing apoptosis in ameloblastoma. Oral oncol 42: 39-45.

Gilbert LC, Rubin J, and Nanes MS. (2005) The p55 TNF receptor mediates TNF inhibition of osteoblast differentiation independently of apoptosis. Am. J. Physio.; 288(5): E1011-E1018.

Gupta S. A. (2002) decision between life and death during TNF-alpha induced signaling. J. Clin. Immunol. 22 (4): 185-194.

Haranaka, K., Satomi, N., Sakurai, A., and Nariuchi, H. (1985) Purification and partial amino acid sequence of rabbit tumor necrosis factor. Int J Cancre. 36: 395-400.

Harnois DM, Que FG, Celli A, LaRusso NF, and Gores GJ. (1997) Bcl-2 is over expressed and alters the threshold for apoptosis in a cholangiocarcinoma cell line. Hepatology 26: 884-890.

Higuchi, M. and Aggarwal BB. (1994) Differential roles of two types of the TNF receptor in TNF induced cytotoxicity, DNA fragmentation, and differentiation. J. Immunol, 152 : 4017-4025.

Justin L. Mott, Steve F. Bronk, Ruben A. Mesa, et al.(2008) BH3-only protein mimetic obatoclax sensitizes cholangiocarcinoma cells to Apo2L/TRAIL-induced apoptosis Mol Cancer Ther 7: 2339-2347.

Kauffmann-Zeh, A., and others (1997) Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB Nature 385: 544–548.

Kerr JF, Winterford CM, and Harmon BV. (1994) Apoptosis: its significance in cancer and cancer therapy. Cancer 73 : 2013-2026.

Khan, S.A. and others. (2005) Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. Gut 51(supp. VI): vi1-vi9.

Kobayashi S, Werneburg NW, Bronk SF, Kaufmann SH, and Gores GJ. (2005) Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells. Gastroenterology 128: 2054–2065.

Kulik, G., Klippel, A., and Weber, M. J. (1997) Phosphatidylinositol 3-kinase mediates epidermal growth factor-induced activation of the c-Jun N-terminal kinase signaling pathway Mol. Cell Biol. 17: 1595–1606.

Locksley, RM. KN. And Lenardo MJ. (2001) The TNF and TNF receptor superfamilies:integrating mammalian biology. . Cell Adhes Commun. 104: 487-501.

MacEwan DJ. (2002) TNF ligands and receptors--a matter of life and death. Br J Pharmacol. 135(4): 855-875.

Marie-Francoise D., Chantal F. and Pierre L. (1989) Synergistic cytotoxic effects of recombinant human tumor necrosis factor, interferons and heat-stress. Cancer res. 49: 5618-5622.

McDermott, MF. and others. (1999) Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. Cell 2; 97(1) : 133-44.

Michels J, Johnson PW, and Packham G. (2005) Mcl-1. Int J Biochem Cell Biol 37: 267–271.

Miles DW, Happerfield LC, and Naylor MS. (1994) Expression of tumour necrosis factor (TNF-alpha) and its receptors in benign and malignant breast tissue. Int. J. Cancer. 56: 777-782.

Misseri R, Meldrum DR, Dinarello CA, Dagher P, Hile KL, and Rink RC. (2005) TNF-alpha mediates obstruction-induced renal tubular cell apoptosis and proapoptotic signaling. Am. J. Physiol. Renal. Physiol. 288(2) : F406-F411.

Mizokami A, Gotoh A, Yamada H, Keller ET, and Matsumoto T. (2000) Tumor necrosis factor-alpha represses androgen sensitivity in the LNCaP prostate cancer cell line. J. Urol. 164; 800-805.

Morimoto H, Safrit JT, and Bonavida B. (1991) Synergistic effect of tumor necrosis factor-alpha and diphtheria toxin-mediated cytotoxicity in sensitive and resistant human ovarian tumor cell lines J. Immunol. 147: 2609-2616.

Nakashima J, Tachibana M, and Ueno M. (1998) Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. Clin. Cancer Res. 4: 1743-1748.

Natoli, G. CA. and Ianni A. (1997) Activation of SAPK/JNK by TNF receptor 1 through a noncytotoxic TRAF2-dependent pathway. Cellular signalling. 275: 200-203.

