We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK
CITATION
INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Characterization of Acute-Phase Proteins (Apps)

Sin Tak Chu and Ying Chu Lee
*Academia Sinica &National Taiwan University, Taipe,
Taiwan*

1. Introduction

Upon Injury, a complex biological response of tissues is initiated to protect the organism and remove the injurious stimuli then trigger the healing process. Inflammation is a part of this complex response. The local response to tissue injury or infection is acute inflammation. Without inflammation, wounds and infections would never heal. This response is called the acute-phase response. After the beginning of inflammation, a large number of changes in the physiological system occur and last for 1 or 2 days; the system then returns to normal for 4 to 7 days provided there is no further stimulation. This systemic response is called acute-phase reaction (APR), also called acute-phase response. APR is characterized by fever and by an increased number of peripheral white blood cells. At the same time, cellular and biochemical changes occur in liver or other cells. One of the important events in acute-phase response is the change of the protein molecules in the plasma, known as the acute-phase proteins (APPs) (1–6).
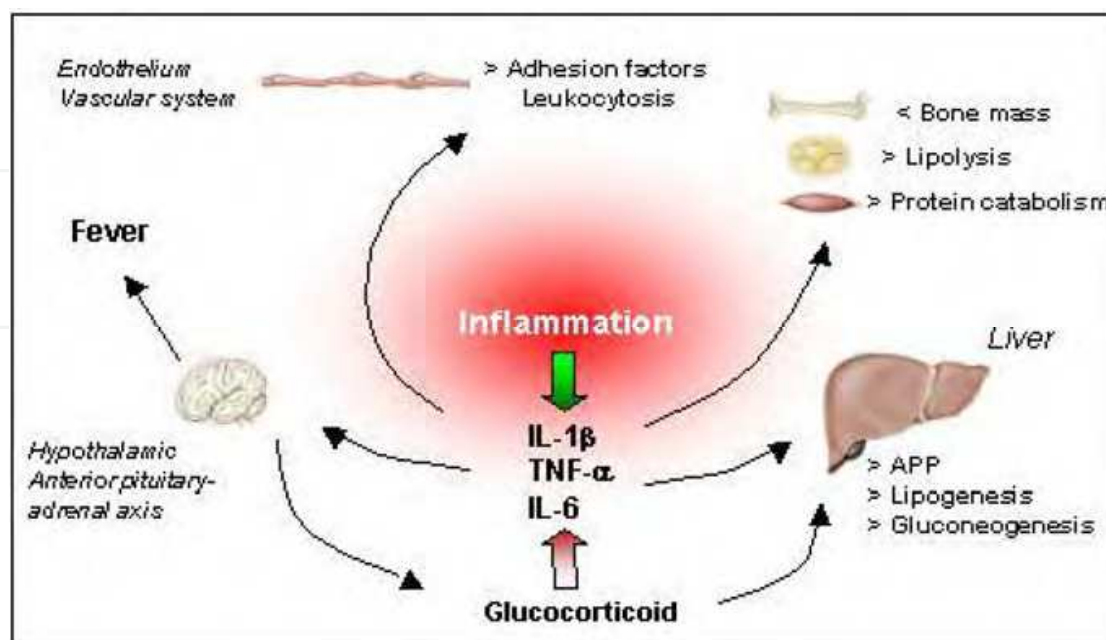

Fig. 1. The acute phase reaction. Green arrow indicates the promoting activity. Red arrow indicates the inhibitory activity.

The level of acute-phase proteins changes rapidly in response to inflammation, and these proteins serve as useful indicators of stress and disease. The APPs are mainly synthesized and secreted from the liver, under cytokine stimulation (Fig.1 is from ref.7). These cytokines can drive the production of anti-inflammatory glucocorticoids by regulating the hypothalamic-pituitary-adrenal (HPA).
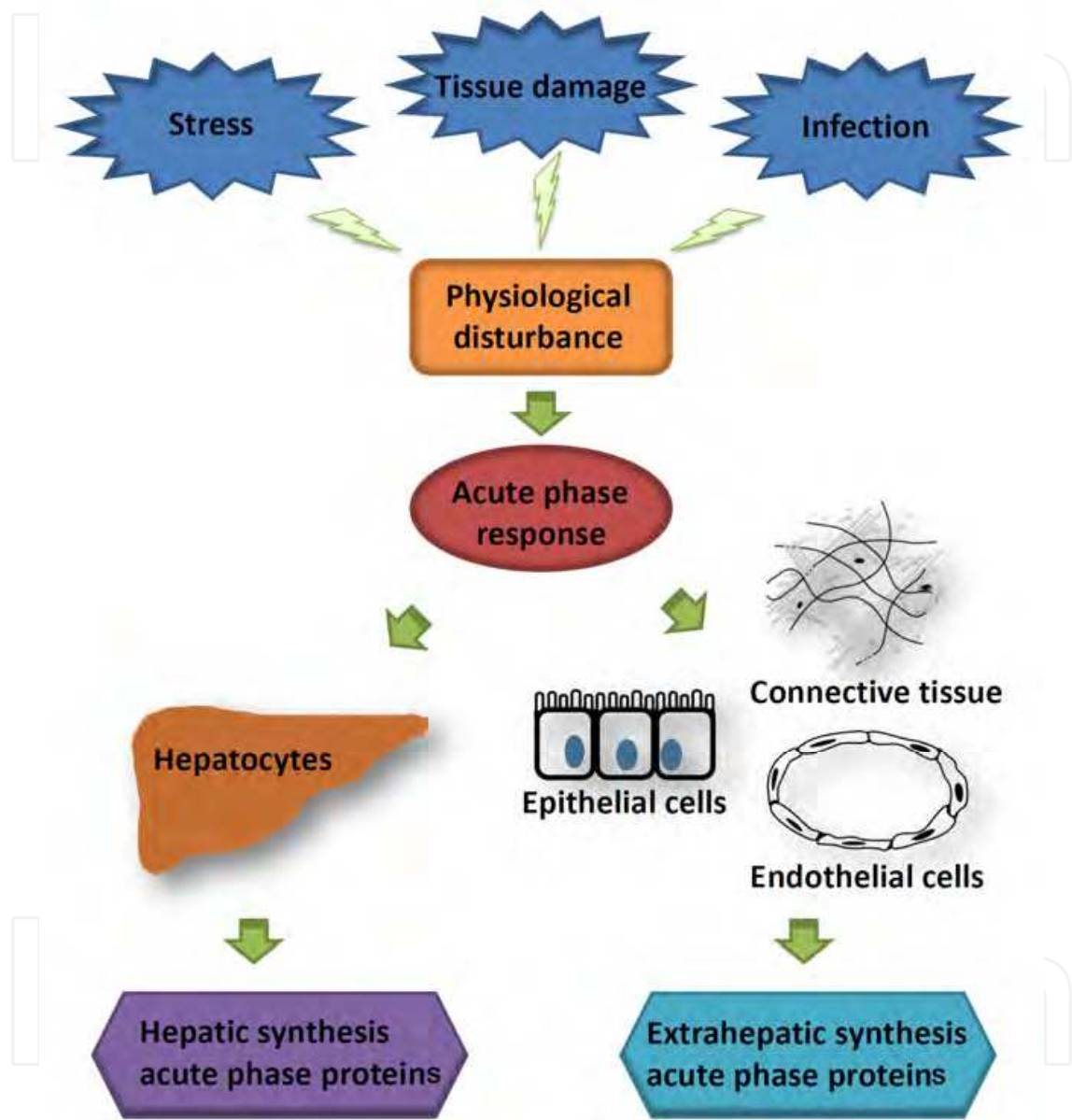

Fig. 2. The summary of acute phase protein production.

The three most common APPs are C-reactive protein (CRP), serum amyloid A (SAA) and serum amyloid P (SAP). Many other APPs have been found, and they all play important roles. According to the report (7), the APPs are stimulated by the release of cytokines such as IL-6 (Fig. 1), which is induced locally and systemically. APPs have showed a correlation with markers of oxidative stress (8). If the tissue damage stimulation has repeated pulses, the acute-phase reaction can become chronic. The chronic inflammation can continuously provide the increasing serum APPs. Some APPs would be expressed in the liver, stimulated

by injury; APP expression might also be a promoter of a benign or malignant tumor, such as SAA in ovarian tumors, and may trigger the tissue disorder. Acute-phase SAA is synthesized in the liver; extrahepatic production of SAA has been observed in several mammalian species (9). Based on these findings, the production of APPs can be summarized as in Fig. 2.

