World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i38.5360

World J Gastroenterol 2012 October 14; 18(38): 5360-5368
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Increased tumor necrosis factor receptor 1 expression in human colorectal adenomas

Kunihiro Hosono, Eiji Yamada, Hiroki Endo, Hirokazu Takahashi, Masahiko Inamori, Yoshitaka Hippo, Hitoshi Nakagama, Atsushi Nakajima

Kunihiro Hosono, Eiji Yamada, Hiroki Endo, Hirokazu Takahashi, Masahiko Inamori, Atsushi Nakajima, Division of Gastroenterology, Yokohama City University School of Medicine, Yokohama 2360004, Japan
Yoshitaka Hippo, Hitoshi Nakagama, Division of Cancer Development System, National Cancer Center Research Institute, Tokyo 1040045, Japan
Author contributions: Hosono K performed most of the research and wrote the paper; Yamada E, Endo H and Takahashi H performed the endoscopies; Inamori M analyzed the data; Hippo Y, Nakagama H and Nakajima A were major contributors in revising the manuscript critically for important intellectual content.
Supported by Grant-in-Aid for Research from the Third-Term Comprehensive Control Research for Cancer Center of the Ministry of Health, Labor and Welfare, Japan; Grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan (KIBAN-B); and the "Collaborative Development of Innovative Seeds" Grant Program from the Japan Science and Technology Agency
Correspondence to: Dr. Atsushi Nakajima, Professor, Division of Gastroenterology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 2360004, Japan. nakajima-tky@umin.ac.jp
Telephone: +81-45-7872640 Fax: +81-45-7843546
Received: March 6, 2012 Revised: June 7, 2012
Accepted: June 15, 2012
Published online: October 14, 2012

Abstract

AIM: To determine the expression statuses of tumor necrosis factor (TNF)-α, its receptors (TNF-R) and downstream effector molecules in human colorectal adenomas.

METHODS: We measured the serum concentrations of TNF-α and its receptors in 62 colorectal adenoma patients and 34 healthy controls. The protein expression of TNF-α, TNF-R1, TNF-R2 and downstream signals of the TNF receptors, such as c-Jun N-terminal kinase (JNK), nuclear factor-κ B and caspase-3, were also investigated in human colorectal adenomas and in normal colorectal mucosal tissues by immunohistochemistry. Immunofluorescence confocal microscopy was used to investigate the consistency of expression of TNF-R1 and phospho-JNK (p-JNK).

RESULTS: The serum levels of soluble TNF-R1 (sTNF-R1) in adenoma patients were significantly higher than in the control group (3.67 ± 0.86 ng/mL *vs* 1.57 ± 0.72 ng/mL, $P < 0.001$). Receiver operating characteristic analysis revealed the high diagnostic sensitivity of TNF-R1 measurements (AUC was 0.928) for the diagnosis of adenoma, and the best cut-off level of TNF-R1 was 2.08 ng/mL, with a sensitivity of 93.4% and a specificity of 82.4%. There were no significant differences in the serum levels of TNF-α or sTNF-R2 between the two groups. Immunohistochemistry showed high levels of TNF-R1 and p-JNK expression in the epithelial cells of adenomas. Furthermore, a high incidence of co-localization of TNF-R1 and p-JNK was identified in adenoma tissue.

CONCLUSION: TNF-R1 may be a promising biomarker of colorectal adenoma, and it may also play an important role in the very early stages of colorectal carcinogenesis.

Key words: Tumor necrosis factor-α; Tumor necrosis factor receptor 1; c-Jun N-terminal kinase; Colorectal adenoma; Biomarker

Peer reviewers: Peter Laszlo Lakatos, MD, PhD, 1st Department of Medicine, Semmelweis University, Koranyi S2A, H1083 Budapest, Hungary; Annika Lindblom, Professor, Molecular Medicine and Surgery, Karolinska Institutet, S17176 Stockholm, Sweden; Manuel Romero-Gomez, Professor, Department of Medicine, University of Sevilla, Avda. de Bellavista, 41014 Sevilla, Spain

Hosono K, Yamada E, Endo H, Takahashi H, Inamori M, Hippo Y, Nakagama H, Nakajima A. Increased tumor necrosis factor receptor 1 expression in human colorectal adenomas. *World J Gastroenterol* 2012; 18(38): 5360-5368 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i38/5360.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i38.5360

INTRODUCTION

The proinflammatory cytokine, tumor necrosis factor (TNF), plays an important role in diverse cellular events, such as the induction of other cytokines' expression, cell proliferation, differentiation, necrosis and apoptosis[1,2]. Many of the TNF-induced cellular responses are mediated by one of two specific cellular membrane receptors, tumor necrosis factor receptor 1 (TNF-R1) and TNF-R2, both of which belong to the TNF receptor superfamily[3]. In response to TNF treatment, activation of nuclear factor-κ B (NF-κB), a transcription factor, as well as mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase, p38, and c-Jun N-terminal kinase (JNK) have been reported in most cell types and, in some cases, after apoptosis or necrosis had been induced[4,5].

Overexpression of TNF-α and its receptors often accompanies tumor development and progression, and their important role in the pathogenesis of cancer is now increasingly recognized. Several studies have reported on the status of TNF-α expression in various malignancies. For example, TNF-α mRNA and protein have been detected in both malignant and stromal cells in human ovarian cancer biopsies[6,7]. The plasma levels of TNF-α have been reported to be increased in some cancer patients, especially those with poor prognoses[8-11]. Blood TNF-α concentrations have been reported to be higher in prostate cancer patients with advanced, cachectic disease[12]. These findings suggest that TNF-α might also have an important role in carcinogenesis. We now know that many of the cells and mediators of inflammation that have been detected in human and experimental cancers and inflammatory conditions increase the risk of cancer development[13-16]. There is strong evidence to suggest that cancer-related inflammation aids in the proliferation and survival of malignant cells, stimulates angiogenesis and metastasis[17-19], subverts adaptive immunity[20-22], and modulates their responses to hormones and chemotherapy[23,24].