Naylor MS, Stamp GWH, Foulkes WD, Eccles D, and Balkwill FR. (1993) Tumor necrosis factor and its receptors in human ovarian cancer. J. Clin. Invest. 91: 2194–2206.

Parkin DM, Whelan SL, Ferlay J, Teppo L, and Thomas DB. (2002) Cancer incidence in five continents, vol. VIII. Lyon: IARC Press [IARC Scientific Publications No. 155].

Pisetsky DS. (2000) Tumor necrosis factor blockers in rheumatoid arthritis. N. Engl. J. Med. 342:810-811.

Raff M. (1998) Cell suicide for beginners. Nature 396: 119-22.

Rath PC AB. (1999) TNF-induced signaling in apoptosis. J Clin Immunol Cell Biol. 19: 350-364.

Sarin A, Conan-Cibotti M, and Henkart PA. (1995) Cytotoxic effect of TNF and lymphotoxin on T lymphoblasts. J. Immunol. 155 : 3716-3718.

Shaib YH, Davila JA, McGlynn K, and El Serag HB. (2004) Rising incidence of intrahepatic cholangiocarcinoma in the United States: a true increase? J. Hepatol. 40 : 472–477.

Simpson, L., and Parsons, R. (2001) PTEN: life as a tumor suppressor. Exp. Cell Res. 264: 29–41

Takada, H. CN. And Mirtsos C (2003) Role of SODD in regulation of tumor necrosis factor responses. Mol Cell Biol. 23: 4026-4033.

Taniai M, Grambihler A, and Higuchi H. (2004) Mcl-1 mediates tumor necrosis factor-related apoptosis-inducing ligand resistance in human cholangiocarcinoma cells. Cancer Res 64: 3517–3524.

Terada, T. and Nakanuma Y. (1996) Expression of apoptosis, proliferating cell nuclear antigen, and apoptosis-related antigens (bcl-2, c-myc, Fas, Lewis(y) and p53) in human cholangiocarcinomas and hepatocellular carcinomas. Pathol Int. 46: 764-770.

Thakar J, Schleinkofer K, Borner C, and Dandekar T. (2006) RIP death domain structural interactions implicated in TNF-mediated proliferation and survival. Proteins. 63(3): 413-23.

Tracey KJ, Fong Y, and Heses DG. (1987) Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 330: 662-664.

Tracey KJaC, A. (1993) Tumor necrosis factor, other cytokines and disease. Annu. Rev. Cell. Biol. 9: 317-343.

Utaisincharoen P., Ubol S., Tangthawornchaikul N., Chaisuriya P., and Sirisingha S. (1999) Binding of tumor necrosis factor-alpha (TNF-alpha) to TNF-RI induces caspases(s)-dependent apoptosis in human cholangiocarcinoma cell lines. Clin Exp Immunol, 116: 41-47.

Vandenabeele, P. DW. and Beyaert R. (1995) Two tumour necrosis factor receptors: structure and function. Trends cell biol. 5: 392-399.

VandenBerghe T, Denecker G, Brouckaert G, VadimovischKrysko D, D'Herde K, and Vandenabeele P. (2004) More than one way to die: methods to determine TNF-induced apoptosis and necrosis. Meth Mole Med. 98: 101-26.

Wang CY, Mayo MW, and Balwin AS Jr. (1996) TNF and cancer therapy-induced apoptosis potentiation by inhibition of NF-κB. Science 274: 784-787.

Watanabe, N. and others. (1988) Synergistic cytotoxic and antitumor effects of recombinant human tumor necrosis factor and hyperthermia. Cancer Res 48: 650-653.

Wingfield P., Pain RH, and Craig S. (1987) Tumor necrosis factor is a compact trimer. FEBS Lett. 211: 179-184.

Wong, GHW. and Goeddel DV. (1994) Fas antigen and p55 TNF receptor signal apoptosis through distinct pathways. J Immunol. 152: 1751-1755.

APPENDIX

CHEMICAL SUBSTANCES AND EQUIPMENT

All chemicals used in this study were analytical grade. The chemical are listed alphabetically below.

