CHAPTER 1

Introduction

1.1 Historical Background

Cancer is a condition where uncontrollable cells growth severely affects human health around the world. There are several multi-step processes that are involved in cancer progression, transformation, cancer cell growth, invasion and metastasis. However, the most deleterious stage is invasion and metastasis. Additionally, cancer cell invasion and metastasis are the serious steps and the most aggressive phenotype of cancers patients. The ability of cancerous cells to metastasize in the body is responsible for approximately 90% of cancer patients who ultimately die from metastasis (1, 2).

There are several major steps involved in cancer metastasis: migration, intravasation, transport, extravasation, and metastatic colonization. A key event in cancer metastasis is the capability of the empowered cancer cells to go through the blood circulation (3). The metastasis process is entailed on cells adhesion to the outside of the vascular basement membrane, local degradation of extracellular matrix (ECM), and migration through the damaged basement membrane and enter to the blood circulation (4). It also requires proteolytic enzymes to degrade extracellular matrix and assists the migration including serine/cysteine proteases, urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMPs) (5).

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The uPA/uPAR system is an important system in tumor metastasis. The high expression of uPA has been detected in various kinds of tumors, for instance, breast, ovarian, prostate and gastric cancers. This system is concerned with poor prognosis in many cancers (6). The uPA system composes of uPA, uPA receptor and plasminogen inhibitor type 1 and type 2 (PAI-1 and -2). After uPA binds with its uPAR, active uPA can convert inactive plasminogen to active plasmin, which plays an essential role in ECM degradation.

Additionally, uPA/uPAR complex also activates the signal transduction pathways leading to increased cancer cells migration (7-9).

MMPs are a group of zinc-dependent endopeptidases and causes degradation of ECM components (10). These enzymes are necessary in many physiological conditions for example, tissue development, tissue regeneration, and wound repair. In contrast, they are also involved in pathologic conditions such as rheumatoid arthritis, osteoarthritis, and atherosclerosis and tumor progression. MMPs are not only involved in cancer metastasis but also involved in cancer angiogenesis, growth and survival (11, 12). The correlation of tumor progression and the overexpression of MMP family (MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-11 and MT1-MMP) has been studied in animal and clinical studies (13-15). For instance, increased MMP levels have been correlated with increased tumor aggressiveness (16).

Normal healthy cells generally express very low level of MMPs; however, there is over expression of MMP are found during tissue remodeling. There are several biological cues which regulate MMPs. These include transcriptional regulation by growth factors and cytokines, posttranscriptional regulation due to changes in mRNA stability, posttranslational regulation by activation of the secreted latent form, and inhibition by endogenous inhibitors (TIMPs) (17-20). These levels of regulation precisely control expression and activity MMP under normal physiological conditions (21). However, in pathological conditions such as metastasis, the opportunity for deregulation is elevated. Some growth factors and cytokines secreted by inflammatory cells, stromal cells and tumor cells regulate the expression of MMPs. Some studies have reported that there was a cross talk between tumor cells, stromal cells, and inflammatory cells during the cancer cell invasion process (22, 23).

Inflammation responses play a function at different steps of tumor development: initiation, promotion, malignant conversion, invasion, and metastasis. Several cytokines are also essentially involved in the inflammatory process, especially pro-inflammatory mediators (e.g., nitric oxide (NO) and pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin-1 beta (IL-1β) and interleukin-6 (IL-6). These pro-

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inflammatory cytokines are associated with carcinogenesis, invasion and metastasis. One of the profound connection between inflammation and cancer is nuclear factor-kappaB (NF-κB). This cytokine stimulates proliferation and survival signaling in premalignant cells via the STAT3 and AP-1 pathways (24-26). In turn, inflammatory cell also acts as a factory for MMPs and proteases production. Hence, regulating the expression and the activity of MMPs is considered as target for therapeutic intervention.

One of the problematic events encountered during cancer therapy is the incidence of cancer cells invading and the spreading of tumor cells throughout the body. This is a prominent cause of the poor prognosis in numerous cancers types. Although current anti-cancer chemotherapy utilises cytotoxic agents to interfere with cell division, another recently emerging area is designation of specific chemotherapeutic agents to reduce or inhibit the process of invasion and metastatsis. Many studies have been made to find out the less toxic agents that can block invasion and/or inflammation, also, enhances the survival of cancer patients (27).

Development and strategic use of anticancer drugs or phytochemical compound(s) from medicinal herbs have become one of the most effective ways of controlling malignant disease and noteworthy reservoir for novel anti-cancer drug discovery. The medication by natural remedies can provide both in prophylactic and therapeutic effects of tumorigenesis, cancer invasion, migration and chronic inflammation-driven diseases (28, 29). The biological activities of medicinal plants are attributed mostly to their bioactive chemicals. The phytochemicals such as a polyphenol, flavonoid, anthocyanin or proanthocyanidin are high contently found in colored rice. These phytochemicals have beneficial properties against various types of free radical, anti-carcinogenic, anti-inflammatory, anti-proliferative and anti-angiogenic properties (30, 31).

Red rice consumption is substantially growing presently due to their identity as healthy functional food ingredients. Vast amounts of studies have shown that pigment of red rice is composed of bioactive phytochemicals. These phytochemicals include vitamin E derivatives such as the four analogues (-δ, -γ, -β and -α) of tocopherols, tocotrienols (T3), γ-oryzanol, phenolic acids and flavonoids. These phytochemicals are understood to

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exert important roles in protecting against degenerative diseases. All of them are superb powerful antioxidants that can scavenge free radicals, giving an anticancer and anti-inflammatory effect. Moreover, previous studies have shown that red rice exhibits greater antioxidant activity and higher phenolic contents than non-pigmented and light brown rice (32-34).

From the multiple beneficial properties of red rice including antioxidant, anti-tumor activity, anti-inflammation and anti-invasion effects, it may potentially to be used as therapeutic substance for cancers. However, there is no evidence whether which active phytochemical(s) component in red rice extract affects invasion and inflammation of human cancer cells**.** In order to search for the active component in red rice, bio-guided fractionation technique will be employed to identify the active component(s) of whole grains of red rice extract. Taken together, the investigation of the phytochemical components in whole grains of red rice extract will be carried out, researching the effect on anti-inflammation, and anti-invasion will be tested in human invasive cancer cells cultures.

1.2 Literature reviews

1.2.1 The hallmarks of cancer

Human tumor development requires six hallmark biological capabilities. These hallmarks are the underlying principles for understanding the complexities of neoplastic disease. They comprise of signaling concerned with cell proliferation, suppression of cell growth, anti-apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis (Figure 1.1) (35).

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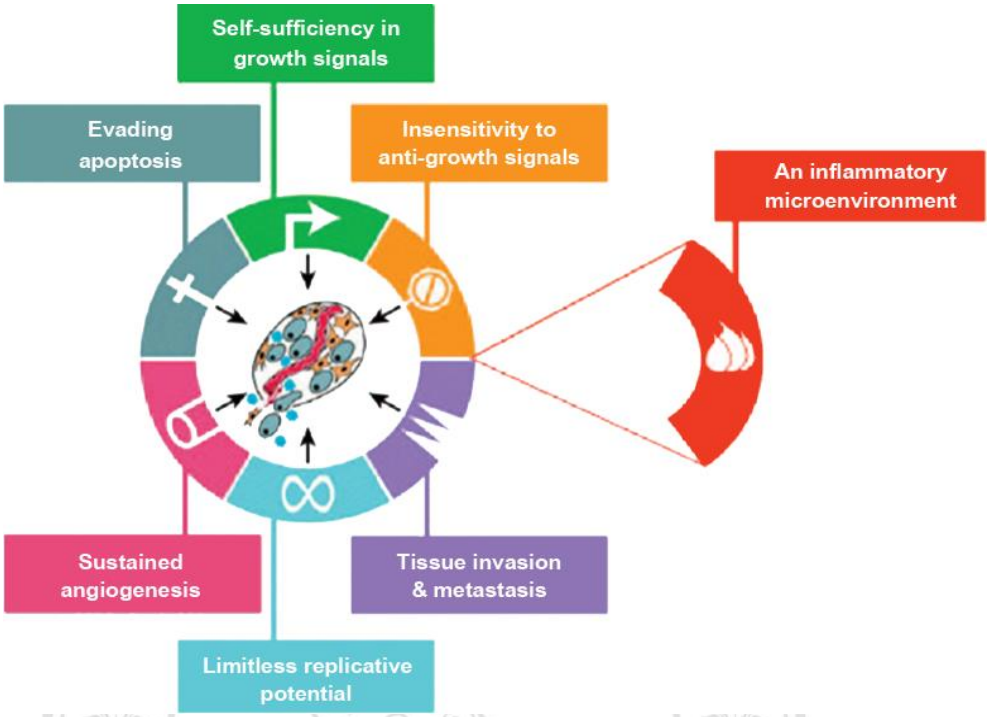

Figure 1.1 The hallmark biological capabilities of cancer (36)

Recent studies have shown the implication of an inflammatory microenvironment and cancer progression. During the inflammation process, inflammatory cells can produce the metabolites of arachidonic acid, cytokines, and chemokines, which take part in recruiting inflammatory cells to the site of injury, generating more ROS/NOS reactive free radical species. These molecules can induce cyclooxygenase-2 and inducible nitric oxide synthase; iNOS, unusual expression of inflammatory cytokines (tumor necrosis factor; TNF), interleukin (IL-1, IL-6), MMPs and cell adhesion molecules such as ICAM-1 and VCAM-1 (37, 38).