In colitis-related colon cancer, TNF-α has been demonstrated to promote carcinogenesis, and anti-TNF-α drugs are being used therapeutically[25,26]. Moreover, serum TNF-α is known to serve as an important pathophysiologic marker for the presence and severity of inflammatory bowel disease[27]. However, TNF-α has a short half-life (20 min); therefore, accurate measurement of the blood levels is difficult[28]. There is some debate about whether TNF-α can actually be measured using some sort of physiologically active substance[28,29]. On the other hand, TNF-R1 and TNF-R2 are released into the blood as soluble TNF-R1 (sTNF-R1) and soluble TNF-R2 (sTNF-R2) after proteolysis of their extracellular domains in response to activators, including TNF-α itself[30,31]. Their presence in the peripheral blood is reflective of an inflammatory response occurring within the body. sTNF-R1 and sTNF-R2 also have very long half-lives; thus, they are more stable than TNF-α[32,33]. These receptors may provide better serum biomarkers than TNF-α. Spoettl *et al*[34] found that serum sTNF-R1 levels were significantly increased in ulcerative colitis (UC) patients compared with that of healthy controls. However, there have been few reports of the association between TNF-R1 and TNF-R2 and sporadically occurring colorectal neoplasms[35]. Thus, the association of these receptors with the risk of colorectal adenoma has not yet been fully clarified. The aim of this study was to investigate the serum levels of TNF-α, sTNF-R1 and sTNF-R2 in adenoma patients and also to investigate the expressions of downstream molecules in the TNF-α signaling pathway, including TNF-R1, TNF-R2, JNK, NF-κB and caspase-3 in adenoma tissues in comparison to those in normal colorectal mucosa.

MATERIALS AND METHODS

Patients

The study population consisted of 62 consecutive patients with colorectal adenoma who underwent colonoscopy from January 2008 to January 2009 at the Division of Gastroenterology, Yokohama City University School of Medicine. Patients with concomitant diseases, including infectious diseases, inflammatory bowel diseases, autoimmune conditions, allergy or asthma, that were likely to elevate serum TNF-α levels were excluded from the study. The patients ranged in age from 54 to 86 years (mean ± SD: 67.7 ± 8.2 years) and consisted of 39 males and 23 females. The control group consisted of 34 healthy people, matched for age. The control group consisted of people undergoing colonoscopy after positive fecal occult blood test but not found to have adenomas. The study was carried out in accordance with the Declaration of Helsinki (revised 1989) and with the approval of the Ethics Committee of Yokohama City University School of Medicine. Informed consent was obtained from each patient participating in this study and for the use of the obtained data for research purposes.

Serum samples

Before colonoscopy, blood samples were obtained from all participants after an overnight fast to determine the serum concentrations of TNF-α, sTNF-R1 and sTNF-R2. The blood samples were centrifuged at 800 g for 5 min. Serum was separated as soon as possible from the clot of red blood cells by centrifugation to avoid TNF-α production by the blood cells, which would have led to falsely increased values[36]. Then, the serum specimens were stored at -80 ℃ until the biochemical analyses.

Biochemical determinations

Serum concentrations of human TNF-α, sTNF-R1 and sTNF-R2 were determined by enzyme-linked immunosorbent assay (ELISA) kit (R and D Systems Inc., Minneapolis, MN, United States). The minimum limits of detection of the assay were as follows: TNF-α, 1.6 pg/mL; sTNF-R1, 0.77 pg/mL; and sTNF-R2, 0.6 pg/mL. The tests were carried out as described in the product manuals.

Immunohistochemical analysis

Immunohistochemical analysis was performed in the adenomatous polyps retrieved from the study patients (n = 62). Adenomatous polyps removed during colonoscopy, together with normal colorectal mucosal biopsies from the same patients when possible, were examined. Four-micrometer sections were prepared from the formalin-fixed, paraffin-embedded tissues and mounted on slides coated with polylysine. We examined only sporadically occurring polyps by excluding specimens from patients with familial adenomatous polyposis (FAP) or hereditary non-polyposis coli (HNPCC). The protein expression levels were determined in all 62 normal and colorectal adenomatous tissue sections by immunohistochemistry. Sections were deparaffinized in xylene and rehydrated. The sections were then heated in a 750 W microwave three times for 7 min. Inhibition of endogenous peroxidase activity was performed by treating sections with 3% hydrogen peroxide for 10 min. After washing three times with Tris-Buffered Saline (TBS), the blocking of non-specific interactions was accomplished by incubating the samples with blocking serum for 30 min at room temperature. Sections were then probed with the primary antibodies (Table 1) and subsequently incubated with the Histofine simple stain max PO kit for 30 min (Nichirei Laboratories, Tokyo, Japan) in accordance with the manufacturer's instructions. The signals were visualized by treatment with diaminobenzidine (peroxidase substrate kit, Vector Laboratories, Burlingame, CA, United States), and the sections were counterstained with hematoxylin. Six random microscopic fields per sample of approximately 250 cells were counted at a magnification of 400 × under a bright-field microscope. The results were expressed as the percentage of positive cells with separating epithelial cells and stromal cells.

Immunofluorescence confocal microscopy

For immunofluorescence studies, the sections were stained with anti-TNF-R1 rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, United States) at a dilution of 1:50, anti-TNF-R2 rabbit polyclonal antibody (Santa Cruz Biotechnology) at a dilution of 1:50, and anti-p-JNK mouse monoclonal antibody at Thr 183 and Tyr 185 (Santa Cruz Biotechnology) at a dilution 1:50. After thorough washes in TBS, sections were incubated with their respective Alexa594-conjugated anti-rabbit IgG secondary antibodies (Invitrogen, Carlsbad, CA, United States) or Alexa488-conjugated anti-mouse IgG secondary antibodies (Invitrogen) for 1 h, washed, and mounted.

The confocal imaging was carried out on a FV1000-D (Olympus, Tokyo, Japan) confocal laser scanning microscope. Excitation and detection of the samples were carried out in sequential modes to avoid overlapping of signals. Sections were scanned simultaneously at both wavelengths (488/594 nm) with appropriate laser intensity, confocal aperture, and gain. The Black-level setting was kept constant for all samples.

Table 1 Summary of the specific antibodies used and immunohistochemistry

Protein	Antibody (catalog number)	Dilution	Incubation
TNF-α	Rabbit polyclonal (Abcam, ab6671)	1:100	Overnight
TNF-R1	Rabbit polyclonal (Abcam, ab19139)	1:2000	1 h
TNF-R2	Rabbit polyclonal (Abcam, ab15563)	1:50	30 min
p-JNK	Rabbit polyclonal (CST, #9251)	1:100	Overnight
NF-κB	Rabbit polyclonal (CST, #3034)	1:50	Overnight
Caspase-3	Rabbit polyclonal (CST, #9661)	1:200	Overnight

Abcam (Cambridge, United Kingdom). CST: Cell signaling technology (Beverly, MA, United States).

