UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION AND CHARACTERIZATION OF TOLL-LIKE RECEPTOR (TLR) 2 AND 4 MUTATIONS IN COLORECTAL

HOMA DAVOODI

FPSK(p) 2009 9

IDENTIFICATION AND CHARACTERIZATION OF TOLL-LIKE RECEPTOR (TLR) 2 AND 4 MUTATIONS IN COLORECTAL

By
HOMA DAVOODI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2009

This thesis is dedicated to

My husband and my son , my late father, my beloved mother, sister, brothers and my parents in law with love and gratitude and also to the kids who have ability but don't have facilities to obtain education.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

IDENTIFICATION AND CHARACTERIZATION OF TOLL-LIKE RECEPTOR (TLR) 2 AND 4 MUTATIONS IN COLORECTAL

By

HOMA DAVOODI

December 2009

Chairperson: Seow Heng Fong, PhD

Faculty: Medicine and Health Sciences

Toll-like receptors (*TLRs*) are the most important receptors in innate immunity that have been identified as a major class of pattern-recognition receptors. The *TLR* family comprises at least eleven members, these *TLRs* recognize a limited but highly conserved set of molecular structures, so called pathogen-associated molecular patterns (PAMPs). For example, *TLR4* recognizes LPS, which is unique to gram-negative bacteria. Increasing evidence suggest that the ability of certain individuals to respond properly to *TLR* ligands may be impaired by single nucleotide polymorphisms (SNPs) within *TLR* genes. The role of *TLRs* signaling and effect of SNPs mutations on cancer outcome and survival is not exactly determined yet. The objectives of this study were firstly to detect the two most common *TLR2* (Arg677Trp, Arg753Gln) and *TLR4* SNPs (Asp299Gly, Thr399Ile) in colorectal cancer (CRC), secondly to evaluate the *TLR2* and 4 expressions in colorectal cancer cell lines and the effect of polymorphisms on the *TLR4* expression. The cytokine profiles secreted by colorectal cancer cells with mutant and non- mutant *TLR4* and the expression of some signal transduction molecules involved in *TLR4* signalling were also determined.

Lastly, the impact of these SNPs on the cytotoxicity and apoptosis induction of the 5-Fluorouracil (5-FU) was also evaluated. PCR-RFLP was carried out on fifty normal blood samples and sixty human colorectal cancer

paraffin-embedded blocks to determine the incidence of *TLR2* and *TLR4* mutations. The results showed two individuals were heterozygous for the Asp299Gly (D299G) and Thr399Ile (T399I) polymorphisms in the *TLR4* gene. However, all samples in control group were the wild-type form. Since we could not find any *TLR2* mutations in our samples, our study focused on the *TLR4* gene. *In vitro* studies were performed on HCT116 cell line transfected with mutant and wild-type *TLR4* genotype. A series of experiments were conducted to examine the effect of *TLR4* variations on the expression of *TLR4,* LPS responsiveness and the response of the cells to the 5-FU as a chemotherapeutic agent. FACS analysis of *TLR4* expression on transfected HCT116 cells showed that the expression of wild-type was higher than mutant *TLR4*. LPS induced *TLR4* expression on transfected cells and the response of wild-type genotype to the LPS was more significant compared to the mutants. Western blot analysis and Dual Luciferase assay showed that the activity of pNF-kB was higher in cells transfected with plasmid for *TLR4* D299G compared to the other cells. However, the activity of pAKT, pERK1 and pIRAK was higher in wild-type.

The results of cytokine measurements showed that IL-8 levels were increased in wild-type and basal VEGF was high in un-transfected cells. Secreted VEGF levels was decreased by LPS in wild-type cells but increased in un-transfected cells. IL-17 was secreted by transfected cells at a low level and was not significantly affected by LPS. The results of MTT assay showed that the cytotoxicity effect of 5-FU on transfected cells expressing D299G *TLR4* mutant was lower compared to the other cells. 5-FU

increased *TLR4* expression on transfected cells and LPS has a synergistic effect with 5-FU. LPS increased the apoptosis induced by 5-FU and suggesting that it may be useful as an adjuvant in chemotherapy. HMGB1, an endogenous ligand for *TLR4*, was secreted by 5-FU- treated cells and also detected in cell lysate. *TLR4* is functionally active on transfected HCT116 cell line. The increased activity of pNF-kB in cells transfected with plasmid expressing TLR4 D299G may lead to decreased-cytotoxicity effect of 5- FU in this variant. Therefore, it is possible that the CRC patients who harbor this polymorphism tend to be more resistant to drug compared to the wild-type. High level of pNF-kB can also explain the association of this polymorphism with Inflammatory Bowel Disease (IBD), susceptibility to infectious disease and even cancer.

PENGECAMAN DAN PENCIRIAN MUTASI PADA "TOLL-LIKE RECEPTOR" 2 DAN 4 (*TLR*) DALAM KANSER KOLOREKTAL

Oleh

HOMA DAVOODI

Disember 2009

Pengerusi : Seow Heng Fong, PhD

Fakulti : Perubatan Dan Sains Kesihatan

Toll like receptors (*TLRs*) merupakan reseptor penting dalam imuniti semulajadi, yang telah dikenalpasti sebagai komponen utama 'pattern recognition receptor'. *TLR* merangkumi sekurang-kurangnya sebelas komponen dan *TLR* boleh mengenalpasti struktur molekul secara terperinci yang dikenali sebagai pathogen-associated molecular patterns (PAMPS). Sebagai contoh, *TLR4* boleh mengenalpasti LPS, yang merupakan komponen bakteria gram negatif. Kajian telah menunjukkan bahawa kebolehan seseorang individu untuk bertindak balas terhadap ligan *TLR* mungkin terganggu oleh polimorfisme mononukleotida(SNPs) pada gen *TLR*. Peranan utama isyarat *TLRs* dan kesan mutasi SNPs terhadap kanser belum dikenalpasti lagi. Objektif utama kajian adalah untuk mengenalpasti dua *TLR* yang utama iaitu *TLR2*(Arg 677 Trp, Arg 753 Gln) dan *TLR4* SNPs (Asp 299 Gly, Thr 399 Ile) dalam kolorektal kanser (CRC). Kedua, menilai ekspresi *TLR2* dan *TLR4* dalam titisan sel kanser kolorektal dan juga kesan polimorfisme terhadap ekspresi *TLR4*. Mengenalpasti profil sitokin yang dirembes oleh sel-sel kanser kolorektal antara *TLR4* mutan dan bukan mutan.