In addition to the production of acute-phase protein, other physical responses would happen during the acute-phase response. They are listed in Table 1 (Table is from ref.10). Acute-phase phenomena may be included in a large number of behavioral, physiological, biological and nutritional changes. APPs can be induced under all of these phenomena.

Neuroendocrine changes
    Fever, somnolence, and anorexia
    Increased secretion of corticotropin-releasing hormone,
        corticotropin, and cortisol
    Increased secretion of arginine vasopressin
    Decreased production of insulin-like growth factor I
    Increased adrenal secretion of catecholamines
Hematopoietic changes
    Anemia of chronic disease
    Leukocytosis
    Thrombocytosis
Metabolic changes
    Loss of muscle and negative nitrogen balance
    Decreased gluconeogenesis
    Osteoporosis
    Increased hepatic lipogenesis
    Increased lipolysis in adipose tissue
    Decreased lipoprotein lipase activity in muscle and adipose
        tissue
    Cachexia
Hepatic changes
    Increased metallothionein, inducible nitric oxide synthase,
        heme oxygenase, manganese superoxide dismutase, and
        tissue inhibitor of metalloproteinase-1
    Decreased phosphoenolpyruvate carboxykinase activity
Changes in nonprotein plasma constituents
    Hypozincemia, hypoferremia, and hypercupremia
    Increased plasma retinol and glutathione concentrations

Table 1. Other acute phase phenomena

2. Acute-phase proteins (APPs)

Currently, numerous proteins are considered APPs, human APPs are listed in Table 2 (10). (Table 2 is from ref.10). The proteins can serve as inhibitors or mediators of the inflammatory processes. These proteins are a large and varied group of glycoproteins that would appear in the bloodstream and would be unrelated to immunoglobulin being responsive to inflammatory reaction (11).

Proteins whose plasma concentrations increase

Complement system
   C3
   C4
   C9
   Factor B
   C1 inhibitor
   C4b-binding protein
   Mannose-binding lectin
Coagulation and fibrinolytic system
   Fibrinogen
   Plasminogen
   Tissue plasminogen activator
   Urokinase
   Protein S
   Vitronectin
   Plasminogen-activator inhibitor 1
Antiproteases
   α_1-Protease inhibitor
   α_1-Antichymotrypsin
   Pancreatic secretory trypsin inhibitor
   Inter-α-trypsin inhibitors
Transport proteins
   Ceruloplasmin
   Haptoglobin
   Hemopexin
Participants in inflammatory responses
   Secreted phospholipase A_2
   Lipopolysaccharide-binding protein
   Interleukin-1–receptor antagonist
   Granulocyte colony-stimulating factor
Others
   C-reactive protein
   Serum amyloid A
   α_1-Acid glycoprotein
   Fibronectin
   Ferritin
   Angiotensinogen

Proteins whose plasma concentrations decrease

Albumin
Transferrin
Transthyretin
α_2-HS glycoprotein
Alpha-fetoprotein
Thyroxine-binding globulin
Insulin-like growth factor I
Factor XII

Table 2. Human acute phase proteins

2.1 Classification of acute-phase proteins (APPs)

Based on the protein concentration in plasma, APPs can be divided into two classes.

2.1.1 Negative acute-phase protein

APPs are produced by the liver and have an increased concentration in the serum. When APP concentration in the serum is decreased, the APPs are called negative APPs. Albumin, transferrin, transthyretin and retinol-binding protein (vitamin A binding protein, RBP) have been found as negative APPs (12). In chronic inflammation in humans, especially in developing countries, vitamin A deficiency is serious (13, 14). It is well-known to have a negative feedback effect on immunity. As this stress, nutrient deficiency, RBP would be reduced.

2.1.2 Positive acute-phase proteins

When APP concentration in the serum increases, the APPs are called positive APPs. They include such proteins as CRP, mannose-binding protein, α-1 antitrypsin, etc, as listed in Table 3 (Table is from ref.5). The overall changed APP concentration in the serum includes negative and positive APPs. The APP pattern may vary from one species to another. Serum amyloid p-component (SAP) is an APP in mice but not in humans. Age may also be a factor. For example, some APPs exist in the infant stage normally, but they may not be found in adults. Furthermore, some APPs would be increased in some species but decreased in others. Transferrin is a negative APP in most mammalian species, but it is a positive APP in chickens (15). According to the report of González et al., (16), they mentioned that the serum albumin would be decreased significantly at 48h and a lot of APPs would be increased, such as fibrinogen, SAA and Hp etc. However, there has no evidence to conclude the correlation between albumin and induction of positive APPs.

Mammals	Birds
Positive reactants	
TNF-α, IL-1, IL-6, cortisol	TNF-α, IL-1, IL-6, cortisol
SAA, CRP, Hp, AGP, etc.	SAA, CRP, hemopexin, AGP, etc.
Fibrinogen, Ceruloplasmin	Fibrinogen, Transferrin, Ceruloplasmin
Cu	Cu, Ca
Negative reactants	
TTR, RBP	Hp
Albumin, Transferrin	Albumin
Fe, Zn, Ca	Unbound serum iron, Zn

TNF: Tumour necrosis factor; IL: Interleukin; SAA: Serum amyloid A; CRP: C-reactive protein; Hp: Haptoglobin; AGP: α1-acid glycoprotein; Cu: Copper; Ca: Calcium; TTR: Transthyretin; RBP: Retinol binding protein; Fe: Iron Zn: Zinc

Table 3. Major positive and negative acute phase reactants in mammals and birds.

2.2 The function of APPs

The function of APPs has not been completely clarified. In general, the positive APPs serve different physiological functions in the immune system and in regulating and trapping

infected microorganisms and their products. In addition, the alteration of APP production can serve a useful purpose in inflammation, healing to injury or adaptation to infection. For example, the concentration of C-reactive protein (CRP) in serum rose significantly under acute-phase reaction; it is referred to as an acute-phase protein. It can participate in inflammatory response by inducing production of inflammatory cytokines and display anti-inflammatory effects (17). CRP, characterized as a calcium-dependent binding to various substrates, such as DNA or cellular proteins, increases dramatically in response to tissue-destructive processes (18). Currently, CRP has been found in direct stimulation of angiogenesis and may play a role in vessel formation (19). It indicates that APPs have multiple functions in the biosystem.

2.3 Regulation of APPs

Acute-phase reaction (APR) is a systemic response to injury and/or infection associated with endocrine and metabolic changes, including alteration of behavior, body temperature, production of cytokines and induction of APPs. Inflammatory cytokines, produced by inflammatory cells, are induced locally and systemically. The inflammatory mediators activate many cells and trigger a systemic release of cytokines (as pro-inflammatory cytokines). The increased serum-cytokines would result in the production of APPs. IL-1, TNF, INF-α are major and important cytokines for the expression of inflammatory mediators to induce the production of cytokines from the liver, such as IL-6. It is the major mediator of liver secretion of many APPs. In mammals, APR activities are enhanced indirectly by the activation of the pituitary (Fig. 1). As the APR occurring, increase of the glucocorticoids is a result of cytokine secretion from the pituitary. The human acute phase serum amyloid A (SAA), a positive acute phase protein, was up-regulated significantly after stimulation by glucocorticoid and cytokine in human hepatoma cells (20). Besides, many non-hepatic cell types including monocytes, endothelial cells and others have been shown to express SAA in high levels when stimulated with cytokines and glucocorticoids (21). This indicates that cytokines and glucocorticoids regulate the synthesis of APPs (22). Glucocorticoid can be a positive and negative regulator of APP synthesis during APR (Scheme 1 is from ref.22).

To our knowledge, cytokines are released from an injury or pathogen's infection site. The proinflammatory cytokines, TNF, IL-1 and IL-6, are considered the primary mediators of the APR. Upon injury or infection, macrophage or circulating monocytes are activated; they release cytokines locally and stimulate the liver to synthesize the APPs. At the same time, these early responsive pro-inflammatory cytokines can also activate the hypothalamus/adrenal cortex and induce fever and synthesis of glucocorticoids. Glucocorticoids have been proved to enhance the cytokine-dependent APPs' synthesis in the liver during APR. Numerous studies have provided evidences that glucocorticoids clearly show positive effects on APP synthesis during APR by way of a synergistic enhancement of pro-inflammatory cytokine effects (23–25).