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1.2.2 Inflammation and cancer

Inflammation is a complex natural processes that organisms use to fight foreign molecules including allergens, toxic chemicals, pathogen infections or exposure to radiation. A gathering of inflammatory cells at the site of infections is one of the first protective reactions of our immune systems in order to eliminate an initial cause of infections/tissue damages or as a part of

healing processes. The response of the body to tissue injury and physical trauma also triggers a series of these natural defense mechanisms (39).

Acute and chronic inflammation are two stages of inflammation which exist. Acute inflammation is an initial stage of inflammation (innate immunity) and is mediated through the activation of the immune system. This type of inflammation is temporary and is usually beneficial for the host. If the inflammation lasts for a longer period of time, the second stage of inflammation, or chronic inflammation, sets in and may direct the host to various chronic illnesses, including cancer. Prolonged inflammation may lead to a higher the risk of cancer (40, 41).

Locally, persistent inflammation disturbs the homeostatic control of cell signaling pathways, which may lead cells to premalignant and malignant change. Variety of inflammatory immune cells, stromal cells, and cancer cells present in tumor microenvironment can produce pro-inflammatory mediators leading to activate tumor invasion and metastasis. There are two mechanisms which describe the connection between inflammation and cancer (Figure 1.2). The first mechanism is inflammation induced carcinogenesis, known as the extrinsic mechanism. The second one implicates increased the progression of tumor triggered by inflammatory mediators, known as the intrinsic mechanism. Importantly, the inflammatory intrinsic mechanism, caused by the interaction between cytokines and growth factors released from tumor cells or stromal cells, further promotes cancer cell growth by increasing angiogenesis and metastasis (42, 43).

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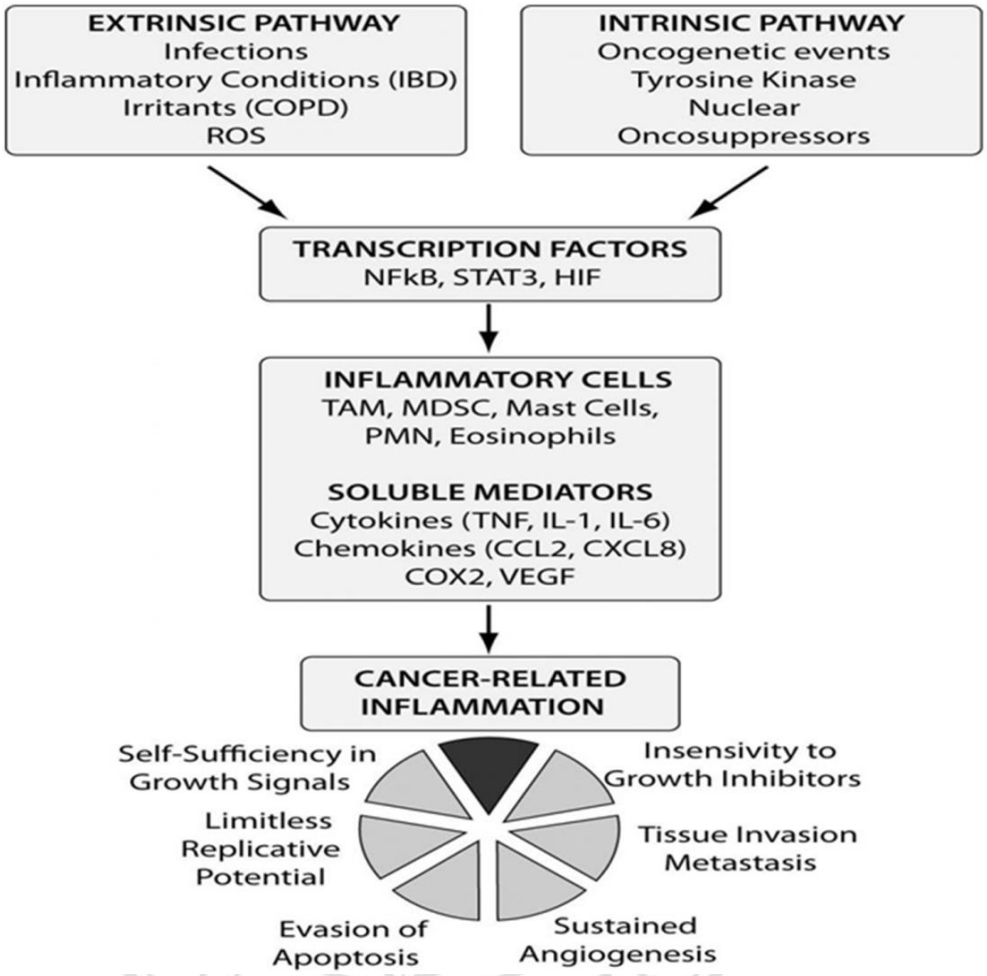

Figure 1.2 Extrinsic and intrinsic cascades relating inflammation and cancer (44)

1.2.3 The biochemical mediators of inflammation and cancer

Inflammation which appears for just a short period of time at the initial stage of the inflammatory response is known as acute inflammation. On the other hand, if the causative agents have persisted for a long duration, the progression of unpleasant symptoms and the events of chronic illnesses can be seen. In addition, the inflammation shows as a major biochemical regulator in tumor promotion and progression by several pathways including alteration of cell cycle arrest, evasion apoptotic cell and stimulation of tumor neovascularization. The key regulatory molecules associated in the development of inflammation to cancer (Table 1.1) are proinflammatory

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cytokines, (IL-1, IL-6, and TNF-α), transcription factors such as nuclear factor-kappa B (NF-κB) and inflammatory enzymes (iNOS, COX-2) (37, 45).

The inflammatory cells can produce the metabolites of arachidonic acid, cytokines, and chemokines, that act as recruitment of inflammatory cells to the injury site and creating the more reactive species. These mediators can stimulate signal transduction pathway as well as induce the transcription factors, such as NF-κB, signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1α (HIF-1α) or activator protein-1 (AP-1), nuclear factor of activated T cells which mediate immediate cellular stress responses (46). Induction of COX-2 and iNOS, overexpression of inflammatory cytokines such as TNF-α, IL-1, IL-6 and chemokines have also been reported to be an essential inflammatory pathway that induce by the stress (Figure 1.3).

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Table 1.1 The regulatory biochemical mediators of inflammation and cancer (37, 47).

Biochemical mediators	**The correlation of inflammation and cancer**
Pro-inflammatory cytokine	- The expression of molecules involved in angiogenesis such as VEGF, VEGFR, IL-8, NO, ICAM-1 and VCAM-l leads to increased angiogenesis - Activation of proinflammatory signaling molecule via NF-κB and JAK/STAT pathway - Induce DNA damage, promote tumor growth, and anti-apoptosis in cancer.
NF-κB	- Mediates inflammation progress - Promotes metastasis and chronic inflammation tumor invasion - Forms a feedback loop between proinflammatory cytokines - Increases the release of proinflammatory mediators and inflammatory signaling activation - Promote the production of ROS
iNOS	- Downstream regulation of NF-κB and proinflammatory cytokines mediated signaling - Regulates angiogenesis and metastasis
NO	- Stimulates cell proliferation, thus promoting tumour growth - Nitration of nucleotide bases leads to DNA damage

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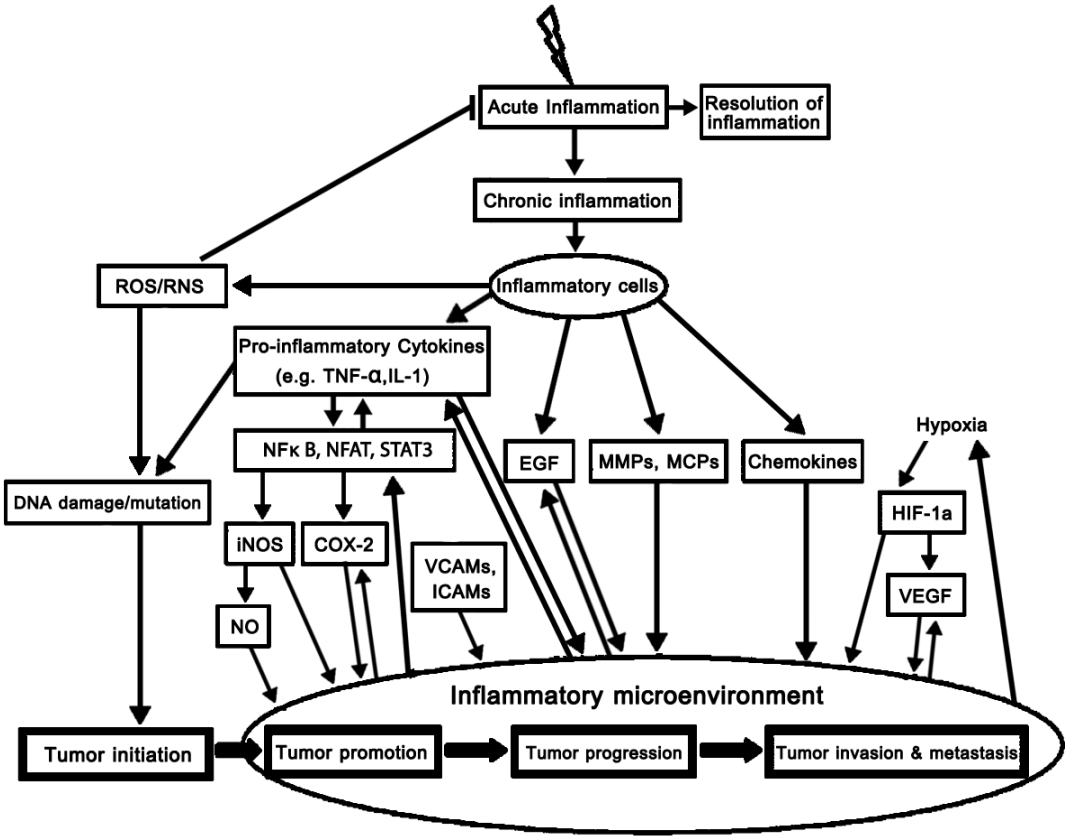