Name of Chemical	Company
Bovine serum albumin	Sigma
Bradford	Biorad
Bromphenol blue	Sigma
DNA marker	New England Biolab
Dithiothreitol (DTT)	Sigma
dNTP mix	Qiagen
EDTA	USB
Ethanol	Merck
Fetal Bovine Serum	Gibco
HAM-12	Gibco
HEPES	Merck
MTT	USB
Taq DNA polymerase	Qaigen
TEMED	USB
TNF-alpha	Pacific Science
TNFRI and RII primers	BSU

Name of Chemical	**Company**
Tris base	USB
Triton X100	USB
Trypsin-EDTA	Gibco
6 well and 96 well tissue culture plates	Corning
24 well Transwell chamber	Corning
25 cm2 and 75 cm2 tissue culture flasks	Corning
60 mm and 100 mm tissue culture dishes	Corning
Autopipette	Gilson
Agarose gel electrophoresis apparatus	Amersham Biosciences
Centrifuge	Hettich
CO_2 Incubator	Heraeus
ELECHYS 2012	Roche
Fluorescent microscope	Olympus, Nikon and Axioimage
Laminar flow	TIF-Filtrations
Light microscope	Meiji
UV gel documentation	Biorad

REAGENTS

HAM-F12 incomplete medium (1 Liter pH 7.4)

HAM-F12	1	pack
HEPES	3.57	g
Sodium bicarbonate	1.17	g
DW make to	1	L

Phosphate Buffer Saline (PBS) (1 Liter, pH 7.4)

NaCl	8	g
$Na_2HPO_4.2H_2O$	1.15	g
KCl	0.2	g
KH_2PO_4	0.2	g
DW make to	1	L

Lysis buffer

RIPA buffer *	755	µl
10X Protease Inhibitor (Roche)	150	µl
1M NaF (Sigma)	50	µl
1M β-glycerophosphate (sigma)	40	µl
0.5 M Na_3VO_4 (Sigma)	4	µl

1M DTT (Amersham)	1	µl
Total Volume	500	µl

* RIPA buffer containing with 150mM Tris-HCl pH 7.4, 150mM NaCl, 2mM EDTA, 0.1% SDS (sodium dodecylsulfate), 1% Sodium Deoxycholate, 1% Nonidet P-40, 2 mM EDTA

Separating solution (12% gel)

29.2:0.8 % Acrylamide : Bisacrylamide solution	4	ml
1.5 M Tris-HCl, pH 8.8	2.5	ml
10% SDS (Sodium Dodecylsulfate)	100	µl
DW	3.28	ml
10% Ammonium persulfate	50	µl
TEMED (Tetramethylethylenediamine)	20	µl
Total volume	10	ml

Stacking solution (4 % gel)

29.2:0.8 % Acrylamide : Bisacrylamide solution	0.8	ml
1 M Tris-HCl, pH 6.8	0.75	ml
10% SDS (sodium dodecylsulfate)	60	µl
DW	4.324	ml
10% Ammonium persulfate	60	µl

TEMED	6	µl
Total volume	4	ml

TBS/T buffer (1 L)

1 M Tris, pH 8	10	ml
5 M NaCl	30	ml
Tween-20	1	ml
DW	959	ml

5X SDS sample buffer

1 M Tris-HCl, pH 6.8	3.125	ml
Glycerol	5	ml
SDS	0.5	g
Bromophenal blue	1.25	ml
DW	0.276	ml
Total volume	10	ml

Separating solution (7.5% gel)

30 : 1 % polyacrylamide:bis-acrylamide	2.5	ml
1.5 M Tris-HCl, pH 8.8	2.5	ml
10% SDS	100	µl

1% gelatin	1	ml
DW	3.8	ml
10% AP	50	µl
TEMED	50	µl
Total Volume	10	ml

Stacking solution (4 % gel)

29.2:0.8 % Acrylamide : Bisacrylamide solution	0.8	ml
1 M Tris-HCl, pH 6.8	0.75	ml
10% SDS (sodium dodecylsulfate)	60	µl
DW	4.324	ml
10% Ammonium persulfate	60	µl
TEMED	6	µl
Total volume	4	ml

RNA primer mixtures (1 Reaction)

1 µg total RNA	n	µl
10 mM dNTP	1	µl
0.5 µg/µl Oligo (dT)12-18 nt	1	µl
DEPC-treated water up to	10	µl