APPs are almost glycosylated and change with their structure of side sugar chain during different inflammatory processes (11). Glycosylation changes in APPs could be markers of disease. As an example, α-1-acid glycoprotein (AGP) is an APP and has expressed more sialyl Lewis X (SLe^x) linkages in pancreatic cancer (PaC) tissue than in inflamed pancreatic tissue; however, the SLe^x linkages were barely detected in healthy pancreatic tissue (26). This reveals that the glycosylation of APPs can regulate the function of APPs.
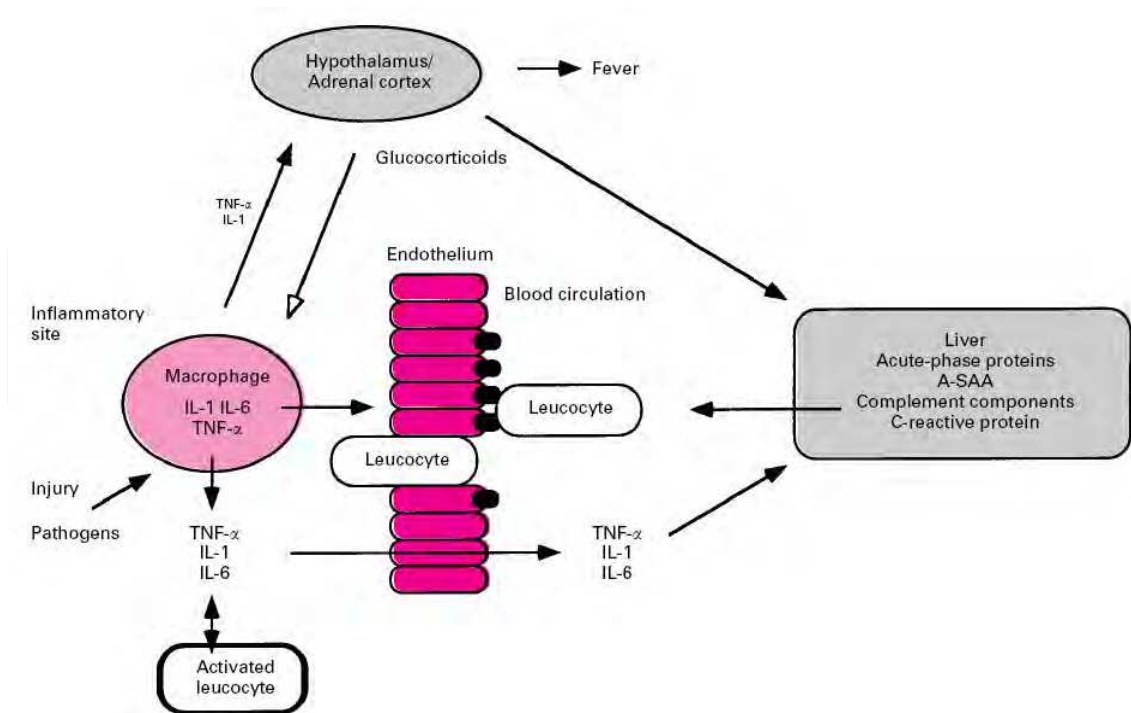
Scheme 1. Activation of the AP response
The cytokine-mediated network linking the inflammatory site and target organs/tissues is shown. Following exposure to the stimulating agent, cytokines produced by macrophages act on neighbouring cells, e.g. leucocytes (monocytes, neutrophils and lymphocytes) and endothelial cells, in the vicinity of the inflamed sites. These cells themselves become activated and express additional cytokines and receptors. Endothelial cells express selectins (●) which recruit circulating leucocytes and platelets from the bloodstream. Cytokines released to the circulation induce the hepatic AP response, which involves the increased synthesis of AP proteins e.g. A-SAA, C-reactive protein and complement components. Stimulation of the central nenrous system by cytokines induces fever and the synthesis of glucocorticoids by the adrenal cortex. Glucoconicoids enhance the hepatic AP response and at the same time feed back to down-regulate the local inflammation (open arrow) and its systemic inflammatory consequences.

3. Acute phase index

During the APR, there is an increase in APPs (positive APPs) and there are decreases in some APPs (negative APPs); the quantification of these proteins provides valuable clinical information in diagnosis and treatment. Numerous researchers have developed the method in animals to assess-nonhealthy animals versus healthy ones, and the calculating index is called the "acute phase index" (6, 27).

$$\text{Nutritional and acute phase index (NAPI)} = \frac{\text{Value of a rapid positive APP} \times \text{Value of a slow positive APP}}{\text{Value of a rapid negative APP} \times \text{Value of a slow negative APP}}$$

(Eq. is from ref.6)

In general, the calculated indexes were significantly higher in nonhealthy animals than in normal ones, and decreased indexes were observed after treatment. For calculating the indexes, the ratio between positive and negative acute-phase proteins has to the determined. If the ratio is combined with those of rapid and slow changes in positive and negative APPs, the acute-phase signal can be enhanced. The index has been used as a prognostic inflammatory for human patients, who might also have a nutritional deficiency. Determination of the index with several APPs can help in monitoring the health of individual subjects.

Before calculating the index, the protein concentrations have been measured quantitatively. Many technologies, such as the protein chip, the protein microarray method or quantitative polymerase chain reaction (qPCR), can be used for APP measurement. These technological developments should have crucial importance in future diagnostics.

4. Acute-phase proteins related to oxidative stress

The reactive oxygen species (ROS) in the environment indicates the stress status. It has been implicated in inflammation. Elevation of ROS induces gene expression, which is involved in inflammatory and acute-phase responses (28, 29). It indicates the stress will trigger the APR and the occurrence of APPs synthesis. For example, patients with pressure sores present a systemic inflammatory response associated with the decrease of ascorbic acid levels, suggesting that the patient may be nutritionally deficient (30). Nutritional deficiency is a kind of stress *in vivo* and triggers the elevation of the ROS level. The high ROS level *in vivo*, known as an acute-phase status, includes alternations of APPs and is related to cell apoptosis. These may contribute to the development of pressure sores in patients and may impede the wound-healing process.

Infections are also associated with elevating the intracellular oxidative stress via the reaction of proinflammatory cytokines. The bactericidal factors and the inflammatory response confer oxidative stress to the cells that may lead to cell apoptosis. This means APR was developed by infections and initiated an increase in oxidative stress and possibly triggered cell apoptosis via APPs (31). For example, LCN2 could induce the increase of intracellular ROS significantly within a short time, upon the protein interaction with the cells and suppression by the ROS inhibitor (Fig. 3) (32).

This protein has been described as being mainly expressed in tissues that may be exposed to microorganisms (33), or detected in acute inflammatory response (34) in keeping with these conditions' stress status. Cowland *et al.* (35) showed that during lung inflammation, the human LCN2 protein (also called Neutrophil Gelatinase Associated Lipocalin; NGAL or human 24p3 protein) synthesis increases in the bronchial epithelial cells, so it was considered as a disease activity marker (36, 37). All of these imply that the LCN2 protein correlates with environmental stress and tissue damage. In cultured cells, the LCN2 protein increment is observed in response to glucocorticoid stimulation, and also in other conditions such as serum deprivation. Elevating the glucocorticoid level in circulation during stress may trigger the LCN2 protein being highly expressed under this stress condition and exert an autocrine control (38) during stress, thus playing a role in cell death (39). Over the years, ROS has been perceived as a biological hazard, causing oxidative damage to the cellular components, and leading to cancer, cell degeneration and disorders related to aging (40).

Based on the descriptions, APPs may initiate the aberrant cell growth under stress conditions.