Figure 1.3 The involvement mechanisms of inflammation in cancer development (37)

1) The key regulatory molecules associated in the development of inflammation to cancer

1.1) Cytokine

Cytokines are principal molecules which associate with the inflammation process. The inflammatory mediators, such as cytokines, growth factors and transcription factors (e.g., NF-κB/STAT) regulate cancer gene expression. Other important products include inflammatory enzymes such as iNOS and COX-2. These inflammatory cytokines regulate the production of reactive oxygen species (ROS/NOS) and the level of eicosanoid (48, 49).

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Both extrinsic and intrinsic pathways of inflammation and associated carcinogenesis are contributed to by a variety of cytokines such as IL-1, IL-6 and TNF-α. Various pro-inflammatory cytokines, especially IL-1, IL-6 and TNF-α, have been related to carcinogenesis by interacting with specific cell surface receptors. These interactions activate the upstream of intracellular kinases with activation of their downstream transcription factors which promote inflammation and cancer (50-52).

1.2) Nuclear Factor-kappa B (NF-κB)

NF-κB is a transcription factor, composed of heterodimer with p52 and p65 subunit of the Rel family. In mammalian cells, it can found five member of homo- and heterodimers such as RelA (p65), RelB and c-Rel, p50/p105 (NF-κB2) and p52/p100 (NF-κB2). The NF-κB signaling pathway is stimulated through the canonical or noncanonical NF-κB kinase. The cytoplasmic NF-κB is inactive, forming complex with IκB. In the extracellular mechanism, when stimulated by cytokines, chemokines or adhesion molecules, IκB is subjected to phosphorylation, ubiquitination, and then proteolytic degradation. This causes a disruption of the complex, allowing it to split into its separate components. After IκB degradation, NF-κB is released and lead to nuclear translocation (53-56). Here, NF-κB binds to its promoter regions of its target genes involved in inflammation (IL-1, IL-6, TNF-α, COX-2 and iNOS), invasion (MMP, uPA, ICAM-1, VCAM-1), angiogenesis (VEGF) and metastasis (Figure 1.4).

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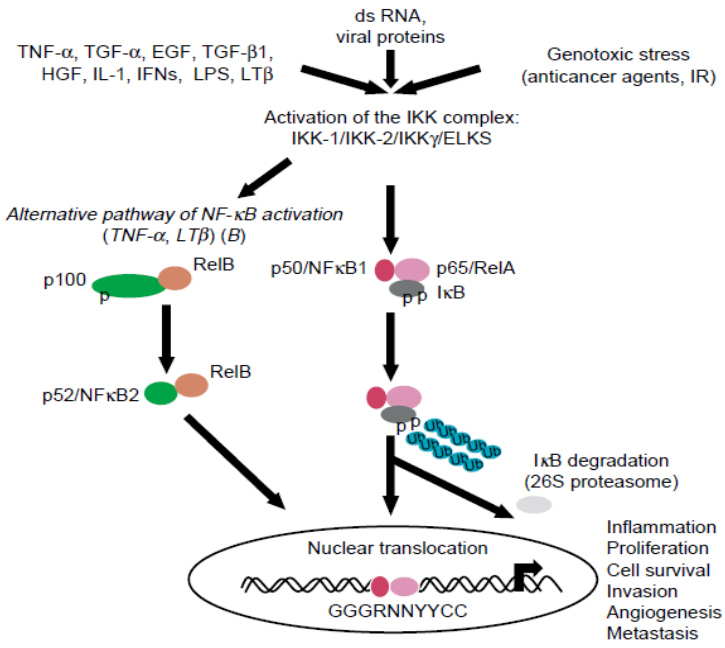

Figure 1.4 The NF-κB activation pathway (57)

1.3) Inducible Nitric Oxide Synthase (iNOS)

Nitric oxide (NO) is an important intracellular and intercellular signalling molecule involved in the regulation of immunological systems. It is free oxygen radical (NO•) and can act as a cytotoxic agent in pathological processes, particularly in inflammatory disorders. The production of NO in the body is catalysed by 130–160 kDa nitric oxide synthases (NOSs). These enzymes have three isoforms; endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) (58).

Inducible NOS (iNOS) was found to be highly expressed in some chronic inflammatory diseases and various types of cancer. It is not found in the resting cells but can be induced by proinflammatory cytokines, infection by bacteria, which can affect the macrophage, hepatocyte, endothelium, monocyte and smooth muscle cells. During periods of prolonged inflammation, NO is an important regulatory molecule for linking the inflammatory response and cancer development. iNOS is

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subjected to induction by proinflammatory cytokines, such as TNF-α and IL-1β, and the transactivation by NF-κB.

Therefore the downstream effect of cytokines and NF-κB may link inflammation to cancer metastasis. In previous studies it was shown that the production of peroxynitrite from iNOS could be the mechanism through which NO may indirectly activate pro-MMP-9 along with other MMPs. Therefore, the inhibition of iNOS may be beneficial for the treatment of inflammatory disease (59-62).

1.2.4 Inflammation and cancer invasion

Furthermore, inflammatory cells also produce soluble mediators, for example arachidonic, metabolite, cytokines, and chemokines. The inflammatory cytokines such as TNF-α, IL-1, and IL-6 have the property to regulate MMPs activity via different pathways and induce the expression of MMPs (25). Also, microenvironments within the inflammation have been closely related to cancer progression. The key mediators can activate the signal transduction cascades as well as inducing alterations in the expression of transcription factors, such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3) and activator protein-1 (AP-1). These transcription factors promote immediate cellular inflammatory responses and link inflammation with cancer cell invasion and metastasis (54, 63).

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For example, high expression of IL-6 regulates RAS/RAF/MEK, PI3K, and TNF/TLR/IL-1 signaling pathways. In cancers, activation of these pathways leads to the recruitment and activation of transcription factors including AP-1, NF-κB, CREB and STAT3, resulting from IL-6 expression. Additionally, IL-6 also enhances MMP-2 and MMP-9 expressions via the JAK/STAT pathway (17, 64-66). In contrast, LPS can induce the expression of IL-6 via ERK and JNK, p38 and NF-κB pathways in Human U937 mononuclear phagocytes. The high level of IL-6 induces tumor proliferation in tumor

initiating intestinal epithelial cells through NF-κB-IL-6-STAT3 cascade. Therefore, the expression of IL-6 via JAK/STAT, MAPK and NF-κB pathways can induce matrix degradation by stimulating MMP level which promotes cancer invasion and metastasis (17, 65, 67, 68).

Another cytokine, IL-1β induces the expression of many genes including IL-2, IL-6, IL-8, vascular endothelial growth factor, monocyte chemoattractant protein-1, intercellular adhesion molecule-1, E-selectin, and cyclooxygenase. IL-1β has been reported to induce the MMPs production by upregulating MMP-9 expression via the NF-κB signaling cascade in RAW 264.7 cells. Generating similar results, TNF-α can induce the activation of transcription factors, such as NF-κB, AP-1 and promote the gene expression involved in cell proliferation, progression, invasion and angiogenesis (69-71). The pro-inflammatory cytokines such as IL-1β and TNF-α activate pro MMPs, allowing them to function correctly. The mature MMPs then perform a proteolytic cleavage of EMC and generate small fragments of ECM components that alter their local cell microenvironments. Meanwhile growth factors are released, after which IL-1β is deactivated by the action of MMP-1, -2, -3 and -4. These reaction cascades mediate cell growth and repair processes as well as promoting the spread and metastasis in certain types of cancer (45, 72, 73).

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Increasing the levels of inflammatory mediators promotes the outgrowth of premalignant cells as well as activating the transcription factors, primarily NF-κB. NF-κB, one of key components that is abundant in tumor cells, is stimulated by many agents, for example, phorbol esters, IL-1, TNF-α, lipopolysaccharide (LPS), double-stranded RNA, bacteria, and viral transactivators (74, 75). Following activation, NF-κB transcriptionally regulates many other cellular genes involved in the early immune and inflammatory responses, including TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-12, iNOS and COX-2. The activated NF-κB (Figure 1.5) alters the regulatory

function of cell affecting proliferation, invasion, angiogenesis and metastasis (76).