Table 2 Comparison of the clinical data and measured biochemical parameters in the serum of patients with adenomas and control subjects (mean ± SD)

Parameters	Control (n = 34)	Adenoma (n = 62)	P value
Age (yr)	67.6 ± 15.9	67.7 ± 8.2	NS
Sex (F/M)	14/20	23/39	NS
BMI	23.5 ± 5.0	23.9 ± 3.4	NS
TNF-α (pg/mL)	5.56 ± 1.3	4.83 ± 1.9	NS
sTNF-R1 (ng/mL)	1.57 ± 0.72	3.67 ± 0.86	< 0.001
sTNF-R2 (ng/mL)	2.72 ± 1.15	3.09 ± 0.91	NS

P < 0.05 was denoted significance. NS: Non-significant; TNF-α: Tumor necrosis factor-α; sTNF-R1/2: Soluble tumor necrosis factor receptor 1/2; F/M: Female/male; BMI: Body mass index.

Statistical analysis

Data were expressed as the mean ± SD unless otherwise indicated. The significances of the differences in clinical characteristics between patients with adenomas and controls were evaluated by the χ^2-test for categorical variables and Welch's test for continuous variables. One-way analysis of variance was performed to compare the prevalence of each variable between groups. To assess the diagnostic sensitivity and specificity of the sTNF-R1 and sTNF-R2 measurements, receiver operating characteristic (ROC) curve analysis was performed. P < 0.05 was considered to indicate statistical significance.

RESULTS

Biochemical determinations

Table 2 shows a comparison of the clinical data and laboratory parameters between the 62 patients with adenoma and the 34 normal controls. No differences in

Table 3 Association between the measured biochemical parameters and the number of colorectal adenomas per person

	Control (n = 34)	≤ 3 (n = 34)	≥ 4 (n = 28)	P value
TNF-α (pg/mL)	5.63 ± 1.3	4.31 ± 1.9	4.95 ± 2.0	NS
sTNF-R1 (ng/mL)	1.57 ± 0.72	3.37 ± 0.93	4.05 ± 0.55	< 0.001
sTNF-R2 (ng/mL)	2.72 ± 1.15	3.13 ± 0.88	3.04 ± 0.95	NS

Values are expressed as the mean ± SD; $P < 0.05$ denoted significance. NS: Non-significant; TNF-α: Tumor necrosis factor-α; sTNF-R1/2: Soluble tumor necrosis factor receptor 1/2.

the age, sex or body mass index were found between the two studied groups. The mean serum levels of sTNF-R1 were significantly higher in the adenoma patients compared to the control group patients. On the other hand, there were no significant differences in the serum levels of TNF-α or sTNF-R2 between the two groups. Linear contrast analysis was conducted to evaluate the correlation between each variable and the number of adenomas per person (Table 3). The serum levels of sTNF-R1 were positively correlated with the number of adenomas in the colorectum ($P < 0.001$). As assessed by nonparametric analysis of the ROC curves, sTNF-R1 measurements showed a high diagnostic sensitivity at a value of 0.928 (Figure 1). The best cut-off level for TNF-R1 as 2.08 ng/mL, which showed a sensitivity of 93.4% and a specificity of 82.4%.

Immunohistochemical analysis

Immunohistochemistry showed varying expression intensities of TNF-α, TNF-R1, TNF-R2, p-JNK, NF-κB and caspase-3 in different areas of the samples. The expression of TNF-α in the stromal area of adenomas was significantly higher than that in the stromal areas of the normal mucosa ($P < 0.05$); however, the expression of TNF-α in the epithelial cells of adenomas was not as high as that in the epithelial cells of the normal mucosa (Figure 2). The expression of TNF-R1 in the epithelial cells of the adenomas was significantly higher than that in the epithelial cells of the normal mucosa ($P < 0.05$); however, in the stromal areas, the expression of TNF-R1 was equivalent between the adenomas and the normal mucosa. No significant difference in the expression of TNF-R2 was noted in either the epithelial cells or the stromal areas between the adenomas and the normal mucosa. In relation to the expressions of the downstream molecules in the TNF-α signaling pathway, the expression of p-JNK in both the epithelial cells and stromal areas of the adenoma were significantly higher than those in the normal mucosa ($P < 0.05$) (Figure 3). However, there were no significant differences in the expressions of NF-κB or caspase-3 in either the epithelial cells or stromal areas between the adenomas and the normal mucosa.

To investigate the consistency of TNF-R1 and p-JNK expression, we performed immunofluorescence staining of the adenoma tissues, because the results of immunohistochemistry revealed high levels of TNF-R1 and p-JNK expression in the epithelial cells of the adenomas. The results revealed expression of TNF-R1 and p-JNK co-localized predominantly in the epithelial cells of the adenomas. Merged images of TNF-R1 and p-JNK immunofluorescence staining demonstrated this co-localization (Figure 4). On the other hand, the expression of TNF-R2 was localized predominantly to the stromal area of the adenomas, and merged images of TNF-R2 and p-JNK immunofluorescence staining showed a lack of co-localization.

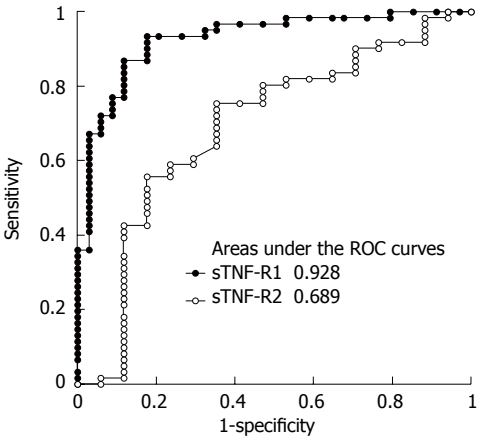

Figure 1 Receiver operating characteristic curves for soluble tumor necrosis factor receptor-1 and 2 in patients with adenomas. ROC: Receiver operating characteristic.