In general, we have known that cytokines are stimulators of most APPs' synthesis during APR. At the same time, APP is also a stimulator of cytokine production as cells are responsive to the APP. Exposure of endometrial carcinoma cell line (RL95-2) to LCN2 for >24 h reduced LCN2–induced cell apoptosis, changed the cell proliferation and up-regulated cytokine secretions, including: interleukin-8 (IL-8), inteleukin-6 (IL-6), monocyte chemotatic protein-1 (MCP-1) and growth-related oncogene (GRO) (Fig. 4) (41). These cytokines may change the growth of the cells.
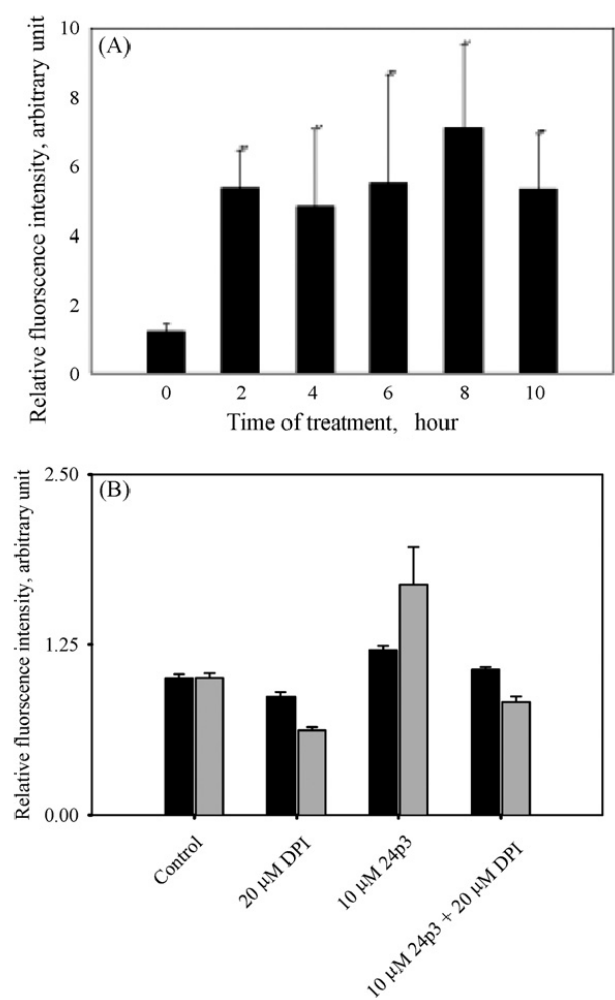

Fig. 3. Induction of ROS by 24p3 protein (also called mouse LCN2) treatment. RL95-2 cells were treated with 5μM 24p3 protein for various time intervals, followed by incubation with 20μM DCFH-DA for 15 min. The amount of intracellular ROS can be quantified by detection via a microfluorometer with excitation and emission wavelengths at 485 and 535 nm, respectively. The results were confirmed in multiple experiments and presented as the mean±S.D. (**) $p < 0.01$, (***) $p < 0.001$, $n = 5$. (A) The time course of 24p3 protein effect on RL95-2 cells. (B) The DPI prevents the 24p3 protein from inducing ROS in RL95-2 cells. The black bar indicates the 30-min incubation and the gray bar indicates 60-min incubation of 24p3 protein with the cells.
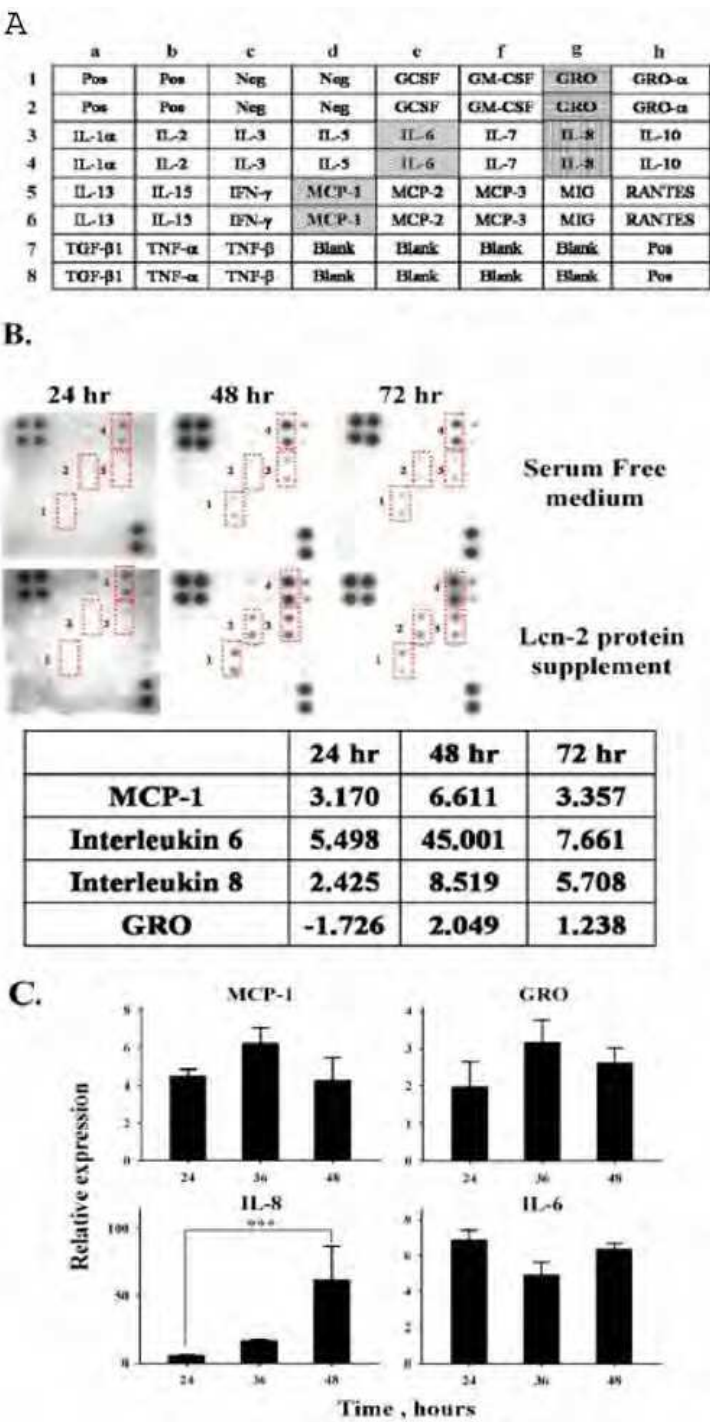
A

	a	b	c	d	e	f	g	h
1	Pos	Pos	Neg	Neg	GCSF	GM-CSF	GRO	GRO-α
2	Pos	Pos	Neg	Neg	GCSF	GM-CSF	GRO	GRO-α
3	IL-1α	IL-2	IL-3	IL-5	IL-6	IL-7	IL-8	IL-10
4	IL-1α	IL-2	IL-3	IL-5	IL-6	IL-7	IL-8	IL-10
5	IL-13	IL-15	IFN-γ	MCP-1	MCP-2	MCP-3	MIG	RANTES
6	IL-13	IL-15	IFN-γ	MCP-1	MCP-2	MCP-3	MIG	RANTES
7	TGF-β1	TNF-α	TNF-β	Blank	Blank	Blank	Blank	Pos
8	TGF-β1	TNF-α	TNF-β	Blank	Blank	Blank	Blank	Pos

B.

	24 hr	48 hr	72 hr
MCP-1	3.170	6.611	3.357
Interleukin 6	5.498	45.001	7.661
Interleukin 8	2.425	8.519	5.708
GRO	-1.726	2.049	1.238

C.

Fig. 4. **Cytokine array analysis of conditioned medium from Lcn-2-treated RL95-2 cells.**
(A) RayBio® Human Cytokine Antibody Array I Map. (B) The signals of cytokine concentrations in conditioned media; significant signals are labeled with Arabic numerals: 1, MCP-1; 2, IL-6; 3, IL-8; and 4, GRO. Relative intensities of these four cytokines are shown in the lower panels with the intensities of values from control serum-free media set as 1. (C) mRNA levels of cytokines in Lcn-2-treated cells. A total of 4×10⁴ cells were incubated with or without 10 µM Lcn-2 in serum-free medium for 24, 36 and 48 h. After incubation, the total RNA isolated from cells was reverse transcribed and amplified by RT-PCR using primers for MCP-1, IL-6, IL-8 and GRO. Levels of mRNA were determined by semi-quantitative RT-PCR.