Mengenalpasti kesan polimorfisme terhadap ekspresi sesetengah molekul yang terlibat dalam isyarat *TLR*4. Seterusnya menilai impak SNPs terhadap kesitotoksikan '5 Flourouracil' (5-FU) dan menilai impak SNPs terhadap apoptosis yang berpunca dari 5- FU. PCR–RFLP dilakukan terhadap lima puluh sampel darah normal dan enam puluh blok paraffin untuk mengenalpasti mutasi *TLR2* dan *TLR4*. Keputusan kajian ini menunjukkan bahawa dua individu adalah heterozigot untuk Asp299Gly dan Thr399Ile polimorfisme dalam gen *TLR4*. Walau bagaimanapun, semua sampel yang digunakan sebagai kawalan adalah jenis liar. Disebabkan tiada mutasi *TLR2* dalam sampel, kajian kami difokuskan pada gen *TLR4*. Kajian *in-vitro* telah dilakukan terhadap titisan sel HCT116 tertransfeksi dengan mutan dan juga genotip *TLR4* jenis liar. Beberapa eksperimen telah dilakukan untuk mengkaji kesan variasi *TLR*4 terhadap ekspresi *TLR*4, tindakbalas LPS dan juga tindakbalas sel terhadap 5-Fu sebagai agen kemoterapi. Analisis 'FACS' terhadap ekspresi *TLR* 4 bagi titisan sel HCT116 tertransfeksi dan juga tindakbalas genotip jenis liar terhadap LPS adalah lebih signifikan berbanding dengan mutan. Analisis 'Western Blot' dan juga 'Dual Luciferase' telah menunjukkan bahawa aktiviti pNF-KB adalah lebih tinggi pada D299G berbanding dengan sel-sel lain. Walau bagaimanapun aktiviti pAKT, pERK1, pIRAK adalah lebih tinggi dalam jenis liar. Keputusan penyukatan sitokin telah menunjukkan bahawa IL-8 adalah tinggi dalam jenis liar dan VEGF adalah tinggi dalam sel yang tidak ditransfeksikan. VEGF telah mengalami pengurangan dengan kehadiran LPS dalam sel jenis liar tetapi tinggi dalam sel yang tidak ditransfeksikan. IL-17 telah dirembes oleh sel yang telah ditransfeksikan pada kadar yang rendah dan tidak memberi kesan yang signifikan terhadap LPS. Keputusan cerakin MTT telah menunjukkan bahawa kesan kesitotoksikan 5- FU pada

mutan D299G *TLR*4 adalah rendah berbanding sel-sel lain. 5-FU telah meningkatkan ekspresi *TLR*4 terhadap sel yang ditransfeksikan dan LPS mempunyai kesan sinergistik. LPS meningkatkan kadar apoptosis yang diaruhkan oleh 5-FU dan boleh digunakan sebagai adjuvan dalam kemoterapi 5-FU. HMGB1 yang merupakan ligan endogenus untuk *TLR4* telah dirembes oleh sel yang dirawat dengan 5-FU dan dikenalpasti dalam media dan lisat sel . *TLR4* adalah aktif terhadap titisan sel HCT116 tertransfeksi Aktiviti yang tinggi oleh p-NFkB dalam D299G boleh mengurangkan kesan kesitotoksikan 5-FU dalam varian ini. Oleh itu, ada kemungkinan bahawa peasakit CRC yang mempunyai polimorfisme ini , adalah lebih rintang terhadap dadah berbanding jenis liar. Kadar pNFkB juga boleh menjelaskan hubungan antara polimorfisme dan 'Inflammatory Bowel Disease'(IBD), kecenderungan mendapat penyakit berjangkit dan juga kanser.

ACKNOWLEDGEMENTS

Firstly, I thank God for providing a way for me to complete this goal. I would like to thank three important groups of people, my supervisory committee, my lab-mates, and my family. First of all, I wish to express my heartfelt appreciation to my supervisor, Prof. Dr.Seow Heng Fong for her invaluable support, guidance and advice throughout this research project. She has also been meticulous in reading and proofreading my document, and for this I am most grateful. I also thank my co-supervisors, Dr. Maha bt. Abdullah and Dr.Sharmili Vidyadaran, as they helped me whenever I needed them. I thank to my dear lab-mates in Molecular Immunology Lab, they were all very friendly and helpful; Leslie Than Thian Lung, Leong Pooi Pooi, Yip Wai Ken, Lim Chooi Ling, Choo Chee Wei, Jee Jap Meng, Ooi Suek Chin, Koh Rhun Yin, Tang Wai Yee, Malini Fonsseka. I also thank all staff members in Molecular Immunology Lab. I am deeply indebted to my husband, Reza Hashemi, who has been a pillar of strength throughout this research project. His advice on matters concerning my work and his support has been instrumental in ensuring the successful completion of this work. Special thanks to my son because of his patience during thesis writing. I particularly want to thank my parents; to the memory of my father, my heartfelt gratitude to my mother for her love, encouragement and patience throughout all these years. Many thanks go to my sister and brothers specially Mr. Davood Davoodi, my parents-in-law, nephews and nieces (particularly Mahta) for their love and support. Last but not least, I am grateful to the Universiti Putra Malaysia for providing me the facilities and financial support during this course.

I certify that a Thesis Examination Committee has met on 7 December 2009 to conduct the final examination of Homa Davoodi on her thesis entitled "Identification and characterization of Toll-like receptor (TLR) 2 and 4 mutations in colorectal cancer" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy (PhD).

Members of the Thesis Examination Committee were as follows:

Sabariah Abdul Rahman, PhD
Associate professor
Institute of Bioscience
University Putra Malaysia
(Chairman)

Chong Pei Pei, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Daud Ahmad Israf Ali, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Kennet Scott, PhD
Senior lecturer
Faculty of Science
University of Auckland
New Zealand
(External Examiner)

BUJANG BIN KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Seow Heng Fong, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairperson)

Maha Bt Abdullah , PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Sharmili Vidyadaran, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate studies
Universiti Putra Malaysia

Date: 11 February 2010

DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and it is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

HOMA DAVOODI
Date: 11 February 2010

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
Abs	Antibodies
Ag	Antigens
Arg	Arginine
ATCC	Ameriacan Type Culture Collection
Caco-2	Human colon carcinoma epithelial cell line
CBA	Cytometric bead array
CD	Crohn's Disease
CD8	Cluster of differentiation 8
CRC	Colorectal cancer
D	Aspartic acid
E. coli	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular Signal-Regulated Kinase
5-FU	5-Fluorouracil
G	Glycine
Gly	Glycine
Gln	Glutamine
HMGB1	High-mobility group box 1
HSP	Heat shock protein
IBD	Inflammatory Bowel Disease
IC_{50}	Median Inhibition Concentration (the concentration of substance that provides 50% inhibition to certain reaction)
I	Isoleucine
IEC	intestinal epithelial cells
IL	Interleukins
IRAK1	IL-1 receptor associated kinase
LPS	Lipopolysaccharide
MAP kinase	Mitogen-activated protein kinase