DISCUSSION

This is the first study to show marked changes in the expression levels of TNF-R1 in colorectal adenoma tissues. The serum sTNF-R1 levels were also significantly higher in colorectal adenoma patients than in the control subjects. To evaluate the possible usefulness of TNF-R1 as a biomarker for colorectal adenoma, we evaluated the correlation between the number of adenomas in the colorectum and the serum sTNF-R1 level. The results showed that the serum sTNF-R1 level positively correlated with the number of colorectal adenomas. A ROC analysis revealed the high diagnostic sensitivity and specificity of sTNF-R1 measurements for diagnosis of colorectal adenoma. These results may suggest that TNF-R1 is a promising biomarker for colorectal adenomas. In addition, our results agreed with the Kaminska *et al*[37] study, which showed that sTNF-R1 had the highest diagnostic sensitivity in colorectal cancer patients. Other previous reports have shown a high diagnostic and prognostic accuracy of serum sTNF-R1 levels for various diseases. Determination of the TNF-R1 levels in several body fluids, including the serum, provides valuable insight into a variety of pathological conditions. For example, in cervical adenocarcinoma patients, serum sTNF-R1 is reported to be a useful marker, especially in the early stages of disease[38]. In patients with breast cancer, serum sTNF-R1 is considered to be an independent and clinically useful

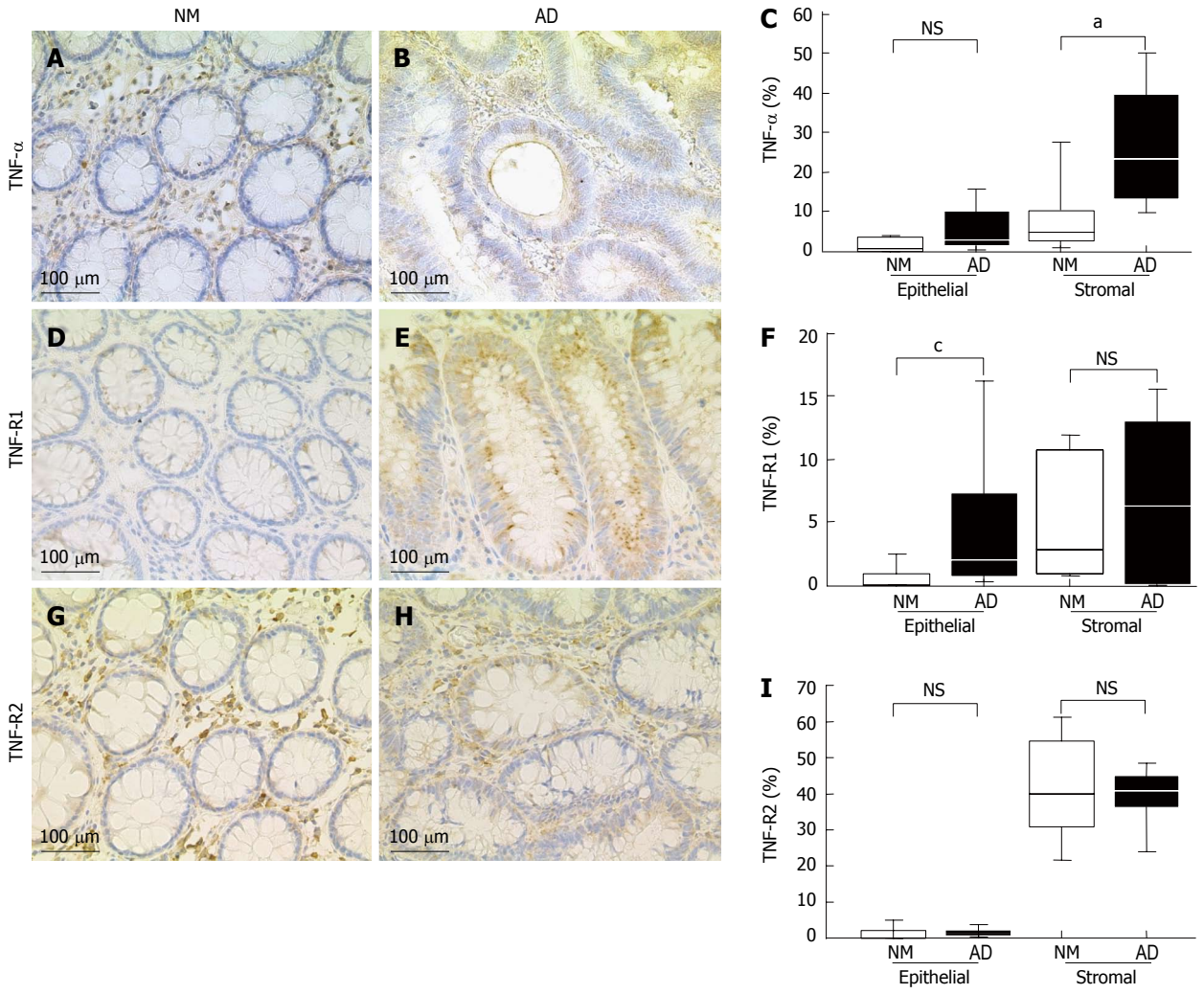

Figure 2 Immunohistochemical analyses in the normal colorectal mucosa and adenoma tissues. A: Tumor necrosis factor (TNF)-α expression in the normal colorectal mucosa; B: TNF-α expression in the adenoma tissues; C: The percentage of TNF-α-positive cells; D: Tumor necrosis factor-receptor 1 (TNF-R1) expression in the normal colorectal mucosa; E: TNF-R1 expression in the adenoma tissues; F: The percentage of TNF-R1-positive cells; G: TNF-R2 expression in the normal colorectal mucosa; H: TNF-R2 expression in the adenoma tissues; I: The percentage of TNF-R2-positive cells. Box plots display median values and interquartile ranges (C, F, I). The non-outlier range is also shown. $^{a}P < 0.05$ between NM and AD in stromal of TNF-α; $^{c}P < 0.05$ between NM and AD in epithelial of TNF-R1. NS: Non-significant; NM: Normal mucosa; AD: Adenoma.

indicator of a poor prognosis[39].

TNFR-1 is an important member of the death receptor family, which is capable of inducing apoptotic cell death[40]. In addition to its involvement in apoptotic signaling, TNF-R1 has been widely studied because it is a dual-role receptor. In addition to inducing apoptosis, it also has the ability to transduce cell survival signals. When TNF-R1 transduces cell survival signals, TNFR-associated factor 2 (TRAF-2) is recruited to the complex, which inhibits apoptosis *via* the cytoplasmic inhibitor of apoptosis protein (cIAP). The binding of TRAF-2 initiates a sequence of phosphorylation steps resulting in the activation of cFos/cJun transcription factors *via* MAPK and JNK[41]. The cFos/cJun transcription factors induce transcription of antiapoptotic, proliferative, immunomodulatory, and inflammatory genes.