However, IL-8 mRNA and protein levels were dramatically increased in LCN2–treated RL95-2 cells. The major focus was to determine the IL-8 effect on LCN2-treated RL95-2 cells. Adding recombinant IL-8 (rIL-8) resulted in decreased caspase-3 activity in LCN2–treated cells, whereas the addition of IL-8 antibodies resulted in significantly increased caspase-3 activity and decreased cell migration. Data indicate that IL-8 plays a crucial role in the induction of cell migration (Fig. 5) (41).
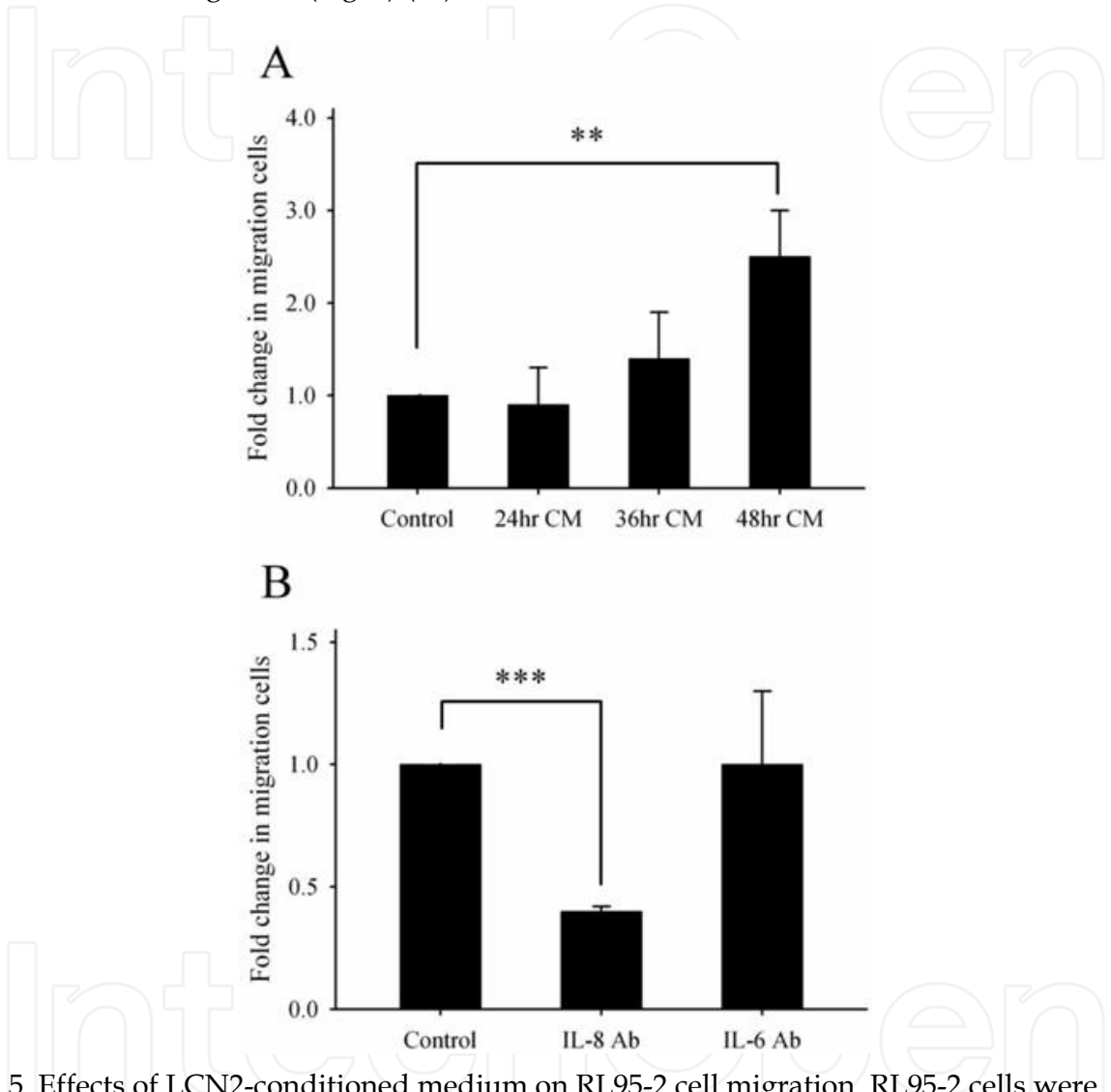

Fig. 5. Effects of LCN2-conditioned medium on RL95-2 cell migration. RL95-2 cells were stimulated with LCN2 for varying lengths of time, and then culture supernatants were collected from conditioned medium. Cytokine antibodies (anti-IL-8 or anti-IL-6) were added to clarify the effect of these cytokines on 48 h CM-induced cell migration. Cell migration was measured using the transwell assay in the absence or presence of conditioned medium. (A) After incubation for 24 h, RL95-2 cells were stained with 0.5% crystal violet, and cells were counted in ten random fields under a microscope at X20 magnification. (B) OD570 values of cells on the lower surface of the membrane extracted with 33% acetic acid after anti-IL-8 neutralization. These data are representative of three independent experiments. The cell number of RL95-2 incubation under 48 h CM for 24 h is as a control experiment. Values are the mean ± SEM. **, P < 0.01; ***, P < 0.001.

Interestingly, LCN2-induced cytokines, secretion from RL95-2 cells, could not show the potent cell migration ability with the exception of IL-8. We concluded that LCN2 triggered cytokine secretions to prevent RL95-2 cells from undergoing apoptosis and subsequently increased cell migration. We hypothesize that LCN-2 increased cytokine secretion by RL95-2 cells, which in turn activated a cellular defense system. This means the APP would secrete from the cell under stress and then promote the secretion of cytokines and enhance cell growth.

5. Acute-phase proteins related to cytokines

Furthermore, tumorigenesis and the invasion capacity of tumor cells are medicated by growth factors, including cytokines, which promote cell proliferation, including the invasion of tumor cells. Cytokines are a part of homeostasis, stress response, inflammation and tumorigenesis (42). IL-6 is known to be a proinflammatory cytokine. The elevation of IL-6 levels in patients with a Ras-induced cancer can trigger the other secreting cytokine, IL-8, an important factor for tumor growth in HeLa cells. It provided the information linking cytokines to tumorigenesis and also hinted at the linkage of APPs to tumorigenesis (43–46). IL-8 can be induced in endometrial cells by an acute-phase protein, LCN2, also an angiogenic factor in some cancers (47). According to the report of Arenberg *et al.*, (37) inhibition of IL-8 expression would reduce the tumor development of human lung cancer in mice. It was announced that the IL-8 plays role in mediating angiogenesis during tumorigenesis of human cancer. IL-8 thereby offers the potential to promote the formation of tumor (48). Based on the result of Lin *et al.* (41), LCN2 could induce IL-8 expression and secretion and enhance cell migration and invasion. This suggests IL-8 as an LCN2-induced tumor factor. It indicates that the LCN2-induced IL-8 secretion from uterine endometrial cells and promotes the cell migration therefore the result provides evidence for APP playing a role in tumorigenesis.

6. Acute-phase proteins as tumorigenic factors or diseases inducers

APPs may initiate tumor formation indirectly via induced cytokines, or/and directly by themselves. To our knowledge, serum amyloid A (SAA) is an APP; it has been found that it may contribute the role in directing and enhancing the tumor process. Especially, numerous studies on SAA had been focused on the tumor progression.

The acute-phase serum amyloid A proteins (SAAs) are multifunctional proteins that would be up-regulated by proinflammatory cytokines during inflammation, infection, trauma or stress. Several biological effects of SAA have been described in relation to inflammation, including cell adhesion, cell migration, tissue infiltration of inflammatory cells, enhancing matrix metalloproteinases (MMPs) increasing expression of cytokines, or stimulating angiogenesis. The liver is the main site for SAA synthesis; however, extra hepatic expression has been found in many normal human tissues (20). In more recent studies, SAA in serum levels were found elevated in a wide range of cancers. SAA is expressed locally in colon carcinoma and also overexpression in endometrial carcinoma and ovarian tumors (49). Breast cancer is one of the most common cancers in women worldwide. Finding the potential biomarkers to identify the types of breast cancer is an important work in progress. LCN2 is a newly identified biomarker for breast cancer; clarification of the possible

mechanisms underlying its role in tumorigenesis is ongoing. LCN2, originally an inflammatory marker in both adipose and liver tissue (50), can be induced by lipopolysaccharides, suggesting that LCN2 is an acute-phase protein as in previous mentioned. However, increased systemic LCN2 levels in several diseases have been reported, such as chronic renal failure, chronic inflammation and some cancers. This may reveal that APPs play multi-functions in the biological system.