MHC	Major histocompatibility complex
MyD88	Myeloid differentiation protein 88
NF-κB	Nuclear factor-kappa B
PAMP	Pathogen-associated molecular pattern
PARP	Poly ADP ribose polymerase
PI3K	phosphatidylinositol 3-kinase
pH	Hydrogen ion concentration
RFLP	Restriction fragment length polymorphism
PRRs	pattern recognition receptors
RT-PCR	Reverse transcription polymerase chain reaction
SNP	Single-nucleotide polymorphism
T	Threonine
TGF-β	The transforming growth factor beta
Thr	Threonine
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
Trp	Tryptophan
UC	Ulcerative Colitis
UV	Ultra violet ray
VEGF	Vascular endothelial growth factor
WB	Western blot
WHO	World Health Organisation
WT	Wild type

Units

°C	Degrees centigrade
g	Unit for measuring centrifugation force
g	Gram
µl	Microlitre
h	Hour
l	Liter
min	Minute
mL	Milliliter
ng	Nanogram
nm	Nanometer
w/v	Weight by volume

Common abbreviations

e. g.	for example
et al.	and others
i.e.	for example

Statistical terms

P	Probability
SD	Standard deviation
SE	Standard error

Chemical elements and compounds

CO_2	Carbon dioxide
DW	Distilled water
EtOH	Ethanol
HCl	Hydrochloric acid
HOAc	Acetic acid
K	Potassium
KH_2PO_4	Potassium di-hydrogen phosphate
K_2So_4	Potassium sulfate
PBS	Phosphate buffered saline
MeOH	Methanol
NaCl	Sodium chloride
NaOH	Sodium hydroxide

CHAPTER I

INTRODUCTION

Toll-like receptors (*TLRs*) are the most important receptors in innate immunity that have been identified as a major class of pattern-recognition receptors. The *TLR* family comprises at least eleven members, these *TLRs* recognize a limited but highly conserved set of molecular structures, so called pathogen-associated molecular patterns (PAMPs). For example, *TLR4* recognizes LPS, which is unique to gram-negative bacteria, and *TLR2* recognizes peptidoglycan found in gram-positive bacteria.

Recognition of pathogen-associated molecular patterns (PAMPs) by *TLRs* activates numerous signal-transduction pathways that target several transcription factors, a major signalling target of the *TLRs* is activation of the transcription factor NF-kB, a key regulator of immune and inflammatory responses, which control the expression of genes encoding cytokines, chemokines and enzymes that regulate innate and adaptive immune responses (Kaleigh, 2002). Recently researchers have shown that chronic or recurrent inflammation has been implicated in the initiation and development of several human cancers including those of the stomach, liver, colon, and urinary bladder. The fact that colorectal cancer is more often in patients suffering from inflammatory bowel disease, such as ulcerative colitis (UC) and Crohn's disease (CD), raises the question of the relationship between chronic inflammation and malignant transformation (Boraska *et al.*, 2006).

It has been reported that *TLRs* are not only expressed in immune cells, but also on endothelial and epithelial cells. In particular, *TLR4* was found in epithelial cell layers that are in contact with the outer environment, such as intestine, lung, gingival, corneal and renal epithelial cells. Bacterial infection could affect the function of these cells through *TLR4* which leads to local infections or inflammatory processes. It was reported that proinflammatory cytokines induced by inflammatory stimuli can counteract immune surveillance and facilitate tumor outgrowth (He *et al.*, 2007). Toll-like receptors are increasingly implicated in disease pathogenesis. Population studies of polymorphisms in genes encoding *TLRs* or their downstream signalling molecules have directly linked these pathways to multiple human disease processes.

The great majority of polymorphisms studied are single nucleotide polymorphisms (SNPs) that occur with a frequency of >1% in the normal population (in contrast to 'mutations' that occur with a frequency of <1%) (El-Omar et *al*., 2008). Hundreds of SNPs have been identified in *TLRs* but the functional consequences of these have not been revealed completely. Many associations have been reported between *TLR* polymorphisms and infectious disease or cancers and it is the interaction between infection and chronic inflammation that most likely mediates the increased risk of cancer development. SNPs in *TLR2* have been reported to alter the susceptibility to various inflammatory and infectious diseases. The two most common *TLR2* SNPs are Arg677Trp and Arg753Gln. The Arg677Trp mutation has been suggested to cause impaired function of the intracellular domain of the *TLR2* protein. This

polymorphism has been reported to be associated with lepromatous leprosy in a Korean population (Kang and Chae, 2001) and recurrent bacterial infections in Turkish children (Kutukculer *et al.,* 2007). The other well-described *TLR2* SNP is also located in the coding region, which is at amino acid position 753 substituting an arginine residue to a glutamine residue. This polymorphism has been reported to increase the risk of gram-negative sepsis in a Caucasian population, In addition to these two SNPs, Boraska *et al*. (2006) reported that the allele frequency of a *TLR2* guanine thymine (GT) microsatellite repeat polymorphism in the second intron has an impact on the susceptibility to sporadic colorectal cancer. Individuals who developed sporadic colorectal cancer tended to have decreased frequency of alleles 20 and 21 GT repeats and increased frequency of alleles with 31 GT repeats (Boraska *et al*., 2006). The *TLR-4* gene consists of three exons mapped to chromosome 9q32–33 two nonsynonymous single nucleotide polymorphisms (SNPs), A896G (rs4986790) and C1196T (rs4986791) in exon 3 lead to Asp299Gly and Thr399Ile amino acid substitutions, respectively. SNP Asp299Gly was later shown to co-segregate with SNP Thr399Ile— both in the third exon of *TLR4*. These SNPs are present in 10% of Caucasian populations and are reported to have a positive correlation with susceptibility to several infectious diseases (including gram-negative sepsis), atherosclerosis, asthma, malaria and also *H. pylori*-induced gastric cancer (El-Omar *et al*., 2008). The SNPs disrupt the normal structure of the extracellular region of the *TLR4* and are therefore hypothesized to decrease responsiveness to lipopolysaccharide (LPS) through alterations in binding. There are some endogenous proteins which bind and stimulate *TLR4,* including HSP60, HSP70, oxidized low-density lipoprotein, surfactant protein A,

hyaluronan breakdown products, fibronectin, b-defensin, and the alarmin high-mobility group box 1 protein (HMGB1). The term 'alarmin,' denotes an array of structurally diverse host proteins rapidly released during infection or tissue damage that have mobilizing and activating effects for host defence and tissue repair. HMGB1 is a highly mobile nuclear protein (non-histone chromatin binding protein) that influences transcription and other nuclear transactions.