TNF-α is a major mediator of cancer-related inflammation[13,42], and most of the pro-tumor actions of TNF-α appear to be mediated by TNF-R1. Mouse experiments have revealed that the development of primary cancers and metastases is attenuated in mice deficient in TNF-R1. For example, TNF-R1-/- mice are resistant to DMBA-TPA carcinogenesis, as are TNF-α-/- mice[43]. Experimental formation of lung and liver metastases was attenuated in TNF-R1-/- mice compared with that in their normal counterparts[44,45]. In wild-type mice with bone marrow cells repopulated using cells from TNF-R1-/- mice, the likelihood of colitis and colon cancer development was reduced[46] suggesting that TNF-α in the tumor microenvironment enhanced tumor development through its actions on TNF-R1-positive myeloid cells. In various pathologic states, it has been reported that the production and release of TNF-R1 may mediate host responses and determine the course and outcome of the disease by binding with TNF-α and competing with cell surface receptors.

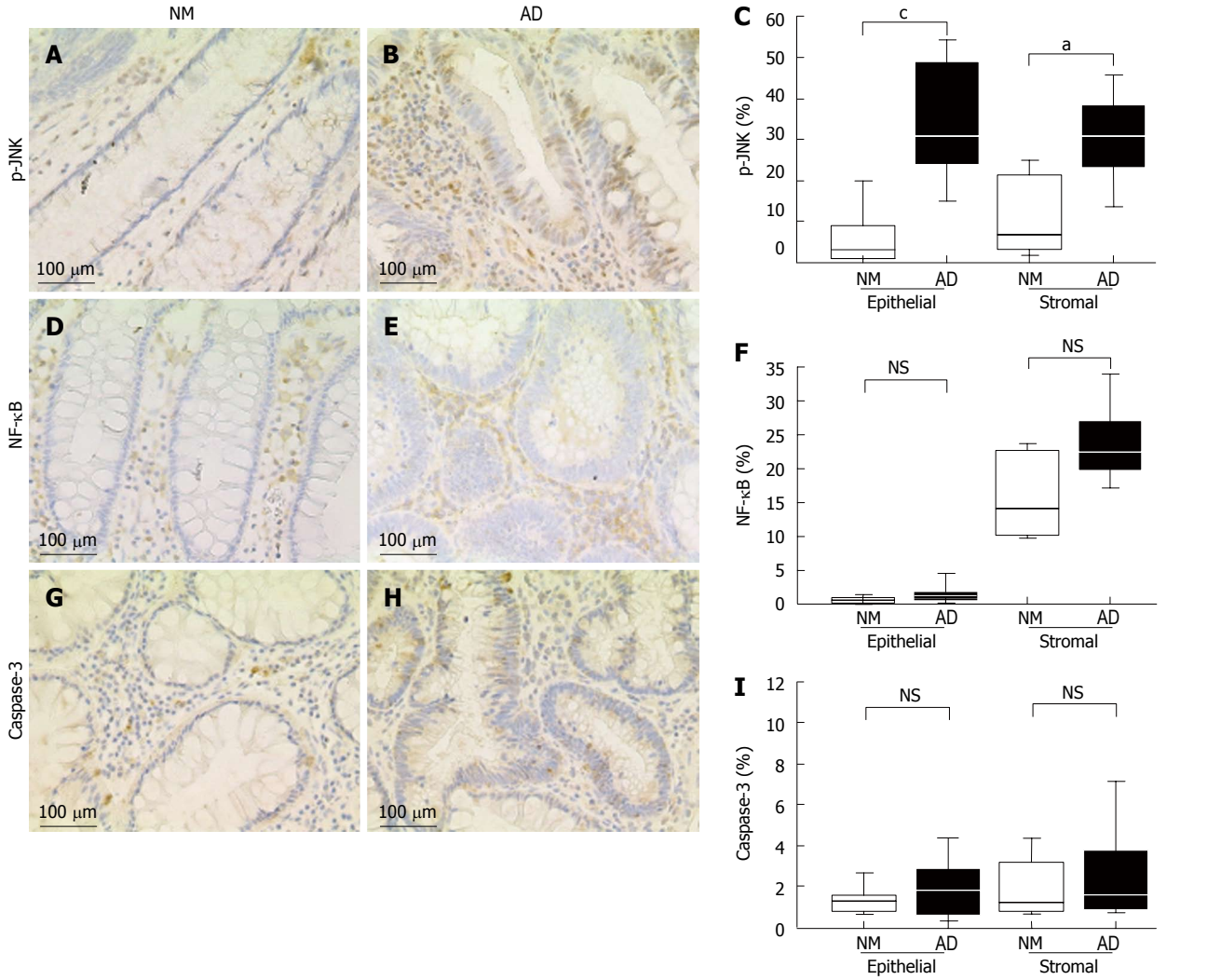

Figure 3 Immunohistochemical analyses in the normal colorectal mucosa and adenoma tissues. A: Phospho-c-Jun N-terminal kinase (p-JNK) expression in the normal colorectal mucosa; B: p-JNK expression in the adenoma tissues; C: The percentage of p-JNK positive cells; D: Nuclear factor-κ B (NF-κB) expression in the normal colorectal mucosa; E: NF-κB expression in the adenoma tissues; F: The percentage of NF-κB-positive cells; G: Caspase-3 expression in the normal colorectal mucosa; H: Caspase-3 expression in the adenoma tissues; I: The percentage of caspase-3-positive cells. Box plots display median values and interquartile ranges (C, F, I). The non-outlier range is also shown. $^{a}P < 0.05$ between NM and AD in stromal of p-JNK; $^{c}P < 0.05$ between NM and AD in epithelial of p-JNK. NS: Non-significant; NM: Normal mucosa; AD: Adenoma.

In addition to the systematic analysis of serum sTNF -R1, we also investigated, by immunohistochemical analysis, the local expressions of downstream molecules in the TNF-α signaling pathway in normal colorectal mucosa and adenoma tissues. The results revealed high levels of TNF-R1 and p-JNK expression in the epithelial cells of adenomas. Moreover, we identified co-localization of TNF-R1 and p-JNK in adenomas by immunofluorescence confocal microscopy. In the past, expression of p-JNK in adenomas has only been described by Hardwick *et al*[47]. These authors found that the expression of p-JNK was observed mainly in stromal T-lymphocytes, and epithelial cells were not stained. The discrepancy in our results was presumably due to differences in the immunohistochemical staining protocol used. The Hardwick study used phosphate- buffered saline (PBS) as the wash buffer, while we used TBS. Additionally, different antibodies were used in the two studies, which may have resulted in the absence of phospho-JNK staining in the epithelial cells of their study.