6.1 Endometrial hyperplasia

Human LCN2 (NGAL) is also found in human endometrial hyperplasia, a uterine disorder disease. Up-regulation of NGAL protein and mRNA was much higher in endometrial hyperplasia than in adenomyosis. Endometrial carcinoma is more often associated with endometrial hyperplasia (55%) than with endometrial adenomyosis (16%) (51). It seems to
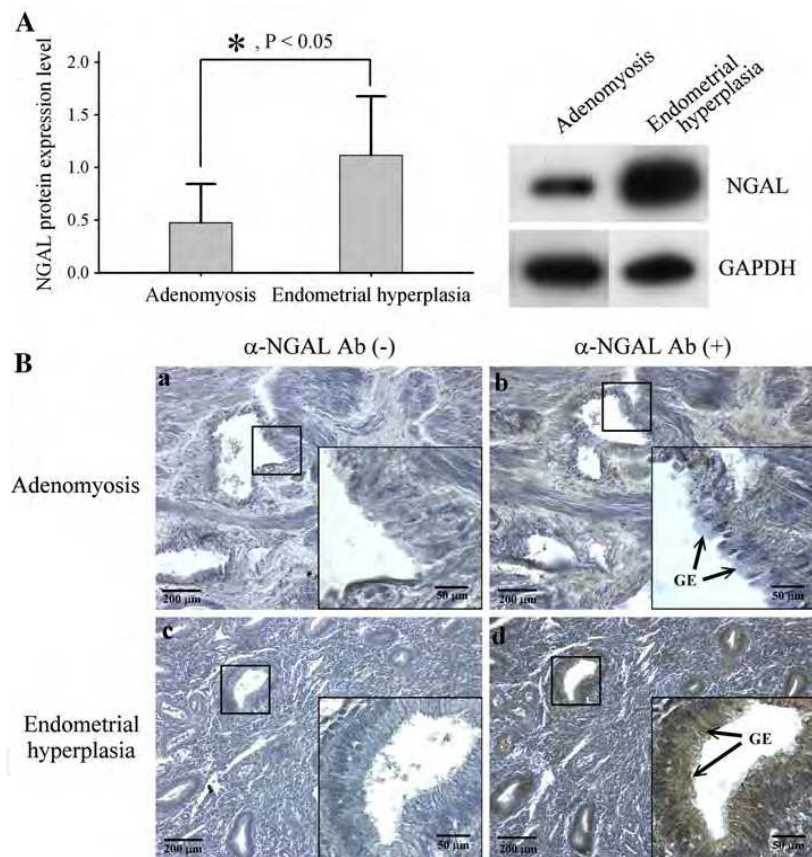

Fig. 6. Western blot analysis of NGAL in biopsy samples of endometrial disorders and immunohistochemical staining of endometrial adenomyosis and hyperplasia samples with anti-NGAL. A. Immunoblotting with anti- NGAL and anti-GAPDH. The data represent the mean ± SEM from all biopsies (eight adenomyosis samples and 27 hyperplasia samples), and were calculated using the ratio of NGAL to the internal control (GAPDH). The right panel shows the signal after immunoblotting. B. NGAL immunoreactivity was not observed in adenomyosis in the presence of the NGAL antibody (b). Similarly, no NGAL expression was observed in either tissue in the absence of NGAL antibody (a, c). However, in the presence of NGAL antibody, strong NGAL expression was observed as a light brown color in the cytoplasm of glandular epithelia (GE) in endometrial hyperplasia (arrows). The nuclei were stained with hematoxylin (blue). Magnification x100 and x400 (inset).

indicate that endometrial hyperplasia itself presents a higher risk for progressing to endometrial carcinoma compared to adenomyosis. Therefore, we asked whether the cancer marker NGAL was responsive to tumorigenic transformation of endometrial hyperplasia and if it was expressed at high levels. Immunohistochemical analysis revealed that NGAL expression in the glandular epithelia was strongly elevated in endometrial hyperplasia compared to adenomyosis (Fig. 6) (52). Some studies have shown that NGAL can be a marker for ovarian, breast, bladder, and pancreatic cancers, and that it is a survival factor for thyroid neoplastic cells (53–55).

Consistent with our findings, a significant increase in NGAL expression in endometrial hyperplasia may be a part of the tumorigenic process. Therefore, we propose an autocrine function for NGAL, which may play a role in uterine disorders or carcinomas. Previous studies have suggested that NGAL overexpression may be required for tumorigenesis by promoting tissue invasion (56). Based on these studies, it seems likely that NGAL is related to the transition from endometrial hyperplasia to endometrial carcinoma. The data showed that NGAL expression was significantly increased in endometrial hyperplasia compared to adenomyosis and correlated positively with COX-2 expression (r = 0.42). The increased COX-2 expression in hyperplasia may signify an early step in carcinogenesis. These uterine disorders may be inflammatory disorders and may trigger COX-2 gene expression. COX-2 is also important during tumorigenic transformation of hyperplasia (57, 58), where it decreases endometrial cell apoptosis and increases angiogenesis. It is the evidence for the correlation of APP with disease.

6.2 Endometriosis

Endometriosis, which usually develops in pelvic organs such as the ovaries and may contribute to infertility, is an estrogen-dependent disease characterized by the presence of endometrium-like tissue outside the uterine cavity (59). A relationship between ovarian endometriosis and certain types of ovarian cancer has been suggested, and endometriosis is believed to increase cancer risk. Endometriosis is similar to cancerous tumors in that it requires angiogenesis for expansion (60–62). Epithelial-mesenchymal transition (EMT) is a process whereby epithelial cells are converted to a mesenchymal phenotype and may be essential for the migration, invasion and relocalization of epithelial cells (59). EMT also can be induced by other signals, including the acute stress response (63). We hypothesized that EMT might be involved in the development of endometriosis. LCN2 is an oxidative stress factor that responds to environmental stress and triggers changes in cellular physiology. This signaling pathway may be activated under physiological as well as pathophysiological conditions (64). Cannito *et al.* found that intracellular ROS also are involved in the regulation of EMT (64), and Yanga *et al.* (65) found that LCN2 is associated with breast cancer progression *via* EMT. In addition, LCN2 also triggered cell migration and invasion (Fig. 7) (unpublished data), and this effect might contribute to the development of ectopic endometrial tissue implantation. Based on our evidence, we propose that LCN2 induces EMT in endometrial epithelial cells under nutrient-deprived conditions and thereby promotes the development of endometriosis.

In summary, during inflammation or stress, APPs can be induced by proinflammatory mediators and trigger the changes in cell physiological balance. Actually, cell apoptosis and cell proliferation are involved in the APPs triggered pro-inflammatory (cell apoptosis) or anti-inflammatory (wound-healing). According to Khatami's theory (66-68), acute phase

response is a highly regulated immune response to achieve a well-balance of cell death and cell growth in biological system; and indicates the APPs reaction is a kind of "Yin-Yang" doctrine. Therefore, the regulation of inflammatory response could initiate the challenge to the balance of tumoricidal versus tumorigenesis in immune system. The alteration of balance is considered as factor for causing the diseases; however, the regulatory pathways of APPs in disease formation remain unanswered. Future elucidation of the complex network of APPs, cytokines and pathological conditions is essential. The knowledge of APPs might be useful for providing an important method to monitor mammalian health.
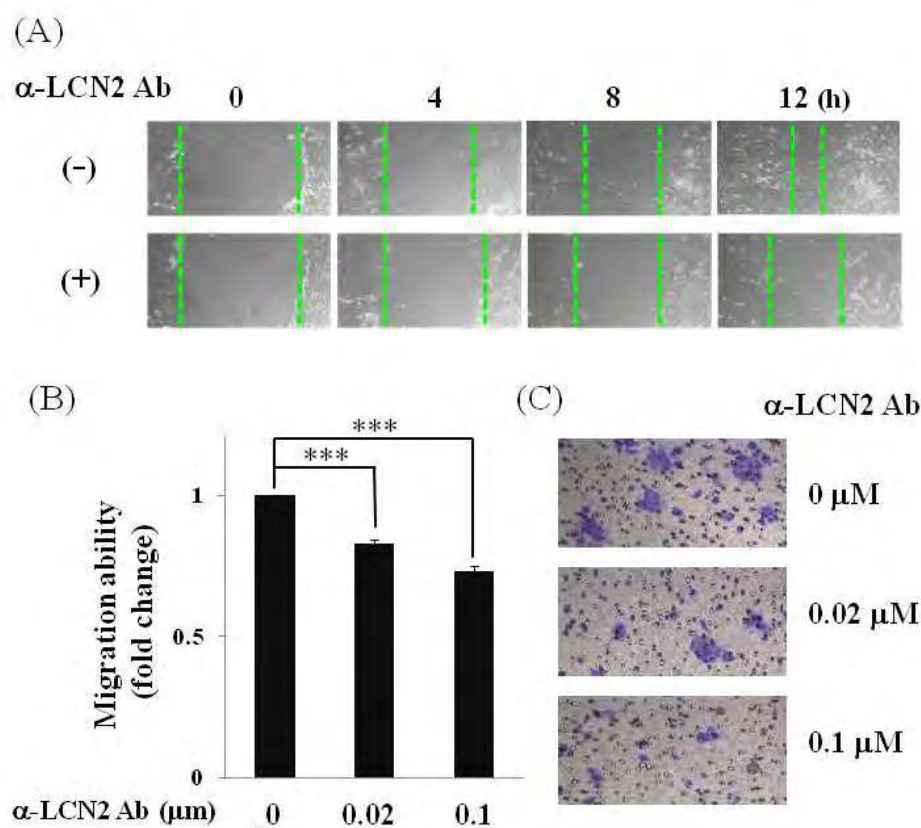
Fig. 7. Endometrial epithelial cell migration assays. Primary endometrial epithelial cells were harvested and then cultured in 1% FBS/DMEM/F12 medium for 48 h. The medium was collected and centrifuged to remove the suspended cells and cell debris and was used as a conditioned medium. Conditioned medium with or without 0.02 μM LCN2 antibody was used for wound-healing experiments and the Transwell assay. A, Wound-healing assay. Endometrial epithelial cells (5 × 10^4) were cultured in 24-well plates for 48 h until near confluence ($\sim$90%). A sterile 200-μl pipette tip was used to scratch through the cells to simulate a wound. After conditioned medium was added, the scratches were observed microscopically over a 12-h period. The green lines indicate the edge of each side of the scratch to show cell migration. B, C, Transwell assay. Endometrial epithelial cells (1 × 10^5) in 100 μl 1% FBS/DMEM/F12 medium were added to Transwell units. Conditioned medium (400 μl) was then added, and cells were allowed to migrate for 24 h in a 37°C, 5% CO_2 incubator. After incubation, the cells in the membrane insert were stained as described in the text and visualized (C); cells were then counted (B).