Apetoh *et al*. (2007) reported that dying tumor cells promoted by cancer therapies trigger an immune response in a *TLR4*-dependent way and that *TLR4* triggering requires HMGB1 released by dying tumor cells. In this study they also showed that in the breast cancer patients the metastasis-free survival was significantly longer in the cohort carrying the normal allele of *TLR4* compare to the patients who carrying the Asp299Gly polymorphism (50% of relapse in mutated versus 37.4% in nonmutated patients at 10 years). Similar results were obtained by analyzing the second missense mutation (Thr399Ile) that co-segregates with the Asp299Gly substitution. Importantly, mutant *TLR4* also reduced the interaction of endogenous *TLR4* with HMGB1, suggesting that it acts as a dominant-negative form of *TLR4* with respect to the binding capacity to HMGB1 (Apeteh *et al*., 2007). Experimental evidence suggests that the neoplastic process may interfere with *TLR* signalling pathways to advance cancer progression. Current *in vivo* genetic studies highlight a more fundamental and direct role for epithelial cell *TLRs* and NF-kB in tumour development. Multiple chronic inflammatory diseases have been associated with cancer development including inflammatory bowel disease, hepatitis and chronic Helicobacter pylori

infection predisposing to colorectal, hepatocellular and gastric cancer respectively. *TLR4* is induced by proinflammatory cytokines and is highly expressed in IECs, resident macrophages, and dendritic cells in inflamed mucosa of IBD patients. Dendritic cell maturation and the development of adaptive immunity seem to require correct *TLR4* signalling.

Disruption of *TLR4* signalling could create an inappropriate innate and adaptive immune response necessary to eradicate pathogens which would result in a more severe inflammation. Studying SNPs in molecules involved in bacterial recognition will be essential to understand individual responses to bacterial components and define genetic backgrounds at risk of IBD (Franchimont *et al*., 2004). Now *TLRs* are hot targets for drug development. The industry has spent hundreds of millions of dollars developing new substances that they hope will kill cancer by *TLR* activation **(**Schmidt, 2006). The most important *TLRs* against bacterial cell walls components are *TLR2* & 4 and mutation in these genes decrease the response to bacterial components, impact gut homeostasis and results in impairment of *TLRs* activation which may affect on cancer development and progression.

Hypotheses

- TLR4 mutant alleles are more frequent in colorectal cancer (CRC) patients compared to the control group.
- The expression of mutant TLR4 on colorectal cancer cells is lower compared to the wild-type.
- Mutant TLR4 allele affect on cytokine profiles and some molecules involved in TLR4 signaling to induce inflammatory response.
- CRC patients with mutant TLR4 genotype are more resistant to 5-Fu chemotherapy compared to the wild-type.

Objectives

The objectives of this study were to:

1. Determine the *TLR2* and *TLR4* polymorphisms in colorectal cancer.
2. Evaluate the *TLR2* and 4 expressions in colorectal cancer cell lines and the effect of polymorphisms on the *TLR4* expression.
3. Determine the cytokine profiles secreted by colorectal cancer cells with mutant and non-mutant *TLR4*.
4. Determine the effect of polymorphisms on the expression of some molecules which are involved in *TLR4* signalling.
5. Determine the impact of these SNPs on the cytotoxicity of the 5-Fluorouracil.
6. Determine the impact of these SNPs on the apoptosis induced by 5-Fluorouracil.

REFERENCES

Achyuta, B. R., U. C. Ghoshalb, N. Moorchung, and B. Mittal. 2007. Association of Toll-like receptor-4 (Asp299Gly and Thr399Ileu) gene polymorphisms with gastritis and precancerous lesions.Hum Immunol.68: 901-907.

Agnese, D. M., J. E. Calvano, S. J. Hahm, S. M. Coyle, S. A. Corbett, S. E. Calvano, and S. F. Lowry. 2002. Human Toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of Gram-negative infections. J Infect Dis. 186: 1522-1525.

Ahmad-Nejad, P., H. Hacker, M. Rutz, S. Bauer, R. M. Vabulas, and H. Wagner. 2002. Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. Eur J Immunol. 32: 1958-1968.

Akira, S. 2003. Toll-like receptor signaling. J Biol Chem. 278: 38105-38108.

Akira, S., and K. Takeda. 2004. Toll-like receptor signalling. Nat. Immunol. 4: 499-511.

Akira, S., and H. Hemmi. 2003. Recognition of pathogen-associated molecular patterns by *TLR* family. Immunol Lett. 85:85-95.

Akira, S., T. Taga, and T., Kishimoto. 1993. Interleukin-6 in biology and medicine. Adv Immunol. 54: 1-78.

Alpay, H. C., E. O. Etem, I. Kaygusuz, H. Yüce, T. Karlidag, E. Keles, I. Orhan, and S. Yalcin. 2009. Evaluation of the polymorphism in the Toll-like receptor 4 (*TLR4*) genes of tympanosclerosis patients. Auris Nasus Larynx. (Article in Press).

An, H., Y., Yu, M., Zhang, H., Xu, R., Qi, X., Yan, S., Liu, W., Wang, Z., Guo, J., Guo, Z., Qin, X., Cao. 2002. Involvement of ERK, p38 and NF-kB signal transduction in regulation of *TLR2*, *TLR4* and *TLR9* gene expression induced by lipopolysaccharide in mouse dendritic cells.Immunology. 106; 1:38-45.

Andersson, U., H. Wang, K. Palmblad, A. C. Aveberger, O. Bloom, H. Erlandsson-Harris, A. Janson, R. Kokkola, M. Zhang, H. Yang, and K. J. Tracey. 2000. High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med. 192: 565-570.

Apetoh, L., A. Tesniere, F. Ghiringhelli, G. Kroemer, L. Zitvogel. 2008. Molecular interactions between dying tumor cells and the innate immune system determine the efficacy of conventional anticancer therapies. Cancer Res. 68:4026-30.

Apetoh, L., F. Ghiringhelli, A. Tesniere, A. Criollo, C. Ortiz, R. Lidereau, C. Mariette, N. Chaput, J. P. Mira, S. Delaloge, F. André, T. Tursz, G. Kroemer,

and L. Zitvogel. 2007. The interaction between HMGB1 and *TLR4* dictates the outcome of anticancer chemotherapy and radiotherapy. Oncogene. 2: 244-252.

Arbour, N. C., E. Lorenz, B. C. Schutte, J. Zabner, J. N. Kline, M. Jones, K. Frees, J. L. Watt1 and D. A. Schwartz. 2000. *TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet. 25: 187-191.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1999. Short Protocols in Molecular Biology. 4th ed. John Wiley & Sons, New York, NY.

Balkwill F., and L. M. Coussens. 2004. Cancer: an inflammatory link. Nature. 431: 405-6.

Bettelli, E., T. Korn, V. K. Kuchroo. 2007. Th17: the third member of the effector T cell trilogy. Curr Opin Immunol. 19: 652-657.

Bochud, P.Y., T. R. Hawn, and A. Aderem . 2003. Cutting edge: a toll-like receptor 2 polymorphism that is associatedwith lepromatous leprosy is unable to mediate mycobacterial signaling. J Immunol. 170: 3451-3454.

Bonaiuto, C., P. P. McDonald, F. Rossi, and M. A. Cassatella. 1997. Activation of nuclear factor-κB by β-amyloid peptides and interferon-γ in murine microglia. J. Neuroimmunol. 77: 51-56.

Boraska Jelavić T, Barisić M, Drmic Hofman I, Boraska V, Vrdoljak E, Peruzović M, Hozo I, Puljiz Z, Terzić J. 2006. Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. Clin Genet. 70:156-60.