Reaction mixtures (1 Reaction)

10X RT buffer	2	µl
25 mM $MgCl_2$	4	µl
0.1 M DTT	2	µl
RNase Out	1	µl
Total Volume	9	µl

STATISTICAL ANALYSIS

Table 3 Multiple Comparisons of MTT assay of KKU-100 cell line

	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-1.3333	4.86864	.960	-16.2717	13.6050
		3.00	60.6667(*)	4.86864	.000	45.7283	75.6050
	2.00	1.00	1.3333	4.86864	.960	-13.6050	16.2717
		3.00	62.0000(*)	4.86864	.000	47.0617	76.9383
	3.00	1.00	-60.6667(*)	4.86864	.000	-75.6050	-45.7283
		2.00	-62.0000(*)	4.86864	.000	-76.9383	-47.0617
Scheffe	1.00	2.00	-1.3333	4.86864	.963	-16.9483	14.2817
		3.00	60.6667(*)	4.86864	.000	45.0517	76.2817
	2.00	1.00	1.3333	4.86864	.963	-14.2817	16.9483
		3.00	62.0000(*)	4.86864	.000	46.3850	77.6150
	3.00	1.00	-60.6667(*)	4.86864	.000	-76.2817	-45.0517
		2.00	-62.0000(*)	4.86864	.000	-77.6150	-46.3850
Dunnett t (2-sided)(a)	1.00	3.00	60.6667(*)	4.86864	.000	46.7290	74.6044
	2.00	3.00	62.0000(*)	4.86864	.000	48.0623	75.9377

* The mean difference is significant at the .05 level.

a Dunnett t-tests treat one group as a control, and compare all other groups against it.

Group 1 = control, Group 2 = TNF-alpha, Group 3 = Wortmannin

Dependent Variable: KKU100

Table 4 Multiple Comparisons of MTT assay of KKU-213 cell line

	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-21.3333(*)	4.07340	.005	-33.8316	-8.8350
		3.00	34.3333(*)	4.07340	.000	21.8350	46.8316
	2.00	1.00	21.3333(*)	4.07340	.005	8.8350	33.8316
		3.00	55.6667(*)	4.07340	.000	43.1684	68.1650
	3.00	1.00	-34.3333(*)	4.07340	.000	-46.8316	-21.8350
		2.00	-55.6667(*)	4.07340	.000	-68.1650	-43.1684
Scheffe	1.00	2.00	-21.3333(*)	4.07340	.006	-34.3978	-8.2689
		3.00	34.3333(*)	4.07340	.000	21.2689	47.3978
	2.00	1.00	21.3333(*)	4.07340	.006	8.2689	34.3978
		3.00	55.6667(*)	4.07340	.000	42.6022	68.7311
	3.00	1.00	-34.3333(*)	4.07340	.000	-47.3978	-21.2689
		2.00	-55.6667(*)	4.07340	.000	-68.7311	-42.6022
Dunnett t (2-sided)(a)	1.00	3.00	34.3333(*)	4.07340	.000	22.6722	45.9945
	2.00	3.00	55.6667(*)	4.07340	.000	44.0055	67.3278

* The mean difference is significant at the .05 level.

a Dunnett t-tests treat one group as a control, and compare all other groups against it.

Group 1 = control, Group 2 = TNF-alpha, Group 3 = Wortmannin

Dependent Variable: KKU213

BIOGRAPHY

PROJECT LEADER

NAME	Dr. Panthip Rattanasinganchan
EDUCATION	Bachelor of Science (Medical Technology)
	Mahidol University
	Master of Science (Medical Technology)
	Mahidol University
	Doctor of Philosophy (Biochemistry)
	Mahidol University
WORKPLACE	Department of Clinical chemistry, Faculty of Medical
	Technology, Huachiew Chalermprakiet University
	Tel. 0-2312-6300 # 1500

CO-WORKER

NAME	Associate Professor Dr. Rutaiwan Tohtong
EDUCATION	Ph.D. (Molecular Genetics) The Ohio State University, Ohio,
	USA
WORKPLACE	Department of Biochemistry, Faculty of Science, Mahidol
	University
	Tel. 0-2201-5596