7. References

[1] Eckersall PD, Bell R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet J. 2010; 185(1): 23-7.

[2] Jahangiri A. High-density lipoprotein and the acute phase response. Curr Opin Endocrinol Diabetes Obes. 2010;17(2): 156-60.

[3] Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. Comp Med. 2009; 59(6): 517-26.

[4] Winsauer G, de Martin R. Resolution of inflammation: intracellular feedback loops in the endothelium. Thromb Haemost. 2007; 97(3): 364-9.

[5] Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B. 2005; 6(11): 1045-56.

[6] Jain S, Gautam V, Naseem S. Acute-phase proteins: as diagnostic tool. J Pharm Bioall Sci. 2011; 3(1): 118-27.

[7] Ceciliani F, Giordano A, Spagnolo V. The systemic reaction during inflammation: the acute-phase proteins. Prot Pept Lett. 2002; 9(3): 211-23.

[8] Mezzano D, Pais EO, Aranda E, Panes O, Downey P, Ortiz M, Tagle R, González F, Quiroga T, Caceres MS, Leighton F, Pereira J. Inflammation, not hyperhomocysteinemia, is related to oxidative stress and hemostatic and endothelial dysfunction in uremia. Kidney Int. 2001; 60(5): 1844-50.

[9] Upragarin N, Landman WJ, Gaastra W, Gruys E. Extrahepatic production of acute phase serum amyloid A. Histol Histopathol. 2005; 20(4): 1295-307.

[10] Gabay C, Kushner I. Acute phase proteins and other systemic response to inflammation. New England J Med. 1999; 340(6): 448-55.

[11] Kaimierczak MT, Sobieskab M, Wiktorowiczb K, Wysocki H. Changes of acute phase proteins glycosylation profile as a possible prognostic marker in myocardial infarction. Intl J Cardiol. 1995; 49: 201-7.

[12] Ingenbleek Y, Young V. Transthyretin (prealbumin) in health and disease: nutritional implications. Ann Rev Nutr. 1994; 14: 495-533.

[13] Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2000; 72: 1170-78.

[14] Baeten JM, Richardson BA, Bankson DD, Wener MH, Kreiss JK, Lavreys L, et al. Use of serum retinol-binding protein for prediction of vitamin A deficiency: effects of HIV-1 infection, protein malnutrition, and the acute phase response. Am J Clin Nutr. 2004; 79: 218-25.

[15] Hallquist NA, Klasing KC. Serotransferrin, ovotransferrin and metallothionein levels during an immune response in chickens. Comp Biochem Physiol Biochem Mol Biol. 1994; 108: 375-84.

[16] Feˊlix H. D. Gonzaˊ lez, Fernando Tecles, Silvia Martıˊnez-Subiela, Asta Tvarijonaviciute, Laura Soler Vasco, Joseˊ J. Ceroˊn Acute phase protein responses in goats. J Vet Diagn Invest. 2008; 20: 580-584.

[17] Xia D, Samols D. Transgenic mice expressing C-reactive protein are resistant to endotoxemia. Pro Natl Acad Sci USA. 1997; 94: 2575–2580.

[18] Du Clos TW, Marnell L, Zlock ' LR, Burlingame RW. Analysis of the binding of c-reactive protein to chromatin subunits. J Immunol. 1991; 146(4): 1220-25.

[19] Turu MM, Slevin M, Matou S, West D, Rodríguez C, Luque A, Grau-Olivares M, Badimon L, Martinez-Gonzalez J, Krupinski J. C-reactive protein exerts angiogenic effects on vascular endothelial cells and modulates associated signalling pathways and gene expression. BMC Cell Biol. 2008; 9: 47.

[20] Jensen LE, Whitehead AS. Regulation of serum amyloid A protein expression during the acute phase response. Biochem J. 1998; 334: 489-503.

[21] Thorn CF, Whitehead AS. Differential glucocorticoid enhancement of the cytokine-driven transcriptional activation of the human acute phase serum amyloid A genes, SAA1 and SAA2. J Immunol. 2002; 169: 399-406.

[22] Kumon Y, Suehiro T, Hashimoto K, Sipe JD. Dexamethasone, but not IL-1 alone, upregulates acute-phase serum amyloid A gene expression and production by cultured human aortic smooth muscle cells. Scand. J. Immunol. 2001; 53: 7-12.

[23] Baumann H, Jahreis GP, Morella KK, Wonf K-A, Pruitt SC, et al.Transcriptional regulation through cytokine andg lucocorticoid response elements of rat acute phase plasma proteing enes by C/EBP and JunB. J Biol Chem. 1991; 266(30): 20390-99.

[24] Ševaljević L, senović E, Vulović M, Mačvanin M, Žakula Z, Kanazir D, Ribarac-Stepić N. The responses of rat liver glucocorticoid receptors and genes for tyrosine aminotransferase, alpha-2-macroglobulin and gamma-fibrinogen to adrenalectomy-, dexamethasone- and inflammation-induced changes in the levels of glucocorticoids and proinflammatory cytokines. Biol Signals Recept. 2001; 10: 299-309.

[25] Yeager MP, Guyre PM, Munck AU. Glucocorticoid regulation of the inflammatory response to injury. Acta Anaesthesiol Scand. 2004; 48: 799-813.

[26] Sarrats A, Saldova R, Pla E, Fort E, Harvey DJ, Struwe WB, de Llorens R, Rudd PM, Peracaula R. Glycosylation of liver acute-phase proteins in pancreatic cancer and chronic pancreatitis. Proteomics Clin. Appl. 2010; 4: 432–48.

[27] Martinez-subiela S, Ceron JJ. Evaluation of acute phase protein indexes in dogs with leishmaniasis at diagnosis, during and after short-term treatment. Vet. Med. – Czech. 2005; 50(1): 39–46.

[28] Keyse SM, Emslie EA. Oxidative stress and heat shock induce a human gene encoding a protein-tyrosine phosphatase. EMBO J. 1991; 10(8): 2247-58.

[29] Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. Nature. 1992; 359(6396): 644-7.

[30] Cordeiro MBC, Antonelli ÉJ, da Cunha DF, Júnior AAJ, Júnior VR, Vannucchi H. Oxidative stress and acute-phase response in patients with pressure sores. Nutrition. 2005; 21: 901–7.

[31] Cossarizza A. Apoptosis and HIV infection: about molecules and genes. Curr Pharm Des. 2008;14(3): 237-44.

[32] Lin HH, Li WW, Lee YC, Chu ST. Apoptosis induced by uterine 24p3 protein in endometrial carcinoma cell line. Toxicology. 2007; 234(3): 203-15.

[33] Friedl A, Stoesz SP, Buckley P, Gould MN, Neutrophilgelatinase-associated lipocalin in normal and neoplastic human tissues. Cell type-specific pattern of expression. Histochem J. 1999; 31: 433–41.

[34] Xu SY, Pauksen K,Venge P. Serum measurements of human neutrophil lipocalin (HNL) discriminate between acute bacterial and viral infection. Scand. J Clin Lab Invest. 1995; 55: 125–31.

[35] Cowland JB, Sørensen DE, Sehested M, Borregaard N. Neutrophil Gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 but not by TNF-α. J Immnunol.2003; 171: 6630–39.

[36] Kjeldsen L, Cowland JD, Borregaard N. Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse. Biochem Biophys Acta. 2000; 1482: 272–83.