Our results suggest that the TNF-R1/p-JNK pathway is upregulated in adenomas and that this pathway may play an important role in adenoma formation, which represents the very early stages of colorectal carcinogenesis because this pathway is not upregulated in the normal colorectal mucosa. Expression of JNK in colon cancer has already received some attention[48,49]; studies have found increased activity of JNK in both rat models of colon cancer and human colorectal tumors. In addition, Zhang *et al*[50] indicated that the TNF-R1/JNK signaling cascade can functionally promote tumorigenesis of human epithelial cancers, such as squamous cell carcinomas. Moreover, a recent study conducted by our group showed that JNK/c-Jun may play an important role in promoting colorectal carcinogenesis and epithelial cell proliferation under high-fat dietary conditions[51].

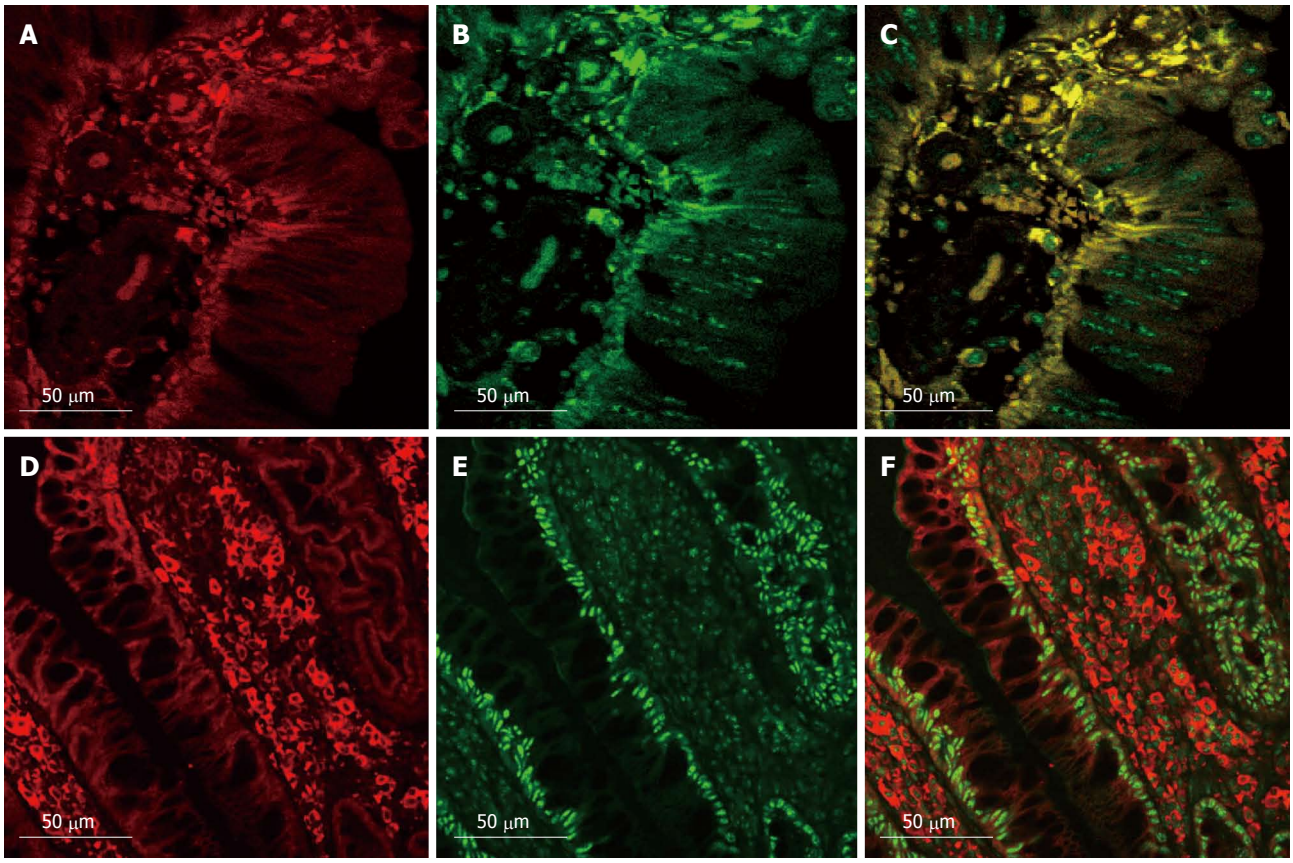

Figure 4 Confocal laser microscopic analyses of colorectal adenoma sections. A: Tumor necrosis factor receptor 1 (TNF-R1) expression; B: Phospho-c-Jun N-terminal kinase (p-JNK) expression; C: Merged image of A and B. Co-localized expression of TNF-R1 and p-JNK was noted predominantly in the epithelial cells of adenomas. Merged images showing co-localization; D: TNF-R2 expression; E: p-JNK expression; F: Merged image of D and E. Expression of TNF-R2 was localized predominantly to the stromal area of the adenoma. Merged images showed a lack of co-localization.

Our study had several important novelties. We showed for the first time that the serum levels of sTNF-R1 may have high diagnostic sensitivity and specificity as a biomarker for colorectal adenoma. Second, TNF-R1 was relatively undetectable in the normal colorectal mucosa, whereas adenomas showed high expression levels of TNF-R1. In addition, co-expression of p-JNK with TNF-R1 was observed in adenomas, although a previous study reported that the epithelial cells of adenoma showed a lack of p-JNK staining[47]. These results suggest that the TNF-R1/JNK pathway may play an important role in the development/progression of colorectal adenoma.

The present study also had some limitations. First, our research target was colorectal adenoma and not colorectal cancer. Thus, further studies targeting colorectal cancer are needed. Secondly, the control population was small. Third, different cell death receptors are able to activate JNK. Therefore, the increased p-JNK expression in adenoma tissue may be related to TNF-R1 or other effectors. The expression of cell death receptors may also be altered in inflammatory cells, which may certainly be involved in cancer progression. Fourth, it was considered that TNF-R1 may directly stimulate JNK activation, but this would also be difficult to prove.

In conclusion, this is the first study to report elevated serum levels of sTNF-R1 in patients with adenomas compared to a control group. We also found, by immunohistochemical analysis, enhanced expression of TNF-R1 in colorectal adenomas. These results suggest that TNF-R1 may not only be a promising biomarker of colorectal adenoma but also plays an important role in the very early stages of colorectal carcinogenesis. More studies are needed to elucidate the exact functions of TNF-R1 in colorectal adenomas.

ACKNOWLEDGMENTS

We thank Machiko Hiraga for her technical assistance.

COMMENTS

Background

The proinflammatory cytokine, tumor necrosis factor (TNF)-α, is a major mediator of cancer-related inflammation. However, little has been reported on the relationship between TNF-α and sporadically occurring colorectal neoplasms. Studies on human colorectal adenoma were performed to determine the expression statuses of TNF-α and its receptors.