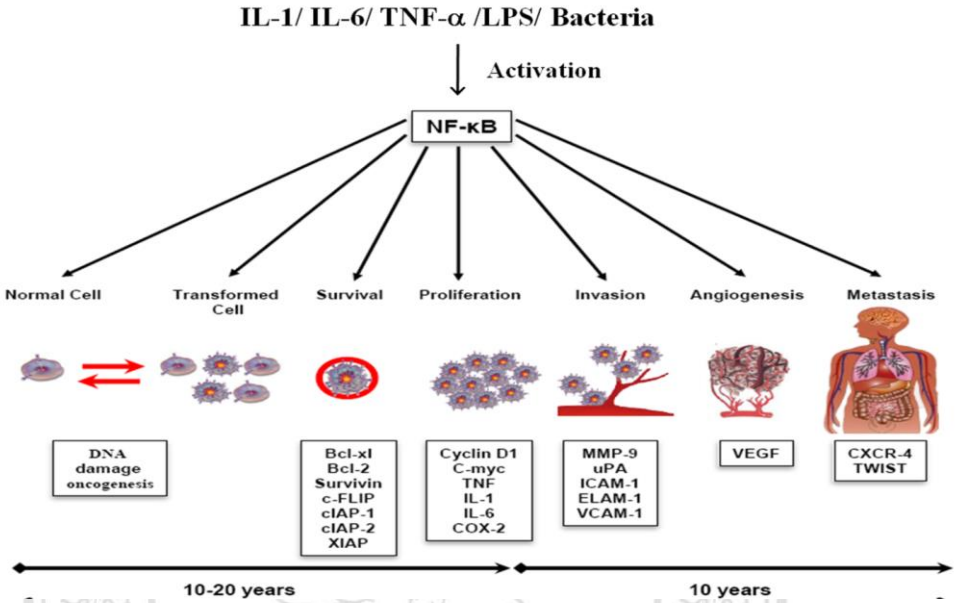

Figure 1.5 The NF-κB activated inflammatory pathway in the transformed cell and the survival in tumor proliferation, invasion, angiogenesis, and metastasis (77)

Therefore, the classic gelatinolytic MMPs produced by inflammatory cells are MMP-2 (gelatinase A) and MMP-9 (gelatinase B). These enzymes are also produced by a variety of other inflammatory cells, which are likely to be the dominant source in inflamed tissues. Several studies have agreed, regarding the connection between pro-inflammatory cytokines and the function of MMPs. The conventional action of MMPs involves in degradation of extracellular, progression. They also regulate various aspects of general inflammation as well as a cancer-related one, considering the extent of their diverse actions (78, 79).

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1.2.5 Cancer metastasis and invasion

Carcinogenesis consist of three steps; initiation, promotion and progression. The critical process of cancer progression, especially, cancer invasion and metastasis, show the aggressive phenotypes which are a major causes of death and the obstacle for successful treatment (80).

1) Cancer metastasis

Cancer metastasis is the migration of cancer cells to the secondary sites within the body. This process involves the proteolytic degradation of basement membrane, stromal invasion, adhesion, angiogenesis, cell proliferation, migration, and anti-apoptosis. It is the major cause of death in cancer patients and form a severe clinical problem. This process composes a long series of sequential, interrelated steps (27).

The major steps in the pathogenesis of metastasis are illustrated in Figure 1.6 and described as follows; Firstly, primary tumors remain vascular until they invade the local epithelial basement membrane. The tumor cell may produce angiogenic factor and allowing it to grow more than 1–2 mm in diameter in size at the primary site. Secondly, angiogenesis (formation of new blood supply for tumor growth) occurs when a tumor receives oxygen and nutrients supply which is created by hypoxic environment and angiogenic growth factors. The growing tumor cells further proliferate and transform into metastatic phenotypes and the escape of tumor cells from the primary site. Next detachment/Invasion is the process by which the tumor passes through the basement membrane and extracellular matrix surrounding the tumor. The detachment of these tumor cells from the primary tumor mass occurs when they acquire an invasive phenotype that results in the loss of cell-cell adhesion and cell-extracellular matrix adhesion followed by proteolytic degradation of the ECM. It is believed that degradative enzymes including serine, thioproteinases, heparinases and metalloproteinases (MMP) such as MMP2 and 9 play an essential role

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in the invasion. Embolism or circulation is the intravasation of the tumor cells into the blood vessel (or lymphatic), prior to hematogeneous dissemination to distant organ sites. Small tumor cell aggregates are detached but the vast majority of circulating tumor cells are rapidly destroyed. A few tumor cells that can aggregate with host cells and survive the circulation must arrest in the capillary beds of organs, either by adhering to capillary endothelial cells. After which adhesion of the circulating tumor cells to the endothelial cell lining at the capillary bed of the target organ site. The fifth step is extravasation is the invasion of the tumor cells through the endothelial cells layer and surrounding basement membrane and target organ such as heart, lung and bone. Finally, growth of secondary tumor occurs at the target organ (1, 3, 81, 82).

After extravasation, cancer cells lodge themselves at the secondary sites, where the cells must also proliferate and colonize for successful metastasis. These processes are controlled by various metastasis promoters such as autocrine motility factor (AMF), hepatocyte growth factor (HGF), transforming growth factor-β (TGFβ), MMPs, uPA and they must be well coordinated to establish successful distant metastasis (82, 83).

Finally, the tumor cells pass through the basement membrane and spread into the adjacent stromal connective tissue. The steps of attachment, degradation, and invasion are repeated within the ECM during tumor growth and spread. In the step of tumor cell invasion, passing through the extracellular matrix is a key process in cancer cell metastasis. This is composed of cell adhesion, ECM degradation and cell migration (84).

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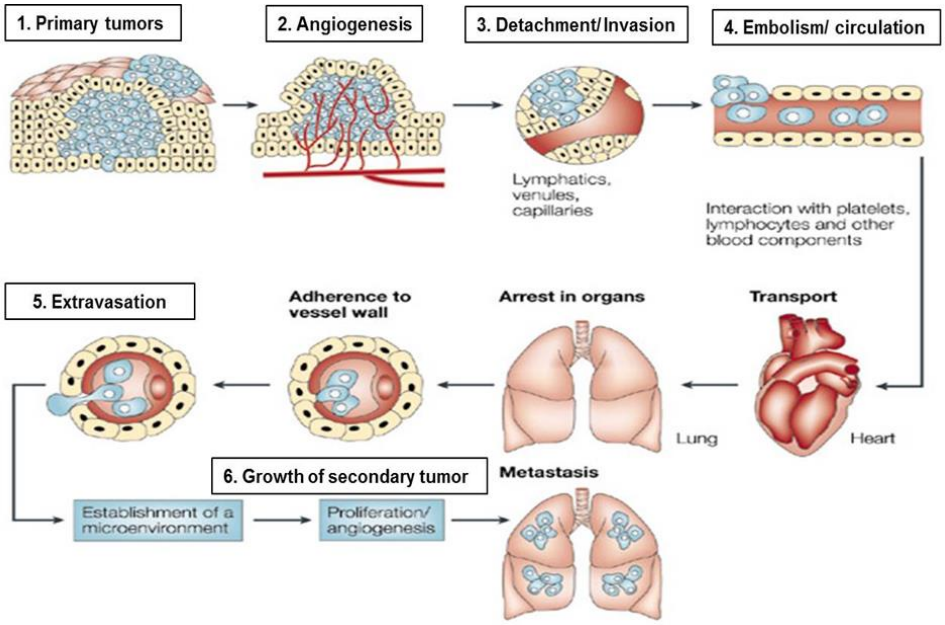

Figure 1.6 Steps of cancer metastasis (85)

2) Cancer Invasion

Cancer invasion is the process by which the cells are enabled to enter into a blood vessel and be transported through the circulation, reaching to a secondary site. It involves in changes of cell behavior, in particular changes in motility and the production of proteolytic enzymes that will degrade the surrounding tissue (86).

The invasion of tumor cell comprise of the secretion of substances to destroy the basement membrane and ECM, especially collagen, proteoglycans, fibronectin, laminin, and glycoproteins (87). These are also related with expression or suppression of protein involved in the control of motility and migration.

3) The significance of ECM proteases for tumor invasion and metastasis

Proteases in the group of ECM degradation enzymes are important in normal physiologic conditions, including ovulation, mammary gland involution and cancer cell growth that are normally under regulatory control. In metastasis, control mechanisms are lost then the dissolution of the components of ECM is occured by the hydrolytic enzymes,

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produced by the tumor cells or by cells surrounding the tumor. The key proteases involved in ECM degradation are the serine proteases (plasmin), uPA, cathepsins and the MMPs as these enzymes function at neutral pH (88). A special kind of proteases, Matrix metalloproteinase (MMPs), a family of zinc and calcium dependent proteolytic enzymes, is essential to invasion, migration, metastasis of carcinogenesis (89).