[37] Hemdahl A-L, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, Thor´en P, Hansson GK. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler. Thromb. Vasc. Biol. 2006; 26:136–42.

[38] Bigsby RM. Progesterone and dexamethasone inhibition of estrogen-induced synthesis of DNA and complement in rat uterine epithelium: effects of antiprogesterone compounds. J Steroid Biochem Mol Biol. 1993; 45: 295–301.

[39] Sivridis E, Giatromanolaki A. New insights into the normal menstrual cycle-regulatory molecules. Histol Histopathol. 2004; 19: 511–6.

[40] Molavi B, Mehta JL. Oxidative stress in cardiovascular disease: molecular basis of its deleterious effects, its detection, and therapeutic considerations. Curr Opin Cardiol. 2004; 10: 387–99.

[41] Lin HH, Liao CJ, Lee YC, Hu KH, Meng HW, Chu ST. Lipocalin-2-induced cytokine production enhances endometrial carcinoma cell survival and migration. Int J Biol Sci. 2011; 7(1): 74-86.

[42] Campbell IL. Cytokine-mediated inflammation, tumorigenesis, and disease-associated JAK/STAT/SOCS signaling circuits in the CNS. Brain Research Reviews. 2005; 48: 166– 177.

[43] Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM. Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. J Clin Invest. 1996; 97: 2792-2802.

[44] Chen Z, Malhotra PS, Thomas GR, Ondrey FG, Duffey DC, Smith CW, et al. Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. 1999; 5: 1369–79.

[45] Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, M. Balbay D, et al. Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. Clin Can Res. 2000; 6: 2104–2119.

[46] Ancrile B, Lim K-H, Counter CM. Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Gen Devel.2007; 21: 1714–19.

[47] CatalánV, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Silva C, Rotellar F, et al. Up-regulation of the novel proinflammatory adipokines lipocalin-2, chitinase-3 like-1 and osteopontin as well as angiogenic-related factors in visceral adipose tissue of patients with colon cancer. J Nutr Biochem. 2011; 22: 634-41.

[48] Ancrile BB, O'Hayer KM, Counter CM. Oncogenic Ras–Induced Expression of Cytokines: A New Target of Anti-Cancer Therapeutics. Mol Intervent. 2008; 8(1): 22-7.

[49] Urieli-Shoval S, Finci-Yeheskel Z, Dishon S, Galinsky D, Linke RP, Ariel I,Levin M, et al. Expression of serum amyloid A in human ovarian epithelial tumors: implication for a role in ovarian tumorigenesis. J Histochem Cytochem.2010; 58(11): 1015–23.

[50] Leng X, Wu Y, Arlinghaus RB. Relationships of lipocalin 2 with breast tumorigenesis and metastasis. J Cell Physiol. 2011; 226: 309–314.

[51] Boruban MC, Altundag K, Kilic GS, Blankstein J. From endometrial hyperplasia to endometrial cancer: insight into the biology and possible medical preventive measure. Eur J Can Prev. 2008; 17: 133–8.

[52] Liao C-J, - Huang YH, Au H-K, Wang L-M, Chu ST. The cancer marker neutrophil gelatinase-associated lipocalin is highly expressed in human endometrial hyperplasia. Mol Biol Rep. 2011; 15 May.

[53] Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N, Friedl A. Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. Breast Cancer Res Treat. 2008; 108: 389–97.

[54] Lim R, Ahmed N, Borregaard N, Riley C, Wafai R, Thompson EW, Quinn MA, Rice GE. Neutrophil gelatinase-associated lipocalin (NGAL) an early-screening biomarker for ovarian cancer:NGAL is associated with epidermal growth factor-induced epithelial-mesenchymal transition. Int J Cancer. 2007; 120: 2426–34.

[55] Iannetti A, Pacifico F, Acquaviva R, Lavorgna A, Crescenzi E, Vascotto C, etal. The neutrophil gelatinase-associated lipocalin (NGAL), a NF-kappaB-regulated gene, is a survival factor for thyroid neoplastic cells. Proc Natl Acad Sci USA. 2008; 105: 14058–63.

[56] Arlinghaus R, Leng X. Requirement of lipocalin 2 for chronic myeloid leukemia. Leuk Lymphoma. 2008; 49: 600–3.

[57] Fosslein E. Molecular pathology of cyclooxygenase-2 in cancer-induced angiogenesis. Ann Clin Lab Sci. 2001; 31: 325–48.

[58] Bakhle YS. COX-2 and cancer: a new approach to an old problem. Brit J Pharmacol. 2001; 134: 1137–50.

[59] Bulun SE. Endometriosis. N Engl J Med. 2009; 360: 268–79.

[60] Somigliana E, Vigano P, Parazzini F, Stoppelli S, Giambattista E, Vercellini P. Association between endometriosis and cancer: a comprehensive review and a critical analysis of clinical and epidemiological evidence. Gynecol Oncol. 2006; 101: 331–41.

[61] Melin A, Lundholm C, Malki N, Swahn ML, Sparen P, Bergqvist A. Endometriosis as a prognostic factor for cancer survival. Int J Cancer. 2010, DOI: 10.1002/ijc.25718

[62] Taylor RN, Lebovic DI, Mueller MD. Angiogenic factors in endometriosis. Ann NY Acad Sci. 2002; 955: 89–100.

[63] Vargha R, Bender TO, Rieenhuber A, Endemann M, Kratochwill K, Aufricht C. Effects of epithelial to mesenchymal transition on acute stress response in human peritoneal mesothelial cells. Nephrol Dial Transplant. 2008; 23: 3494–500.

[64] Cannito S, Novo E, Di Bonzo LV, Busletta C, Colombatto S, Parola M. Epithelial–mesenchymal transition: from molecular mechanisms, redox regulation to implications in human health and disease. Antioxid Redox signal. 2010; 12: 1383–430.

[65] Yanga J, Bielenberg DR, Rodigd SJ, Doiron R, Cliftone MC, Kungf AL, Stronge RK et al. Lipocalin 2 promotes breast cancer progression. Proc Natl Acad Sci. 2009; 106: 3913–18.

[66] Khatami M. Yin and Yang' in inflammation: duality in innate immune cell function and tumorigenesis. Expert Opin Biol Ther. 2008; 8: 1461-72.

[67] Khatami M. Inflammation, aging, and cancer: tumoricidal versus tumorigenesis of immunity: a common denominator mapping chronic diseases. Cell Biochem Biophys. 2009; 55: 55-79.

[68] Khatami M. Unresolved inflammation: 'immune tsunami' or erosion of integrity in immune-privileged and immune-responsive tissues and acute and chronic inflammatory diseases or cancer. Expert Opin Biol Ther. 2011; Jun 11.
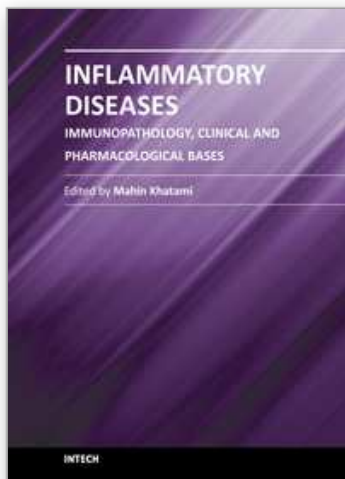
Inflammatory Diseases - Immunopathology, Clinical and Pharmacological Bases
Edited by Dr Mahin Khatami

ISBN 978-953-307-911-0
Hard cover, 396 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

This book is a collection of comprehensive reviews contributed by experts in the diverse fields of acute and chronic inflammatory diseases, with emphasis on current pharmacological and diagnostic options. Interested professionals are also encouraged to review the contributions made by experts in a second related book entitled "Inflammation, Chronic Diseases and Cancer"; it deals with immunobiology, clinical reviews, and perspectives of the mechanisms of immune inflammatory responses that are involved in alterations of immune dynamics during the genesis, progression and manifestation of a number of inflammatory diseases and cancers, as well as perspectives for diagnosis, and treatment or prevention of these disabling and potentially preventable diseases, particularly for the growing population of older adults around the globe.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sin Tak Chu and Ying Chu Lee (2012). Characterization of Acute-Phase Proteins (Apps), Inflammatory Diseases - Immunopathology, Clinical and Pharmacological Bases, Dr Mahin Khatami (Ed.), ISBN: 978-953-307-911-0, InTech, Available from: http://www.intechopen.com/books/inflammatory-diseases-immunopathology-clinical-and-pharmacological-bases/characterizaton-of-acute-phase-proteins-apps-

INTECH
open science | open minds