Bowling, W.M., D. G. Hafenrichter, M. W. Flye, and M. P. Callery. 1995. Endotoxin tolerance alters phospholipase C-gamma 1 and phosphatidylinositol-3-kinase expression in peritoneal macrophages. J. Surg Res. 58: 592-598.

Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal Biochem. 72:248-54.

Brat, D. J., A. C. Bellail, E. G. Van Meir. 2005. The role of interleukin-8 and its receptors in gliomagenesis and tumoral angiogenesis. Neuro Oncol.7: 122-33.

Brat, D. J., and E. G. Van Meir. 2001. Glomeruloid microvascular proliferation orchestrated by VPF/VEGF: A new world of angiogenesis research. Am J Pathol. 158: 789-796.

Calabresi, P., and A. D. Welch. 1962. Chemotherapy of neoplastic diseases. Annu Rev Med. 3: 147-202.

Cario, E., and D. K. Podolsky. 2000. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (*TLR3*) and *TLR4* in inflammatory bowel disease. Infect Immun. 68: 7010-7017.

Cario, E., and D. K. Podolsky. 2005. Intestinal epithelial tollerance versus in tollerance of commensals. Mol Immunol. 42: 887-893.

Chen, K., J. Huang, W. Gong, P. Iribarren, N. M. Dunlop, J. M. Wang. 2007a. Toll-like receptors in inflammation, infection and cancer. Int Immunopharmacol. 7: 1271-1285.

Chen, R., A. B. Alvero, D. A. Silasi, and G. Mor. 2007b. Inflammation, cancer and chemoresistance: taking advantage of the Toll-like receptor signaling pathway. Am J Reprod Immunol. 57: 93-107.

Cheng, I., S. J. Plummer, G. Casey, and J. S. Witte. 2007. Toll-Like Receptor 4 Genetic Variation and Advanced Prostate Cancer Risk. Cancer Epidemiol Biomarkers Prev. 16:352-355.

Child, N. J., I. A. Yang, M. C. Pulletz, K. de Courcy-Golder, A. L. Andrews, V. J. Pappachan, and J. W. Holloway. 2003. Polymorphisms in Toll-like receptor 4 and the systemic inflammatory response syndrome. Biochem. Soc. Trans. 31: 652-653.

Coley, W. B. 19991. The treatment of malignant tumors by repeated inoculations of erysipelas.With a report of ten original cases. Clin Orthop Relat Res. 262: 3-11.

Cooke, G. S., S. Segal, and A. V. Hill. 2002. Toll-like receptor 4 polymorphisms and atherogenesis. N. Engl. J. Med. 347: 1978-1980; author reply 1978-1980.

Correia, J. D., and R. J. Ulevitch 2002. MD-2 and *TLR4* N-linked glycosylations are important for a functionallipopolysaccharide receptor. J Biol Chem. 277:1845-1854.

Coussens, L. M, and Z. Werb. 2002. Inflammation and cancer. Nature 420:860-867.

De Angelis, P. M., B. Fjell, K. L. Kravik, T. Haug, S. H. Tunheim, W. Reichelt, M. Beigi, O. P. Clausen, E. Galteland, and T. Stokke. 2004. Molecular characterizations of derivatives of HCT116 colorectal cancer cells that are resistant to the chemotherapeutic agent 5-fluorouracil. Int J Oncol. 24: 1279-1288.

De Waal, M., R., H. Yssel, M. G. Roncarolo, H. Spits, and J. E. de Vries. 1992. Interleukin-10. Curr Opin Immunol. 4:314-320.

El-Omar, E. M., M.T. Ng, and G. L. Hold. 2008. Polymorphisms in Toll-like receptor genes and risk of cancer. Ontogeny. 27: 244-252.

Ferwerda, B., M. B. McCall, K. Verheijen, B. J. Kullberg, A. J. van der Ven, J. W. Van der Meer, and M. G. Netea. 2008. Functional consequences of toll-like receptor 4 polymorphisms. Mol Med. 6: 346-352.

Fitzgerald, K. A., D. C. Rowe, B. J. Barnes, D. R. Caffrey, A. Visintin, E. Latz, B. Monks, P. M. Pitha, D. T. Golenbock. 2003. LPS-*TLR4* signaling

toIRF-3/7 and NF-kappaB involves the toll adapters TRAM and TRIF. J Exp Med. 198:1043-1055.

Franchimont, D., S. Vermeire, H. El-Housni, M. Pierik, K. Van Steen, T. Gustot, E. Quertinmont, M. Abramowicz, A. Van Gossum, J. Devie re, P. Rutgeerts. 2004. Deficient host-bacteria interactions in inflammatory bowel disease? The Toll-like receptor (*TLR*)-4 Asp299Gly polymorphism is associated with Crohn's disease and ulcerative colitis. Gut. 53: 987-992.

Frederick , M. A., R. Brent , R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, K. Struhl. 2002. Short Protocols in Molecular Biology. Fifth Edition, 2 Volume Set. Page Count: 1512.

Galluzzi, L., M. C. Maiuri, I. Vitale, H. Zischka, M. Castedo, L. Zitvogel, and G. Kroemer. 2007. Cell death modalities: classification and pathophysiological implications. Cell Death Differ. 14: 1237-43.

Garay, R. P., P. Viens, J. Bauer, G. Normier, M. Bardou, J. F. Jeannin, and C. Chiavaroli. 2007. Cancer relapse under chemotherapy: Why *TLR2*/4 receptor agonists can help. Eur J Pharmacol. 563: 1-17.

Gergely, P. Jr., A. Blazsek, Z. Weiszhár, B. Pazár, and G. Poór. 2006. Lack of genetic association of the Toll-like receptor 4 (*TLR4*) Asp299Gly and Thr399Ile polymorphisms with spondylarthropathies in a Hungarian population. Rheumatology. 45: 11946.

Ghosh, S., M. J. May, and E. B. Kopp. 1998. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. Annu Rev Immunol. 16: 225-260.

Greer, C. E, C. M. Wheeler, and M. M. Manos. 1994. Sample preparation and PCR amplification from paraffin-embedded tissues. Genome Res. 3: 113-122.

Guha, M., and N. Mackman. 2002. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J. Biol. Chem. 277:32124-32132.

Haddad, J. J. 2002. Cytokines and related receptor-mediated signaling pathways. Biochem Biophys Res Commun. 297: 700-713.

Haller, D., C. Bode, W. P. Hammes, A. M. A. Pfeifer, E. J. SchiVrin, and S. Blum. 2000. Non-pathogenic bacteria elicit a differential cytokine response by intestinal epithelialcell/leucocyte co-cultures. Gut.47:79-87.

Hamann L, A. Gomma, N. W. J. Schroder, C. Stamme, C. Glaeser, S. Schulz, M. Gross, S. D. Anker, K. Fox, and R. R. Schumann. 2005. A frequent Toll-like receptor (*TLR*)-2 polymorphism is a risk factor for coronary restenosis. J Mol Med. 83: 478-485.