4) Matrix metalloproteinases

Matrix metalloproteinases (MMPs) destroy the ECM components. And other substrates such as cytokines, growth factor receptors, cell–cell and cell–matrix adhesion molecules may also participate to the invasion process. Moreover, the degradation of ECM macromolecules and cell adhesion molecules may function to modulate the cell behavior (3, 90)

MMPs degrade various components of the ECM as well as cell adhesion molecules and growth factors. Currently, there are over 24 different MMPs, of which 23 are found in humans. MMP members divided in two groups based on their cellular localization (secreted versus membrane bound), or in five main groups: collagenases, gelatinases, stromelysins, matrilysins, membrane type, and others, based on their structure and substrate specificity (91, 92). The matrix metalloproteinases family is illustrated in Table 1.2.

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Table 1.2 The matrix metalloproteinases family (93).

Enzyme	MMP	Chromosomal location (Human)
Collagenases		
Insterstitial collagenase; Collagenase 1	MMP-1	11q22-q23
Neutrophil collagenase; Collagenase 2	MMP-8	11q21-q22
Collagenase 3	MMP-13	11q22.3
Collagenase 4	MMP-18	Not found in humans
Gelatinases		
Gelatinase A	MMP-2	16q13
Gelatinase B	MMP-9	20q11.2-q13.1
Stromelysins		
Stromelysin 1	MMP-3	11q23
Stromelysin 2	MMP-10	11q22.3-q23
Matrilysins		
Matrilysin 1	MMP-7	11q21-q22
Matrilysin 2	MMP-26	11p15
Stromelysin 3	MMP-11	22q11.2
Membrane-type MMPs (A) Transmembrane type		
MT1-MMP	MT1-MMP	MT1-MMP
MT2-MMP	MT2-MMP	MT2-MMP
MT3-MMP	MT3-MMP	MT3-MMP
MT5-MMP	MT5-MMP	MT5-MMP
(B) GPI-anchored		
MT4-MMP	MT4-MMP	MT4-MMP
MT6-MMP	MT6-MMP	MT6-MMP

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MMPs are believed to play an important role in the sequential, interrelated steps necessary for tumor growth and metastasis. The expression of MMPs in tumors is regulated in a paracrine manner by growth factors and cytokines secreted by inflammatory cells as well as by tumor or stromal cells. Studies have suggested continuous crosstalk between tumor cells, stromal cells and inflammatory cells during the invasion process (92).

Many MMPs are found to be involved in cancer, especially MMP-2 and MMP-9. They are over-xpressed in a variety of malignant tumors and their expression and activity are often associated with tumor aggressiveness and a poor prognosis. Elevated levels of MMP-2 and/or MMP-9 are found in breast, brain, ovarian, pancreas, colorectal, bladder, and prostate.

5) Matrix metalloproteinases -2 and -9

The matrix metalloproteinases -2 and -9, also called the gelatinases, have been accepted as major proteolytic degradation contributors as shown in Table 1.3. MMP-2 is abundantly expressed in normal fibroblasts, endothelial and epithelial cells as well as in many transformed cells whereas MMP-9 expression is found in normal leukocytes as well as in transformed cells (10, 23)

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Table 1.3 Gelatinase substrate

Substrate	MMP-2/gelatinase A	MMP9/ gelatinase B
Substrate	MMP-2/gelatinase A	MMP9/gelatinase B
ECM substrates	Collagens I, IV, V, VII, X and XI	Collagens III, IV and V
	Gelatin	Gelatin
	Tenascin	Elastin
	Elastin	Vitronectin
	Fibronectin	Entactin
Other substrates	proTGF-β	proTGF-β
	proIL-1β	proTNF-α
	proTNF-α	IL-2Rα
	proHB-EGF	ICAM-1
	FGFR-I	EGFR-1
	IGFBP-3, -5, -6	Kit ligand
	CXCL12/SDF-1	CXCL1/GRO-α
	CCL7/MCP-3	CXCL4/PF4
	CX3CL1/fractalkine	CXCL8/IL-8
	KISS-1	CXCL9/MIG

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Gelatinases play a critical role in both physiological and pathological states, especially cancer. The gelatinases are required in invasive processes during reproduction, growth and development, leukocyte mobilization and inflammation and wound healing (94, 95). MMP-2 and MMP-9 are highly similar enzymes in many respects, but significant differences exist in the regulation of expression, glycosylation, proenzyme activation and substrate selectivity.

First, MMP-2 is a nonglycosylated protein with 72-kDa, whereas the MMP-9 is 92-kDa, consisting of two N-glycosylated domain, the catalytic domain and a number of O-linked glycans (96). Second, MMP-9 exists in plasma as a monomer, complexes with neutrophil-lipocalin and as a dimer, whereas MMP-2 is strictly monomeric and their differential regulation of expression (94, 97).

Typically, MMP-2 is rather constitutively expressed with only modest up or downregulation under various conditions. Instead, MMP- 9 expression is highly inducible and under the control of growth factors, chemokines and other stimulatory signals (98). Their activity can be traced to the promoter elements of the gelatinases. The promoter of MMP-9 is similar to most other MMPs, whereas MMP-2 promoter lacks many of the inducible promoter elements such as binding sites for the NF-κB, AP-1 and E26 transformation specific (99) transcription factors. This may explain the lack of MMP-2 up-regulation by treated with agents such as TPA, TNF-α, IL-8 and hepatocyte growth factor (100). Their selective association with TIMPs varies such as TIMP-1 forms a specific complex with pro-MMP-9 and this complex formation inhibits activation of pro MMP-9. Another one, TIMP-2 also forms complexes with pro-MMP-2 which low TIMP-2 levels are associated with MT1-MMP-mediated MMP-2 activation (101, 102).

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1.2.6 Cell adhesion molecules in cancer metastasis

Cell adhesion molecules are located on the surface of the cells, their role is to mediate cell–cell adhesion and contact between the cell and the extracellular matrix. They are divided into five groups based on their structural and functional properties: cadherins, mucins, selectins, integrins, and the immunoglobulin superfamily. All five groups have found to be involved in tumor progression, particularly the last family; the group of interest regarding breast cancer metastasis (103, 104).

Intercellular adhesion molecule-1 (ICAM-1, also called CD54), is a member of the immunoglobulin (Ig)-like family. It is an inducible surface glycoprotein that mediates adhesion-dependent cell-to-cell interactions. This molecule is expressed on the cell surface of many cell types such as; vascular endothelial cells, thymic and mucosal epithelial cells and dermal fibroblasts. The extracellular domain of ICAM-1 is essential for the trans-endothelial migration of leukocytes from the capillary bed into the tissue, and ICAM-1 may also assist movement (or retention) of cells through the extracellular matrix.

The ICAM-1 expression can be up-regulated in response to a variety of cytokines such as, tumor necrosis factor (TNF)-α), interleukins (IL-1, IL-11) and interferon-γ (IFN-γ). It is also associated with inflammatory and immune responses once activated. Apart from the role of ICAM-1 in leukocyte adhesion and cancer cell invasion, several lines of evidence in this study also show that ICAM-1 plays an important role in tumorigenesis and metastasis. Previous studies have shown that cell invasion and tumor metastasis can be induced by ICAM-1, thus associating this molecule with myeloma, hepatoma, lung, osteosarcoma and breast cancer cell invasion and metastasis (105-109).

Expression of ICAM-1 adhesion molecules in cancer cells have been shown to play important roles in the induction of inflammation and can recruit inflammatory cells such as macrophages or lymphocytes. Subsequently these immune cells release trophic factors to enhance cancer cell survival and facilitate instability of tumor environment. Therefore, ICAM-1 might take a center role in terms of tumorigenesis, and the disruption of this molecule may prevent inflammation, tumor invasion and metastasis (110).

1.2.7. The importance of proteases for tumor metastasis

EMC degradation enzymes are well known for their numerous physiological functions including ovulation, mammary gland involution and cancer cell

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growth which are all under tight regulated mechanisms. However, when they lose such a tight control, metastasis could be initiated. One of the important steps required in order to succeed in metastasis progression is the release of proteolytic enzymes from either tumor cells themselves or from cells surrounding the tumor. Following the proteolytic degradation, the basement membrane is disrupted and the small fragments of EMC components are generated. The key proteases involved in ECM degradation are the serine proteases (plasmin), uPA, cathepsins and MMPs. Interestingly, there is considerable evidence pointing out that the increasing levels of uPA and MMPs directly correlates with the tumor stages (111, 112).

1) Urokinase plasminogen activator system

A major system responsible for cell functions such as extracellular proteolysis, cell adhesion, proliferation, chemotaxis as well as cytokine release is called for the urokinase plasminogen activator system (uPAS). It also contributes to the development, implantation, angiogenesis, inflammation and metastasis of tumors. The proteolytic system of uPA consists of the uPA, tissue-type plasminogen activator (tPA), plasmin and the uPA receptor (uPAR) (8, 113).

Regulatory inhibitors of the proteolytic system include the serine proteinase inhibitors (serpin) superfamily and the plasminogen activator inhibitor-1 (PAI-1) and -2 (PAI-2). Both uPA and tPA are activators that catalyze the conversion of plasminogen precursor into active plasmin under two different circumstances. The tPA is primarily needed for generating plasmin in order to help dissolve the blood clot. On the other hand, the uPA is required to generate plasmin which activates MMPs in the extracellular matrix leading to the degradation of matrix proteins (6).

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2) Urokinase plasminogen activator (uPA)

The uPA is one of the serine proteases generated as an inactive 53-kDa single polypeptide chain. It is transformed to its active form by the action of activators including cathepsin B/L, human kallikrein and plasmin. Interestingly, it is especially plasmin that is activated and converted from plasminogen by uPA. This protease is highly specific to its substrate in comparison to other degradative protease such as ECM proteases and plasmin that have a broader range of substrate specification (7).

Indeed, most proteins presented in the ECM, except for native collagen, can be degraded by the plasmin. It can also stimulate the precursor forms of MMPs such as MMP-2, MMP-3, MMP-9, MMP-13 and MMP-14. Following the stimulation of MMPs, further degradation then causes a remodeling of the ECM environments that enhances the releasing of certain growth factors which subsequently facilitate tumor cell proliferation and migration, while apoptosis is suppressed (114).

In addition, uPAR, expressed on the surface of various cell types, function by enhancing the activity of uPA and thus activating the conversion of plasminogen to plasmin. Once the uPA binds to its specific receptor, uPAR, the receptor-bound uPA is considerably more active than its free form. This could be due to the fact that binding of the uPA to uPAR increases the local concentration of this proteolytic activity at the cell surface. Therefore, giving the cell the ability to degrade its surrounding matrix that allows such cells to migrate and invade other tissues. Receptor-bound uPA is, in particular, associated with the migration of white blood cells as well as cancer cell metastasis (8).

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3) Urokinase plasminogen activator receptor (uPAR)

The uPAR, as mentioned above, is responsible for the binding of uPA on the cell surfaces. The binding appears to be a trimeric structure comprising of uPA and two uPAR subunits. The active binding complex subsequently provides a high local concentration of uPA on the cell surface that not only enhances proteolytic efficiency but also induces signal transduction as shown in Figure 1.7. The mature uPAR consists of 283 amino acids and the molecular weight is approximately 55–60 kDa (7, 9).

It is found anchored on the cell membrane by a glycosyl phosphatidyl inositol moiety. According to the lacking of a transmembrane domain of uPAR, other helping partner proteins are needed to complete the signaling mechanism. Upon binding, the uPA-uPAR complex involves multi-steps of enhancement and modulation of cancer cell progression including cell proliferation, cell migration and cell adhesion (115, 116).

4) Plasminogen activator inhibitors (PAIs)

The plasminogen activator inhibitors belong to serine proteinase inhibitor superfamilies that are used to inhibit the activity of uPA and tPA. They are composed of two types: PAI-1 and PAI-2. The PAI-1 is a single chain 43-kDa glycoprotein that is primarily believed to inhibit the activity of uPA-uPAR system by forming a complex with those coupled as uPAR-uPA-PAI-1 complex. After the internalization process, uPA and PAI-1 are destoryed, while the uPAR is carried on endocytosis and reexposed to the cell surface. The PAI-2 has two forms, an intracellular 47-kDa non-glycosylated form and an extracellular 60-kDa glycosylated form (116-119).

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They are translated from the same mRNA and possess similar function as anti-protease activity. The interaction of PAI-2 and uPA, like PAI-1, also falls into a 1 to 1 binding. However, the inhibitory activity of PAI-2 is less effective than that of PAI-1. Although high expression of PAI-2 inhibit apoptosis and enhance the development of cancer, currently, there is no such evidence to show the activity of PAI-2 in terms of controlling cell adhesion or migration (117, 120).

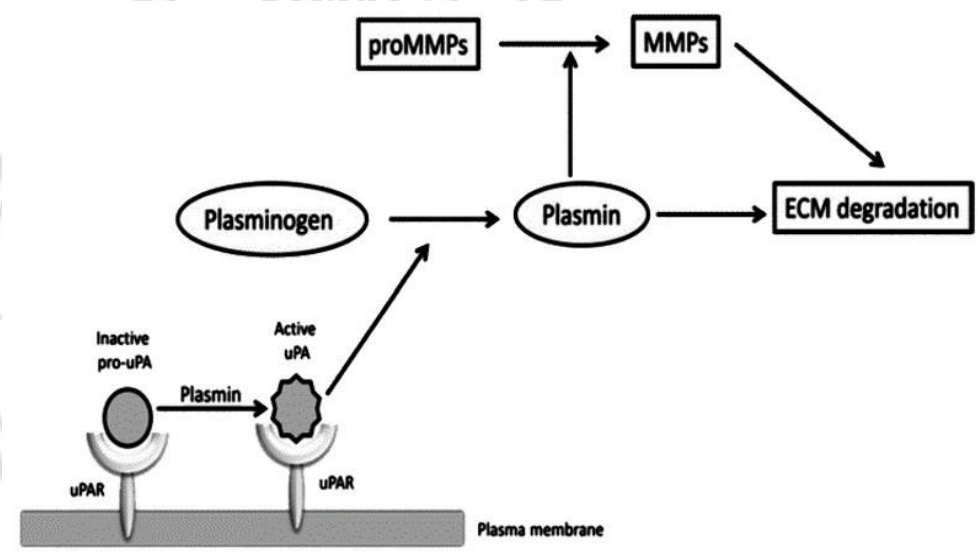

Figure 1.7 Activation of uPA System. uPAR binds to inactive form of uPA first which is later converted to the active form. The active uPA mainly acts as an activator that turns inactive plasminogen into plasmin. The cascade following the activation of plasmin includes the breaking down of ECM components, the activation of latent growth factors or the indirect degradation of ECM through activation of pro-MMPs process (121).

5) Clinical relevant component of uPA system overexpression

The uPAR and uPA are generally overexpressed in tumor associated cells such as macrophages, mast cells, endothelial cells, and fibroblasts (118). Overexpression of these two factors also appears in different types of tumor cell lines and tissues including those of the colon, breast,

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ovary, lung, kidney, liver, stomach, bladder, and bone. Further investigations in tissue samples from patients with colon and breast cancer have also been indicated the strong correlation between the levels of uPA component and metastatic potential in advanced diseases (122).

For example, uPAR and uPA are overexpressed in malignant breast cancer tissues but they do not occur in normal and benign breast tumors. At the leading edge of tumor cells where the invasion is pushed toward the normal tissue, uPAR has been particularly abundantly presented. Over expression of uPA, uPAR and PIA-1 were utilized as biomarkers for several tumor prognoses such as in early breast cancer patients. The elevated levels of uPAR and uPA that typically associate with tumor tissues can become a novel target for anti-metastasis therapy (8, 123).

6) MMP and uPA inhibitors

MMPs and uPA overproduction occurs in tumors at a level that directly correlates with the progression and invasion stage. Thus, inhibition of MMPs, uPA and uPAR expression level represents a promising target for anti-metastatic therapy (Figure 1.8).

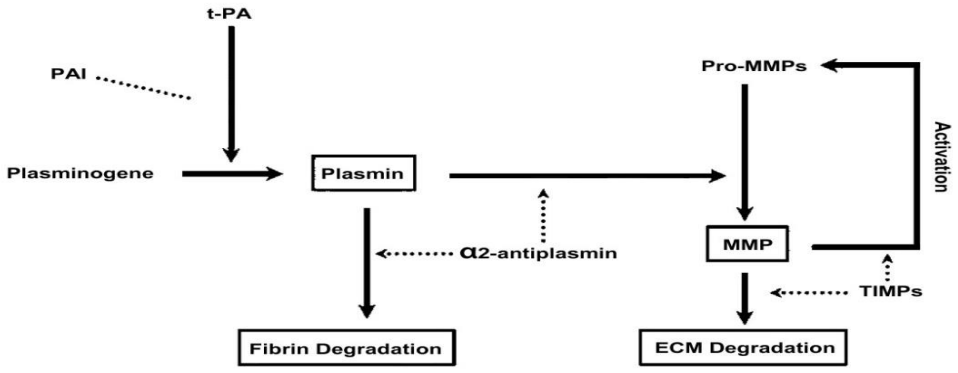

Figure 1.8 Pathway of TIMP (MMP inhibitors) and PAI (uPA inhibitors)

7) MMP inhibitors

There are two major groups of MMP inhibitors which are non-synthetic (e.g. endogenous) and synthetic one. Currently, many powerful MMP inhibitors have been identified. These include hydroxymates, thiols, carbamoylphosphonates, hydroxyureas, hydrazines, β-lactams, squaric acids and nitrogenous ligands (124, 125).

8) Endogenous MMP inhibitors

The endogenous inhibitors that regulate MMP activities are subdivided into two main groups. The first type is a α2 macroglobulin. This macroglobulin is the multisubunit plasma glycoprotein containing four identical subunits. The size of each subunit is 180 kDa. Most proteinases can be inhibited by being entrapped within the macroglobulin. The complex is then rapidly cleared through a process called receptor-mediated endocytosis (126).

The second type of endogenous inhibitors are tissue inhibitors of metalloproteinases (TIMPs). These TIMPs are known for their specific binding to MMPs at a 1:1 ratio. TIMP proteins consist of 184-195 amino acids. TIMPs consist of two domains: the N-terminal domain that can bind to the active site of a MMPs molecule and the C-terminal domain that has the ability to bind to a region of the C-terminal domain of certain MMPs. So far, TIMPs family can be categorized into four members of TIMP-1 to -4 (91, 127-129).

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All four TIMPs can inhibit all forms of active MMPs. But there is a variation in their ability to inhibit each type of MMP. In particular both TIMP-1 and TIMP-2 are play a key role in balancing the degradation of ECM by MMPs in various cancer cell type (130). But, for the sake of a specificity, MMP-9 can be inhibited by TIMP-1 with a high affinity, whilst MMP-2 can be inhibited by TIMP-2. Indeed, from many evidences, TIMPs show their potential applicability as therapeutic

molecules either through gene therapy or direct protein application for cardiovascular disease as well as cancer, yet the study is currently still at the beginning of a developmental stage and requires further investigations (126).

9) Synthetic MMPs inhibitor

Currently, many new drug developments have been focused on inhibitors of MMPs. It is especially MMPIs targeting MMPs in the extracellular space. As shown in Table 1.4, the MMPIs can be grouped into four classes which are (1) peptidomimetics, (2) nonpeptidomimetics, (3) tetracycline derivatives and (4) bisphophonates. Pseudopeptides, is the first synthetic inhibitor that was designed to mimic MMP substrates at the cleavage sites. The MMP activity inhibition occurs through the specific binding of pseudopeptides that contains a zinc-binding hydroxamate portion with the Zn^{2+} ion at the MMP catalytic site. It was the Batimastat (BB-94) that became the first broad-spectrum hydroxamate-based MMPI trial in humans. However, the intraperitoneal administration of Batimastat did not significantly show any responses in the clinical trial (131) Thus further development then moved on to a new peptido-mimetic MMPI, Marimastat (BB-2516). Marimastat can be uptake orally and it can inhibit several class of MMP activity. Those MMP included MMP-1, -2, -3, - 7, -9, -12, and -13. The evidence that show the potency, yet one of the limitation, of Marimastat in order to inhibit numerous MMP is the musculoskeletal pain in the patients who has been treated with this drug for a long period of time. However, as a conventional therapy, Marimastat is extended effectively to use in the patients with the pancreatic carcinoma (132-134).

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Table 1.4 The MMPIs available in clinical cancer therapy (135).

Inhibitors	Class	Specificity	Comments
Marimastat (BB-2516)	Peptido mimetic	Broad spectrum	- Survival benefit in a subset of gastric cancer patients - Survival benefit in glioblastoma multiforme patients in combination with temozolomide
Tanomastat (BAY 12-9566)	Non-peptido mimetic	MMP-2, 3, 9	Development halted because treated patients showed poorer survival than controls
Prinomastat (AG3340)	Non-peptido mimetic	Broad spectrum	survival benefits in NSCL cancer patients No difference in progression of prostate carcinomas
Metastat (COL-3)	Tetracycline derivative	Gelatinases	Multiple mechanisms of action against MMPs
Neovastat (AE-941)	Shark cartilage	Broad spectrum	Multiple mechanisms of action on MMPs Currently recruiting renal-cell carcinoma, multiple myeloma and NSCL cancer patients
BMS-275291	Non-peptido mimetic	Broad spectrum	Currently recruiting NSCL cancer patients
MMI270 (CGS27023A)	Non-peptido mimetic	Broad spectrum	Anti-angiogenic and anti-metastatic effects in animal models Phase I studies in patients with advanced malignancies

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10) The uPA inhibitor

10.1) Plasminogen activator inhibitors (PAIs)

Tissue-type plasminogen activator or tPA and the urokinase-type plasminogen activator or uPA can be inhibited by anti-proteases, especially the inhibitors of the plasminogen activator. These inhibitors belong to serine proteinase inhibitor superfamily and have many subtypes such as PAI-1 and -2.

The PAI-1 is a 45-kDa glycoprotein containing only a single-chain. It has been primarily recognized as the inhibitor for the uPA/uPAR system. Once binding, the PAI-1, uPA and uPAR have form a complex and expose the internal structure. This structure then requires an interaction with a receptor protein, specifically the low-density lipoprotein receptor. Later on, the complex between uPA/PAI-1 is then degraded, while the uPAR itself goes through an endocytosis process and can be able to recycle back once again to outer cell surface (136-138).

The PAI-2 subtype is in two different forms which are the 47-kDa nonglycosylated form and the 60-kDa glycosylated form, found intracellular and extracellular, respectively. They are translated from the same mRNA sequence that contains antiprotease activity. The PAI-2 also forms a complex with uPA at a ratio of 1:1. Cell apoptosis inhibition as well as cancer cell development have been shown to be related to the overexpression of the PAI-2 protein but they do not affect the efficient binding between the PAI-2 and uPA. To our knowledge at present, the PAI-2 acts a lot slower than the PAI-1 and further investigation is needed to explore the role of PAI-2 on a controlling of cell adhesion or cell migration (120).

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10.2) Synthetic uPA inhibitor

The first uPA inhibitors were synthesized and known as WX-UK1 and WX-671, the synthetic inhibitors of serine protease. In preclinical test, the two synthetic inhibitors can be able to perform metastasis formation blockage and also a reduction of primary tumor growth (139). Due to their effectiveness, they are now have already been in the clinical trial phase I/II as a single agent and/or in combination with other chemotherapeutics in order to develop a new treatment for patients at metastatic tumor stage.

Another potent inhibitor has been discovered so far is the Bikunin, a Kunitz-type protease inhibitor. It, effectively and selectively, inhibits trypsin and plasmin, but it shows only moderate inhibitory effect on uPA catalytic activity. Further study on Bikunin has also revealed its inhibitory effect on signaling pathway of the MAPK and PI3K/Akt. Cell growth and invasiveness activity of many tumor cell types can also be potentially inhibited by this synthetic molecule. Oral administration of Bikunin in animal model has recently been examined in ovarian cancer (140).

The DX-1000 is another type of plasmin inhibitor. It is Kunitz domain-based inhibitor that has high inhibitory specificity on tumor growth and *in vivo* metastasis. Although the DX-1000 has shown little unpleasant side effects, it has a short half-life in circulation. The quick clearance of this compound from the body becomes a challenge for a practical use in patients (141).

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1.2.8 Rice

Rice (*Oryza sativa* Linn.) is the main agricultural export product of Thailand as well as being a major component of the staple diet in many Asian countries. The trend of rice consumption had changed over time. In comparison with eating polished white rice in today's tradition, Thais ate home-made, unpolished brown rice in the past, which is richer in fiber and has higher nutritional content. People are currently more concerned about their health, causing clean food and/or organic foods to gradually gain popularity. As rice has been consumed in every meal, it is becoming a target of how to obtain not only how to meet our energy needs but also to increase the nutrition from our daily intake of food.

Rice grains that are dehulled and polished have only their white starchy endosperm left, solely containing starch as an energy source. Many reports indicate phytonutrients contained in brown rice relative to white rice are higher for phenolic acids, dietary fiber, essential fatty acids, phytosterols (γ-oryzanol), vitamin E and vitamin E derivatives (tocopherols and tocotrienols) (142, 143).

In Thailand, there are strains of colored (or pigmented), black, purple and red, that are used in many sweet recipes. On top of those, phytonutrients found in brown rice, current researches also supports the idea that colored rice are rich sources of vitamin E, oryzanol and polyphenols including phenolic acids, flavonoids, proanthocyanidins, anthocyanins. In addition to the colors of the rice, growing conditions as well as geographical differentiation may contribute to the variation of those mentioned bioactive compounds (144-146). This information leads to the belief that wide varieties of colored rice could potentially be served as functional food in order to promote human health.

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Previous studies have shown that red pigmented rice contains vitamin E, oryzanol which are members of the non-polar group. Red rice also contains large amounts of phenolics which are examples of the phenolic acids, as well as flavonoids, including proanthocyanidins as a main component, and low levels of anthocyanin. Thus, the studies on non polar phytochemicals have mainly been observed on red rice, especially tocopherols, tocotrienols and γ–oryzanols such as ferulic acid ester of phytosterols (147, 148). Moreover, the hydrophilic phenolic compounds in rice, exhibit a wide range of physiological properties for example anti-allergenic, anti-cancer, anti-inflammation, anti-artherogenic, antioxidant, anti-microbial, antithrombotic activities and cardioprotection (148-150).

1) Phytochemical components in red rice

1.1) Phenolics, flavonoids and Proanthocyanidin

Phenolic acids are substances which contain a phenolic ring and an organic carboxylic acid functional group. The two derivatives include hydroxybenzoic acid (e.g. gallic, p-hydroxybenzoic, protocatechuic, vanillic and syringic acids) and hydroxycinnamic acid and hydroxycinnamic acid (e.g. ferulic, caffeic, sinapic, p-coumaric, cinnamic acids, and chlorogenic) as shown in Figure 1.9.

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Flavonoids consist of two aromatic rings (A- and B-rings) which create a 15-carbon skeleton. The rings are arranged so that they are interlinked by a three-carbon chain (C6-C3-C6). Flavonoids can be split into the following groups; flavonols, flavanonols, isoflavones, flavones, flavanols (flavan- 3-ols) and flavanones, which normally occur as O- or C-glycosides. Proanthocyanidins, also referred to as condensed tannins, are a class of polymeric phenolic compounds consisting mostly of flavon-3-ol units (catechin, epicatechin, and their 3-O-gallates and epigallates).

Catechin and epicatechin are thought to be abundant in the husk and bran of many pigmented rice varieties (151).

In red rice grains have a high content of phenolics, flavonoids and proanthocyanidin. The phenolic acids in the red rice are vanillic acid, ferulic acid, syringic acid, caffeic acid and p-coumaric acid. While the types of flavonoids include flavonols, flavan-3-ols, flavones and flavanones. In previous studies which regard that bioactivity, they show the red rice extract has the ability to reduce inflammation and anti-radicals activities. These activities are thought to derive from the phenolics, flavonoids and proanthocyanidin contents (33, 152). The extracts also exhibited strong anti-inflammatory and anti-metastasis activities, thought to be from the derivatives of hydroxycinnamic acid, including ferulic acid and coumaric acid (29, 152).

Of these, red rice could be utilized as a staple food due to its enriched of polyphenolic and proanthocyanidin. It is thought that the cause of the antioxidant activity stems from the high amount of hydrophilic phytochemicals present in the rice. In addition, this red rice has the highest content of phytochemicals and many nutritional advantages over white and brown rice (33, 151, 153).

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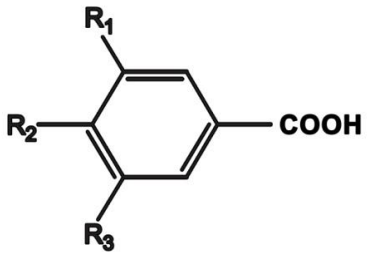
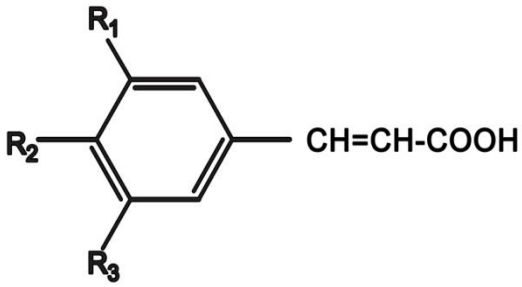


Benzoic acid derivatives	Substitutions		
	R_1	R_2	R_3
Benzoic acid	H	H	H
p-Hydroxybenzoic acid	H	OH	H
Protocatechuic acid	H	OH	OH
Vannilic acid	CH_3O	OH	H
Syringic acid	CH_3O	OH	CH_3O
Gallic acid	OH	OH	OH

b

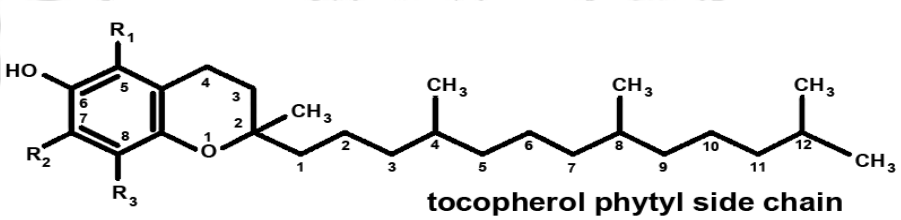


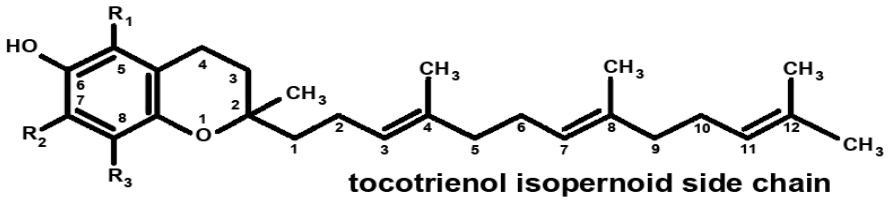
Cinnamic acid derivatives	Substitutions		
	R_1	R_2	R_3
Cinnamic acid	H	H	H
p-Coumaric acid	H	OH	H
Caffeic acid	OH	OH	H
Ferulic acid	CH_3O	OH	H
Sinapic acid	CH_3O	OH	CH_3O

Figure 1.9 The structure of hydroxybenzoic (A) and hydroxycinnamic acid (B) derivatives

1.2) Vitamin E analogs: tocopherols and tocotrienols

Vitamin E derivatives are a group of lipid-soluble substances with a chromanol ring and a hydrophobic side chain including the phytyl group and isoprenyl in the structure of for tocopherols and tocotrienols, respectively. Vitamin E is divided into two groups, tocopherols and tocotrienols, with each form designated as alpha-(α), beta-(β), gamma-(γ) and delta-(δ) according to the number and components of substituent methyl group on the chromanol ring. Tocopherols are composed of four forms with a saturated phytol side chain and the tocotrienols are composed of four forms, each with 3 double bonds in their side chain (Figure 1.10). For example, α-tocopherol, 6-hydroxychroman derivative with methyl groups in the position 2,5,7, and 8 and at carbon 2 has a phytyl side chain attached (151, 154, 155).





compound	R_1	R_2	R_3
α-Tocopherol and α-Tocotrienol	CH_3	CH_3	CH_3
β-Tocopherol and β-Tocotrienol	CH_3	H	CH_3
γ-Tocopherol and γ-Tocotrienol	H	CH_3	CH_3
δ-Tocopherol and δ-Tocotrienol	H	H	CH_3

Figure 1.10 The vitamin E derivatives structure of tocotrienol and tocopherol isoforms

Vitamin E has long been recognized for its protective potential against oxidative stress. Studies upon the other specific biological functions of vitamin E have also been intensively conducted (142). Apart from its anti-oxidative function, γ-tocotrienol exerts anti-inflammatory activity by blocking the function of NF-κB, thus resulting in the suppression of signaling pathways mediated by the inflammatory cytokines (156-158).

Some studies have also reported that the antioxidant activity of tocotrienols have stronger than tocopherols (154, 158). Furthermore, γ- and δ-tocotrienol can potentially regulate the genes expression and activities of protein and enzyme involving metastatic progression (159).

1.3) Phytosterols

Gamma-oryzanol is a steryl ferulates mixture, formed by the esterification of the hydroxyl group of sterols (campesterol, β-sitosterol, stigmasterol) or triterpene alcohols (cycloartenol, cycloartanol, 24-methylenecycloartanol, cyclobranol) along with the ferulic acid carboxylic acid group (Figure 1.11). Compounds containing a double bond in its structure between C5 and C6 or between C7 and C8 are referred to as sterols while sterols with a saturated steroid skeleton are known as stanols (151, 155). Therefore, γ-Oryzanol is one of the most important phytosterols found in rice germ and rice brand. The major γ-oryzanol is 24-methylenecycloartanyl ferulate and some other components available in lesser amounts include cycloartenyl ferulate and γ-sitosteryl ferulate (160).

The anti-inflammatory effects of γ-oryzanol were assessed. It was found to inhibit the secretion of IL-1 or TNF-α and the gene expression of COX-2 through the inhibition of NF-κB activities.

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Moreover, many reports also indicate the potential anti-matastasis property of γ-oryzanol (161, 162).

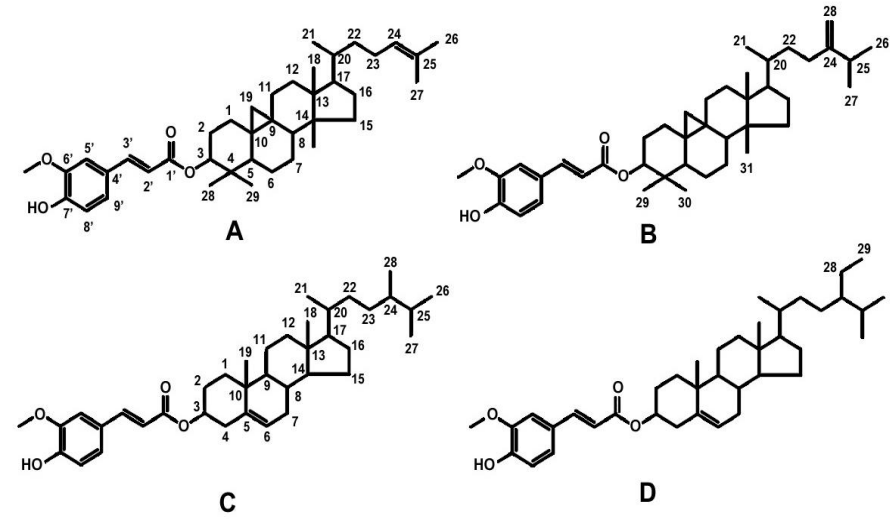

Figure 1.11 The structures of the γ-oryzanol steryl ferulates including cycloartenyl ferulate (A), 24-methylenecycloartanyl ferulate (B), campesteryl ferulate (C) and sitosteryl ferulate (D) (163)

1.3 Objectives

1.3.1 To characterize the active component(s) of red rice extract that inhibit cancer cell invasion

1.3.2 To determine the effect of red rice extract and its active component(s) on the regulation of ECM degradation enzymes in human cancer cells

1.3.3 To determine anti-inflammation effect of red rice extract in RAW264.7 macrophages

1.3.4 To investigate the molecular mechanisms of red rice extract and its active component(s) on cancer cell invasion

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