Research frontiers

Overexpression of TNF-α and its receptors often accompanies tumor development and progression, and their important role in the pathogenesis of cancer is

now increasingly recognized.

Innovations and breakthroughs

The authors show, for the first time, that the serum levels of tumor necrosis factor-receptor 1 (TNF-R1) may have high diagnostic sensitivity and specificity as a biomarker of colorectal adenoma. TNF-R1 was hardly detected in the normal colorectal mucosa, whereas adenomas showed high expression levels of TNF-R1. In addition, co-expression of phospho-c-Jun N-terminal kinase (p-JNK) with TNF-R1 was observed in adenomas. These results suggest that the TNF-R1/JNK pathway may play an important role in the development/progression of colorectal adenoma.

Applications

TNF-R1 may be a promising biomarker of colorectal adenoma, and further studies may show that TNF-R1 expression can be used to screen for adenomas in patients as an alternative or in addition to hemoccult screening or colonoscopy screening.

Terminology

TNF-R1 belongs to the TNF receptor superfamily. In response to TNF treatment, activation of the transcription factor nuclear factor-κ B and mitogen-activated protein kinase, as well as Extracellular Signal-regulated Kinase, p38, and JNK, has been reported in most types of cells and, in some cases, apoptosis or necrosis was also induced.

Peer review

This is an interesting study investigating the importance of TNF-R1/JNK co-expression in colorectal adenoma. The major finding of the study was that serum levels of TNF-R1 were higher in patients with colorectal adenomas, while immunohistochemistry showed high expression of both TNF-R1 and p-JNK in the adenomatous tissues.

REFERENCES

1 **Rothe J**, Gehr G, Loetscher H, Lesslauer W. Tumor necrosis factor receptors--structure and function. *Immunol Res* 1992; **11**: 81-90
2 **Tracey KJ**, Cerami A. Tumor necrosis factor, other cytokines and disease. *Annu Rev Cell Biol* 1993; **9**: 317-343
3 **Tartaglia LA**, Goeddel DV. Two TNF receptors. *Immunol Today* 1992; **13**: 151-153
4 **Smith CA**, Farrah T, Goodwin RG. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell* 1994; **76**: 959-962
5 **Liu ZG**, Han J. Cellular responses to tumor necrosis factor. *Curr Issues Mol Biol* 2001; **3**: 79-90
6 **Naylor MS**, Malik ST, Stamp GW, Jobling T, Balkwill FR. In situ detection of tumour necrosis factor in human ovarian cancer specimens. *Eur J Cancer* 1990; **26**: 1027-1030
7 **Naylor MS**, Stamp GW, Foulkes WD, Eccles D, Balkwill FR. Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. *J Clin Invest* 1993; **91**: 2194-2206
8 **Karayiannakis AJ**, Syrigos KN, Polychronidis A, Pitiakoudis M, Bounovas A, Simopoulos K. Serum levels of tumor necrosis factor-alpha and nutritional status in pancreatic cancer patients. *Anticancer Res* 2001; **21**: 1355-1358
9 **Yoshida N**, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, Kishimoto T, Nakatani T. Interleukin-6, tumour necrosis factor alpha and interleukin-1beta in patients with renal cell carcinoma. *Br J Cancer* 2002; **86**: 1396-1400
10 **Ferrajoli A**, Keating MJ, Manshouri T, Giles FJ, Dey A, Estrov Z, Koller CA, Kurzrock R, Thomas DA, Faderl S, Lerner S, O'Brien S, Albitar M. The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia. *Blood* 2002; **100**: 1215-1219
11 **Bozcuk H**, Uslu G, Samur M, Yildiz M, Ozben T, Ozdoğan M, Artaç M, Altunbaş H, Akan I, Savaş B. Tumour necrosis factor-alpha, interleukin-6, and fasting serum insulin correlate with clinical outcome in metastatic breast cancer patients treated with chemotherapy. *Cytokine* 2004; **27**: 58-65
12 **Pfitzenmaier J**, Vessella R, Higano CS, Noteboom JL, Wallace D, Corey E. Elevation of cytokine levels in cachectic patients with prostate carcinoma. *Cancer* 2003; **97**: 1211-1216
13 **Sethi G**, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. *Front Biosci* 2008; **13**: 5094-5107
14 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545
15 **Balkwill F**, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 2005; **7**: 211-217
16 **Mantovani A**, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436-444
17 **Kulbe H**, Thompson R, Wilson JL, Robinson S, Hagemann T, Fatah R, Gould D, Ayhan A, Balkwill F. The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. *Cancer Res* 2007; **67**: 585-592
18 **Egberts JH**, Cloosters V, Noack A, Schniewind B, Thon L, Klose S, Kettler B, von Forstner C, Kneitz C, Tepel J, Adam D, Wajant H, Kalthoff H, Trauzold A. Anti-tumor necrosis factor therapy inhibits pancreatic tumor growth and metastasis. *Cancer Res* 2008; **68**: 1443-1450
19 **Zins K**, Abraham D, Sioud M, Aharinejad S. Colon cancer cell-derived tumor necrosis factor-alpha mediates the tumor growth-promoting response in macrophages by up-regulating the colony-stimulating factor-1 pathway. *Cancer Res* 2007; **67**: 1038-1045
20 **Kashii Y**, Giorda R, Herberman RB, Whiteside TL, Vujanovic NL. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J Immunol* 1999; **163**: 5358-5366
21 **Prévost-Blondel A**, Roth E, Rosenthal FM, Pircher H. Crucial role of TNF-alpha in CD8 T cell-mediated elimination of 3LL-A9 Lewis lung carcinoma cells in vivo. *J Immunol* 2000; **164**: 3645-3651
22 **Baxevanis CN**, Voutsas IF, Tsitsilonis OE, Tsiatas ML, Gritzapis AD, Papamichail M. Compromised anti-tumor responses in tumor necrosis factor-alpha knockout mice. *Eur J Immunol* 2000; **30**: 1957-1966
23 **van der Veen AH**, de Wilt JH, Eggermont AM, van Tiel ST, Seynhaeve AL, ten Hagen TL. TNF-alpha augments intratumoural concentrations of doxorubicin in TNF-alpha-based isolated limb perfusion in rat sarcoma models and enhances anti-tumour effects. *Br J Cancer* 2000; **82**: 973-980
24 **Seynhaeve AL**, Hoving S, Schipper D, Vermeulen CE, de Wiel-Ambagtsheer G, van Tiel ST, Eggermont AM, Ten Hagen TL. Tumor necrosis factor alpha mediates homogeneous distribution of liposomes in murine melanoma that contributes to a better tumor response. *Cancer Res* 2007; **67**: 9455-9462
25 **Waldner MJ**, Neurath MF. Cytokines in colitis associated cancer: potential drug targets? *Inflamm Allergy Drug Targets* 2008; **7**: 187-194
26 **Rizzo A**, Pallone F, Monteleone G, Fantini MC. Intestinal inflammation and colorectal cancer: a double-edged sword? *World J Gastroenterol* 2011; **17**: 3092-3100
27 **Komatsu M**, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem* 2001; **47**: 1297-1301
28 **Duncombe AS**, Brenner MK. Is circulating tumor necrosis factor bioactive? *N Engl J Med* 1988; **319**: 1227-1228
29 **Hotamisligil GS**, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995; **95**: 2409-2415
30 **Nophar Y**, Kemper O, Brakebusch C, Englemann H, Zwang R, Aderka D, Holtmann H, Wallach D. Soluble forms of tumor necrosis factor receptors (TNF-Rs). The cDNA for the