Hang, J., W. Zhou, H. Zhang, B. Sun, H. Dai, L. Su, and D. C. Christiani. 2004. *TLR4* Asp299Gly and Thr399Ile polymorphisms are very rare in the Chinese population. J Endotoxin Res. 10: 238-240.

Hartford, C. M., and M. E. Dolan. 2007. Identifying genetic variants that contribute to chemotherapy-induced cytotoxicity. Pharmacogenomics. 8: 1159-1168.

Hartford, C. M., and M. E. Dolan. 2007. Identifying genetic variants that contribute to chemotherapy-induced cytotoxicity. pharmacogenomics. 9: 1159-1168.

Hawn, T. R., A. Verbon, M. Janer, L. P. Zhao, B. Beutler, and A. Aderem. 2005. Toll-like receptor 4 polymorphisms are associated with resistance to Legionnaires'disease. Proc Natl Acad Sci. USA. 102: 2487-2489.

He, W., Q. Liu, L. Wang, W. Chen, N. Li, and X. Cao. 2007. *TLR4* signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol. 44: 2850-2859.

Hopkins, P. A., and S. Sriskandan. 2005. Mammalian Toll-like receptors: to immunity and beyond. Clin. Exp. Immunol. 140: 395-407.

Hoption Cann, S., A. J. P. van Netten, and C. van Netten. 2003. Dr William Coley and tumour regression. a place in history or in the future. Postgrad Med J. 79: 672-6 80.

Hori, Y., and T. P. Fan. 1993. Interleukin-8 stimulates angiogenesis in rats. Inflammation. 17: 135-143.

Hu, D. E., Y. Hori, and T. P. Fan. 1993. Interleukin-8 stimulates angiogenesis in rats. Inflammation. 17: 135-143.

Hu, J., R. Jacinto, C. McCall, and L. Li. 2002. Regulation of IL-1 receptor-associated kinases by lipopolysaccharide. J Immunol. 168: 3910-3914.

Huang, B., J. Zhao, J. C. Unkeless, Z. H. Feng. and H. Xiong. 2008. *TLR* signaling by tumor and immune cells: a double-edged sword. Oncogene. 27: 218-224.

Hussain, S. P., and C. C. Harris. 2007. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer. 121: 2373-2380.

Iwasaki, A., and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immuneresponses. Nat Immunol. 5: 987-95.

Izakovicova Holla, L., D. Buckova, A. Fassmann, L. Roubalikova, and J. Vanek. 2007. Lack of association between chronic periodontitis and the Toll-like receptor 4 gene polymorphisms in a Czech population. J Periodont Res. 42: 340-344.

Janeway C. A. Jr. 2001. How the immune system works to protect the host from infection: a personal view. Proc Natl Acad Sci U S A. 98: 7461-8.

Janeway, C. A. Jr., and R. Medzhitov, 2002. Innate immune recognition. Annu Rev Immunol. 20:197-216.

Kaisho, T., and S. Akira. 2004. Pleiotropic function of Toll-like receptors. Microbes Infect. 6: 1388-94.

Kaleigh, S. 2002. Genetic Polymorphism and SNPs. http://www.cs.mcgill.ca Accessed on 27/12/2006.

Kang, E. S., and J. Lee. 2007. Genotypic analysis of Asp299Gly and Thr399Ile polymorphismof Toll-like receptor 4 in systemic autoimmune diseases of Korean population. Rheumatol Int. 27: 887-889.

Kang, T. J., and G. T. Chae. 2001. Detection of Toll-like receptor 2 (*TLR2*) mutation in the lepromatous leprosy patients. FEMS Immunol Med Microbiol 31: 53-58.

Kelly, D., S. Conway, and R. Aminov. 2005. Commensal gut bacteria: mechanisms of immune modulation. Trends Immunol. 26: 326-333.

Kelly, M. G., A. B. Alvero, R. Chen, D. A. Silasi, V. M. Abrahams, S. Chan, I. Visintin, T. Rutherford, and G. Mor. 2006. *TLR*-4 Signalling Promotes Tumor Growth and Paclitaxel Chemoresistance in Ovarian Cancer. Cancer Res. 66: 3859-3868.

Kiechl, S., E. Lorenz, M. Reindl, C. J. Wiedermann, F. Oberhollenzer, E. Bonora, J. Willeit, and D.A. Schwartz. 2002. Toll-like receptor 4 polymorphisms and atherogenesis. N Engl J Med. 347:185-192.

Killeen, S. D., J. H. Wang, E. J. Andrews, and H. P. Redmond. 2006. Exploitation of the Toll-like receptor system in cancer: a doubled-edged sword?. Br J Cancer. 95: 247- 252.

Kinane, D. F., H. Shiba, P. G. Stathopoulou, H. Zhao, D. F. Lappin, A Singh, M. A. Eskan, S. Beckers. S. Waigel, B. Alpert and T. B. Knudsen. 2006. Gingival epithelial cells heterozygous for Toll-like receptor 4 polymorphisms Asp299Gly and Thr399Ile are hypo-responsive to Porphyromonas gingivalis. Genes Immun. 7:190-200.

Kobayashi, K. S., and R. A. Flavell. 2004. Shielding the Double-edged Sword: Negative Regulation of the Innate Immune System. J Leukoc Biol. 75:428-33.

Kobayashi, K., L. D. Hernandez, J. E. Galan, Jr. C. A. Janeway, R. Medzhitov, and R. A. Flavell. 2002. IRAK-M is a negative regulator of Tolllike receptor signaling. Cell. 110: 191-202.

Krynetskaia, N., H. Xie, S. Vucetic, Z. Obradovic, and E. Krynetskiy. 2008. High mobility group protein B1 is an activator of apoptotic response to antimetabolite drugs. Mol Pharmacol. 73: 260-269.

Kutukculer, N., B. S. Yeniay, G. Aksu, and A. Berdeli. 2007. Arg753Gln Polymorphism of the Human Toll-like Receptor-2 Gene in Children with Recurrent Febrile Infections. Genet. 45: 507-514.

Lemaitre, B., E. Nicolas, L. Michaut, J. M. Reichhartand, and J. A. Hoffmann. 1996. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 86: 973-83.

Levy, R. M., K. P. Mollen, J.M. Prince, D. J. Kaczorowski, R.Vallabhaneni, S. Liu, K. J. Tracey, M.T. Lotze, D. J. Hackam, M.P. Fink, Y. Vodovotz, and T.R. Billiar. 2007. Systemic inflammation and remote organ injury following trauma require HMGB1. Am J Physiol Regul Integr Comp Physiol. 293: 1538-1544.

Liang, X. H., W. Cheung, C. K. Heng, D. Y. Wang. 2005. Absence of the Toll-like receptor 4 gene polymorphisms Asp299Gly and Thr399Ile in Singaporean Chinese. Ther Clin Risk Manag. 1: 243-246.

Lien, E., and D. T. Golenbock. 2003. Adjuvants and their signaling pathways: beyond *TLRs*. Nat Immunol. 4: 1162-4.

Lin, W.W., and M. Karin. 2007. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest. 117: 1175-83.

Liu, V. C., L. Y. Wong, T. Jang, A. H. Shah, I. Park, X. Yang , Q. Zhang, S. Lonning, B. A. Teicher, and C. Lee . 2007. Tumor evasion of the immune system by converting CD4+CD25-T cells into $CD4^+$ $CD25^+$ T regulatory cells: role of tumor-derived TGF-*β*. J Immunol. 178: 2883-2892.

Lorenz, E., D. A. Schwartz, P. J. Martin, T. Gooley, M. T. Lin, J. W. Chien, J. A. Hansen, and J. G. Clark. 2001. Association of *TLR4* mutations and the risk for acute GVHD after HLA-matched-sibling hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 7: 384-387.

Lorenz, E., J. P. Mira, K. L. Frees, and D. A. Schwartz. 2002. Relevance of mutations in the *TLR4* receptor in patients with gram-negative septic shock. Arch Intern Med.162:1028-1032.

Luster A. D. 2002. The role of chemokines in linking innate and adaptive immunity. Curr Opin Immunol. 14: 129-35.

Malhotra, D., V. Relhan, B. S. Reddy, and R. Bamezai. 2005. *TLR2* Arg677Trp polymorphism in leprosy: revisited. Hum Genet. 116: 413-415.

Mantovani, A. Cancer: inflammation by remote control. 2005. Nature. 435: 752-753.

Marsik, C., B. Jilma, C. Joukhadar, C. Mannhalter, O. Wagner, G. Endler. 2005. The Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms influence the late inflammatory response in human endotoxemia. Clin Chem. 51: 2178-2180.

Martin, C. R., and W. A. Walker. 2006. Intestinal immune defences and the inflammatory response in necrotising enterocolitis. Semin Fetal Neonatal Med. 11: 369-77.

Medzhitov, R., and C, Jr. Janeway. 2000a. The Toll receptor family and microbial recognition. Trends Microbiol. 8: 452-456.

Medzhitov, R., and C. Jr. Janeway. 2000b. Innate immunity. N Engl J Med. 343:338-44.

Medzhitov, R., P. Preston-Hurlburt, and C. A. Janeway, 1997. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 388: 394-397.

Midgley, R., and D. J. Kerr. 2005. Adjuvant chemotherapy for stage II colorectal cancer: the time is right. Nat Clin Pract Oncol. 2: 364-369.

Miller, A. M., and P. Pisa. 2007. Tumor escapes mechanisms in prostate cancer. Cancer Immunol Immunother. 56: 81-87.

Mumm, J. B., and M. Oft. 2008. Cytokine-based transformation of immune surveillance into tumor-promoting inflammation. Oncogene 27: 5913-5919.

Nakada, T. A., H. Hirasawa, S. Oda, H. Shiga, K. Matsuda, M. Nakamura, E. Watanabe, R. Abe, M. Hatano, and T. Tokuhisa. 2005. Influence of Toll-like receptor 4, CD14, tumor necrosis factor, and interleukine-10 gene polymorphisms on clinical outcome in Japanese critically ill patients. J Surg Res. 129: 322-328.

Nakanishi, C., and M. Toi. 2005. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer. 5: 297-309.

Nicolas, W., J. Schroder, and R. R Schumann. 2005. Single Nucleotide Polymorphisms of Toll-Like, Receptors and Susceptibility to Infectious Disease. Lancet infect Dis. 5: 156-164.

Nishimura, M., and S. Naito. 2005. Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. Biol Pharm Bull. 28: 886-892.

Numasaki, M., J. Fukushi, M. Ono, S. K. Narula, P. J. Zavodny, T. Kudo, P. D. Robbins, H. Tahara, and M. T. Lotze. 2003. Interleukin-17 promotes angiogenesis and tumor growth. Blood. 101: 2620-2627.

O'Neill, L. A. 2006. How Toll-like receptors signal: what we know and what we don't know. Curr Opin Immunol. 18: 3-9.

Ohm, J. E., and D. P. Carbone. 2001. VEGF as a Mediator of Tumor-Associated Immunodeficiency. Immunol Res. 23: 263-72.

Okun, E., K. J. Griffioen, J. D. Lathia, S. C. Tang, M. P. Mattson, and T. V. Arumugam. 2009. Toll-like receptors in neurodegeneration. Brain Res Rev. 59:278-292.

Oostenbrug, L, E., J. P. Drenth, D. J. de Jong, I. M. Nolte, E. Oosterom, H. M. van Dullemen, K. van der Linde, G. J. te Meerman, G. van der Steege, J. H. Kleibeuker, and P. L. Jansen. 2005. Association between Toll-like receptor 4 and inflammatory bowel disease. Inflamm Bowel Dis. 11: 567-75.

Ouburg, S., R. Mallant-Hent, J. B. Crusius, A .A. van Bodegraven, C. J. Mulder, R. Linskens, A. S. Peña, and S. A. Morré. 2005. The Toll-like receptor 4 (*TLR4*) Asp299Gly polymorphism is associated with colonic localisation of

Crohn's disease without a major role for the Saccharomyces cerevisiae mannan-LBP-CD14-*TLR4* pathway. Gut. 54: 439-440.

Papadimitraki, E. D., G. K. Bertsias, and D. T. Boumpas. 2007. Toll like receptors and autoimmunity: a critical appraisal. J Autoimmun. 4: 310-318.

Park, J. S, F. Gamboni-Robertson, Q. He, D. Svetkauskaite, J. Y. Kim, D. Strassheim, J. W. Sohn, S. Yamada, I. Maruyama, A. Banerjee, A. Ishizaka, and E. Abraham. 2006. High mobility group box 1 protein interacts with multiple Toll-like receptors. Am J Physiol Cell Physiol. 290: 917-924.

Paulos, C. M., A. Kaiser, C. Wrzesinski, C. S. Hinrichs, L. Cassard, A. Boni, P. Muranski, L. Sanchez-Perez, D. C. Palmer, Z. Yu, P. A. Antony, L. Gattinoni, S. A. Rosenberg, and N. P. Restifo. 2007. Toll-like receptors in tumor immunotherapy. Cancer Res. 18: 5280-5289.

Pidgeon, G.P., J. H. Harmey, E. Kay, M. Da Costa, H. P. Redmond, and D. J. Bouchier-Hayes. 1999. The role of endotoxin/lipopolysaccharide in surgically induced tumour growth in a murine model of metastatic disease. Br J Cancer. 8: 1311-1317.

Raderer, M., and W. Scheithauer. 1996. Treatment of advanced colorectal cancer with 5-fluorouracil and interferon-α: an overview of clinical trials. Eur J cancer. 31: 1002-1008.

Radstake, T. R., B. Franke, S. Hanssen, M. G. Netea, P. Welsing, P. Barrera, L. A. Joosten, P. L. van Riel, and W. B. van den Berg. 2004. The Toll-likereceptor 4 Asp299Gly functional variant is associated with decreased rheumatoid arthritis disease susceptibility but does not influence disease severity and/or outcome. Arthritis Rheum. 50: 999-1001.

Rallabhandi, P., J. Bell, M. S. Boukhvalova, A. Medvedev, E. Lorenz, M. Arditi, V. G. Hemming, J. C. Blanco, D. M. Segal, S. N. Vogel. 2006. Analysis of 4 polymorphic variants: new insights into *TLR4*/MD-2/CD14 stoichiometry, structure, and signaling. J Immunol. 177: 322-332.

Ranieri, G., R. Patruno, E. Ruggieri, S. Montemurro, P. Valerio, D. Ribatti. 2006. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. 13: 1845-1857.

Robinson, S. C., and L. M. Coussens. 2005. Soluble mediators of inflammation during tumor development. Adv Cancer Res. 93:159-87.

Rock, F. L., G. Hardiman, J. C., Timans, R. A. Kastelein, and J. F. Bazan. 1998. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci. USA. 95: 588-593.

Rosenberg, I. M. 2004. Protein analysis and purification: benchtop techniques, 2nd edn. Birkhauser,. Basel, 520 pp.

Savkovic, D. S., A. Koutsouris, and G. Hecht. 1997. Activation of NF-kB in intestinal epithelial cells by enteropathogenic *Escherichia coli*. Am J Physiol Cell Physiol. 273: 1160-1167.

Schmidt, C. 2006. Immune system's toll-like receptors have good opportunity for cancer treatment. J Natio Cancer Instit. 98:574-575.

Schmoll, H. J., T. Büchele, A. Grothey, and W, Dempke. 1999. Where do we stand with 5-fluorouracil? Semin Oncol. 26: 589-605.

Schröder, N. W., C. Hermann, L. Hamann, U. B. Göbel, T. Hartung, and R. R. Schumann. 2003. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. J Mol Med 81: 368–372.

Starnes, C. O. 1992. Coley's toxins in perspective. Nature. 357: 11-2.

Strengell, M., A. Lehtonen, S. Matikainen, and I. Julkunen. 2006. IL-21 enhances SOCS gene expression and inhibits LPS-induced cytokine production in human monocyte-derived dendritic cells. J Leukoc Biol.79: 1279-85.

Sun, J., A. Turner, J. Xu, H. Grönberg, and W. Isaacs. 2007. Genetic variability in inflammation pathways and prostate cancer risk. Urol Oncol. 25: 250-259.

Sun, Q., Q. Liu, Y. Zheng, and X. Cao. 2008. Rapamycin suppresses *TLR4*-triggered IL-6 and PGE(2) production of colon cancer cells by inhibiting *TLR4* expression and NF-kappaB activation. Mol Immunol. 45: 2929-36.

Suzuki, M., T. Hisamatsu, and D. K. Podolsky. 2003. Gamma interferon augments the intracellular pathway for lipopolysaccharide (LPS) recognition in human intestinal epithelial cells through coordinated up-regulationof LPS uptake and expression of the intracellular Toll-Like Receptor 4–MD-2 complex. Infect Immun. 71: 3503-3511.

Takeda, K., T. Kaisho, and S. Akira. 2003. Toll-like receptors. Annu Rev. Immunol. 21:335-76.

Tal, G., A. Mandelberg, I. Dalal, K. Cesar, E. Somekh, A. Tal, A. Oron, S. Itskovich, A. Ballin, S. Houri, A. Beigelman, O. Lider, G. Rechavi and N. Amariglio. 2004. Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. J Infect Dis. 189: 2057-2063.

Takayama, K., D. H. Mitchell, Z. Z. Din, P. Mukerjee, C. Li and D. L. Coleman. 1994. Monomeric Relipopolysaccharide from *Escherichia coli* is more active than the aggregated form in the *Limulus amebocyte* lysate assay and in inducing Egr-1 mRNA in murine peritoneal macrophages. J Biol Chem. 269: 2241-2244.

Tanaka, S., A. Sakai, K. Kimura, H. Yoshida, H. Fushitani, A. Ogata, A. Miyamoto, M. Fukushima, A. Wada, and N. Tanigawa. 2008. Proteomic analysis of the basic proteins in 5-fluorouracil resistance of human colon cancer cell line using the radical-free and highly reducing method of two-dimensional polyacrylamide gel electrophoresis. Int J Oncol. 33: 361-70.

Texereau, J., J. D. Chiche, W. Taylor, G. Choukroun, B. Comba, J. P. Mira. 2005. The importance of Toll-like receptor 2 polymorphisms in severe infections. Clin Infect Dis. 41: 408-415.

Tulic, M. K., R. J. Hurrelbrink, C. M. Prêle, I. A. Laing, J. W. Upham, P. Le Souef, P. D. Sly, P. G. Holt. 2007. *TLR4* Polymorphisms Mediate Impaired Responses to Respiratory Syncytial Virus and Lipopolysaccharide. J Immunol. 179: 132-140.

Veldhoen, M., R. J. Hocking, C. J. Atkins, R. M. Locksley, B. Stockinger. 2006. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity. 24:179-189.

Wang, J. H., B. J. Manning, Q. D. Wu, S. Blankson, D. Bouchier-Hayes, and H. P. Redmond. 2003. Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. Immunol. 170: 795-804.

Werner, T., and D. Haller. 2007. Intestinal epithelial cell signalling and chronic inflammation: From the proteome to specific molecular mechanisms. Mutat Res. 622: 42-57.

Xia, C., M. Lu, Z. Zhang, Z. Meng, Z. Zhang and C. Shi. 2008. TLRs antiviral effect on hepatitis B virus in HepG2 cells. J Appl Microbiol. 105: 1720-1727.

Yang, X. Y, L. H. Wang, and W. L. Farrar. 2008. A role for PPARγ in the regulation of cytokines in immune cells and cancer. PPAR Res. 961753.

Yoon, H. J., J. Y. Choi, C. O. Kim, Y. S. Park, M. S. Kim, Y. K. Kim, S.Y. Shin, J. M. Kim, and Y.G. Song. 2006. Lack of Toll- like receptor 4 and 2 polymorphisms in Korean patients with bacteremia. J Korean Med Sci. 21: 979-82.

Zarember, K. A., and P.J., Godowski. 2002. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol. 168: 554-561.

Zhao, L., M. J. Kwon, S. Huang, J. Y. Lee, K. Fukase, N. Inohara, D. H. Hwang. 2007. Differential modulation of Nods signaling pathways by fatty acids in human colonic epithelial HCT116 cells. J Biol Chem. 282:11618-11628.

Zitvogel, L., L. Apetoh, F. Ghiringhelli, F. Andre, A. Tesniere, and G. Kroemer. 2008. The anticancer immune response: indispensable for therapeutic success? J Clin Invest. 118:1991-2001.