type I TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor. *EMBO J* 1990; **9**: 3269-3278
31 **Wallach D**, Engelmann H, Nophar Y, Aderka D, Kemper O, Hornik V, Holtmann H, Brakebusch C. Soluble and cell surface receptors for tumor necrosis factor. *Agents Actions Suppl* 1991; **35**: 51-57
32 **Aderka D**, Engelmann H, Shemer-Avni Y, Hornik V, Galil A, Sarov B, Wallach D. Variation in serum levels of the soluble TNF receptors among healthy individuals. *Lymphokine Cytokine Res* 1992; **11**: 157-159
33 **Diez-Ruiz A**, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *Eur J Haematol* 1995; **54**: 1-8
34 **Spoettl T**, Hausmann M, Klebl F, Dirmeier A, Klump B, Hoffmann J, Herfarth H, Timmer A, Rogler G. Serum soluble TNF receptor I and II levels correlate with disease activity in IBD patients. *Inflamm Bowel Dis* 2007; **13**: 727-732
35 **Iwagaki H**, Hizuta A, Kohka H, Kobashi K, Nitta Y, Isozaki H, Takakura N, Tanaka N. Circulating levels of soluble CD30 and other markers in colorectal cancer patients. *J Med* 1999; **30**: 111-121
36 **Leroux-Roels G**, Offner F, Philippé J, Vermeulen A. Influence of blood-collecting systems on concentrations of tumor necrosis factor in serum and plasma. *Clin Chem* 1988; **34**: 2373-2374
37 **Kaminska J**, Nowacki MP, Kowalska M, Rysinska A, Chwalinski M, Fuksiewicz M, Michalski W, Chechlinska M. Clinical significance of serum cytokine measurements in untreated colorectal cancer patients: soluble tumor necrosis factor receptor type I--an independent prognostic factor. *Tumour Biol* 2005; **26**: 186-194
38 **Kotowicz B**, Kaminska J, Fuksiewicz M, Kowalska M, Jonska-Gmyrek J, Gawrychowski K, Sobotkowski J, Skrzypczak M, Starzewski J, Bidzinski M. Clinical significance of serum CA-125 and soluble tumor necrosis factor receptor type I in cervical adenocarcinoma patients. *Int J Gynecol Cancer* 2010; **20**: 588-592
39 **Fuksiewicz M**, Kowalska M, Kotowicz B, Rubach M, Chechlinska M, Pienkowski T, Kaminska J. Serum soluble tumour necrosis factor receptor type I concentrations independently predict prognosis in patients with breast cancer. *Clin Chem Lab Med* 2010; **48**: 1481-1486
40 **Ashkenazi A**, Dixit VM. Death receptors: signaling and modulation. *Science* 1998; **281**: 1305-1308
41 **Natoli G**, Costanzo A, Ianni A, Templeton DJ, Woodgett JR, Balsano C, Levrero M. Activation of SAPK/JNK by TNF receptor 1 through a noncytotoxic TRAF2-dependent pathway. *Science* 1997; **275**: 200-203
42 **Balkwill F**. TNF-alpha in promotion and progression of cancer. *Cancer Metastasis Rev* 2006; **25**: 409-416
43 **Arnott CH**, Scott KA, Moore RJ, Robinson SC, Thompson RG, Balkwill FR. Expression of both TNF-alpha receptor subtypes is essential for optimal skin tumour development. *Oncogene* 2004; **23**: 1902-1910
44 **Tomita Y**, Yang X, Ishida Y, Nemoto-Sasaki Y, Kondo T, Oda M, Watanabe G, Chaldakov GN, Fujii C, Mukaida N. Spontaneous regression of lung metastasis in the absence of tumor necrosis factor receptor p55. *Int J Cancer* 2004; **112**: 927-933
45 **Kitakata H**, Nemoto-Sasaki Y, Takahashi Y, Kondo T, Mai M, Mukaida N. Essential roles of tumor necrosis factor receptor p55 in liver metastasis of intrasplenic administration of colon 26 cells. *Cancer Res* 2002; **62**: 6682-6687
46 **Popivanova BK**, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. *J Clin Invest* 2008; **118**: 560-570
47 **Hardwick JC**, van den Brink GR, Offerhaus GJ, van Deventer SJ, Peppelenbosch MP. NF-kappaB, p38 MAPK and JNK are highly expressed and active in the stroma of human colonic adenomatous polyps. *Oncogene* 2001; **20**: 819-827
48 **Licato LL**, Brenner DA. Analysis of signaling protein kinases in human colon or colorectal carcinomas. *Dig Dis Sci* 1998; **43**: 1454-1464
49 **Licato LL**, Keku TO, Wurzelmann JI, Murray SC, Woosley JT, Sandler RS, Brenner DA. In vivo activation of mitogen-activated protein kinases in rat intestinal neoplasia. *Gastroenterology* 1997; **113**: 1589-1598
50 **Zhang JY**, Adams AE, Ridky TW, Tao S, Khavari PA. Tumor necrosis factor receptor 1/c-Jun-NH2-kinase signaling promotes human neoplasia. *Cancer Res* 2007; **67**: 3827-3834
51 **Endo H**, Hosono K, Fujisawa T, Takahashi H, Sugiyama M, Yoneda K, Nozaki Y, Fujita K, Yoneda M, Inamori M, Wada K, Nakagama H, Nakajima A. Involvement of JNK pathway in the promotion of the early stage of colorectal carcinogenesis under high-fat dietary conditions. *Gut* 2009; **58**: 1637-1643

S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN