CHAPTER II

LITERATURE REVIEWS

Lung cancer

Lung cancer is a severe disease, one of the most common high cancer-related mortality rates with a poor prognosis. The incidence rate of lung cancer has increased throughout the world. Survival rate of these patients is less than 15% and more than 85% die in the first 5-year (33-35).

Lung cancer is divided into board types of lung cancer, comprising of small cell lung cancer or SCLC (approximately 15-20%) and non-small cell lung cancer or NSCLC (80-85% of all lung cancer case). Histological, clinical and neuroendocrine characteristics are used to categorize small cell lung cancer and non-small cell lung cancer. Cigarette smoke is the crucial risk factor of all lung cancer types, nevertheless, lung cancer also is found in non-smoker as adenocarcinoma cell type. Other factors causing lung cancer are not related to smoking include genetic, hormone and virus. As for smoking patients, an ability of DNA repairing is reduced. For example, transcriptional changes are revealed in forms of inflammatory and apoptotic pathway while genetical changes are caused by tissue injuries. If these changes persist, it will result in increased invasion, angiogenesis and metastasis of lung cancer cells as they develop of lung cancer from early stage to advanced stage (33, 36).

Non-small cell lung cancer (NSCLC) is divided into 3 subtypes, including squamous cell carcinoma (SCC), adenocarcinoma cell, and large cell carcinoma. NSCLC has a poor prognosis, and frequently diagnosed at the advance stage. The TNM classification divides NSCLC into 4 stages based on cancer progression. The

treatment of NSCLC is also divided depending on histology and stages of tumor (37). Treatments of NSCL include surgery, radiation therapy or chemotherapy alone or in combination. However, the response rates to single agents is only 20% whereas the response rate of combination chemotherapy is unclear (38). Cisplatin and carboplatin, platinum-based antineoplastic drug, are often used in combination with other anti-cancer agents such as gemcitabine, paclitaxel, docetaxel, etoposide, or vinorelbine (39). The combination of this therapy can produce response rate 17% to 32% and overall survival of 7.4 to 11.3 months (37). Unfortunately, the response of NSCLC therapy is not sensitive at the early stage. Moreover, severe side effect of the cytotoxic chemotherapy – bone marrow suppression and nephrotoxicity – and unsatisfied responses of conventional chemotherapy reflect the need of new therapies or new targeted therapies (38).

Metastasis

The salient cause for more than 90% of lung cancer-associated death is metastasis which is the prior cause of death in most forms of solid tumor (3, 40). The process of metastasis is shown in Figure 1. Metastasis is a complicated process, starting from cancer cells in the primary tumor acquiring invasive properties. For facilitation of the invasion of cancer cells, basement membranes at the invasive site are degraded and extracellular matrix (ECM) are remodeled by protease. After that, disseminating cells invade adjacent tissue, either as individual cells (single cells) or as small clumps (collective cells), into the blood circulation. Cancer cells intravasate into newly formed vessels within or near the tumor. Then, cancer cells are transported through the circulating and arrest in the capillary bed of where they

extravasate. At the site of extravasation of cancer cells, they can grow out to a secondary site, where they can proliferate, and this site also requires ECM remodeling and angiogenesis (41).

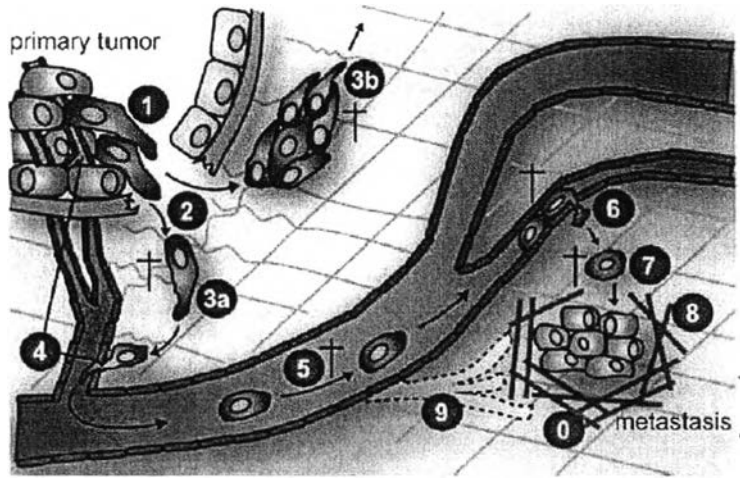


Figure 1 Metastasis process (41).

Migration

As described above, at least five major steps are required for cancer cells to successfully complete the metastasis process (3, 40, 41). Cancer migration was shown to be a critical early step for achieving metastasis (23). Cell migration involves:

(a) Cell membrane protrusion of leading edge-forming lamellipodium and filopodia, arm-like structure, due to the changes in the membrane tension and cycle of actin polymerization and depolymerization;

(b) After cell attachment to the extracellular matrix (ECM) via integrin-FAK containing complex and actin-myosin 2-mediated cell contraction at cell front, release of cell adhesion at the trailing edge leads to cell locomotion;

(c) Contraction of actin cytoskeleton leading to the forward movement of cell body (Figure 2).

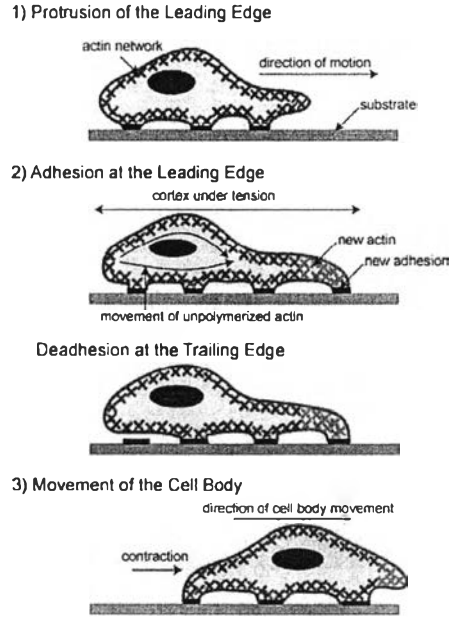


Figure 2 Schematic of cell migration (42).

Indeed, migration of cancer cells is a multi-step process that requires the functions of integrin, focal adhesion kinase (FAK) and Rho family, namely, Rac and Rho proteins (27). Cell migration has also been occurred through integrin-FAK signaling (43, 44). The cytoplasmic portions of integrin recruits the scaffold and signaling proteins to the surface of inner plasma membrane, where they form structure called focal adhesion (FAs). The FAs is a crucial adhesion molecule for migratory activity. During cell migration, FAs associating with ECM generates forces leading to the forward movement of cell body (45). Several proteins have been shown to regulate FAs such as activated FAK (pFAK) (28, 29, 43), activated Rho (Rho-GTP) and activated Rac (Rac-GTP) (27, 31).

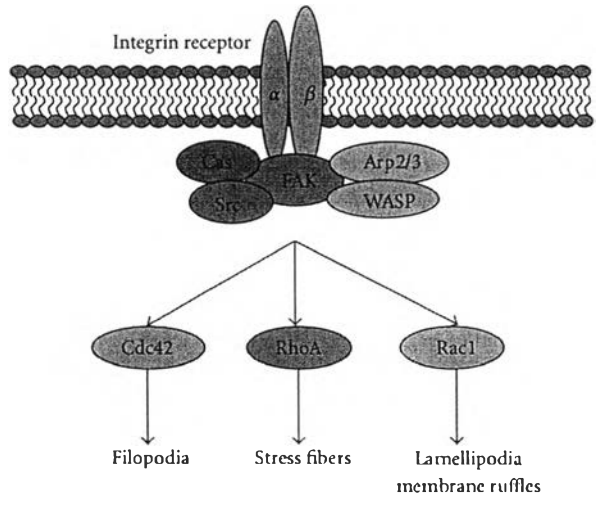


Figure 3 Signaling pathway of FAK and its downstream (46)

Schematic overview of migratory proteins is shown as figure 3. During cell migration, protein FAK is activated by growth factors and integrin activation. FAK is an upstream-molecule of Rho-family (Rho, Rac and cdc42). FAK can stimulate its downstream molecules through a direct interaction or phosphorylation of inhibitor of Rho GTPase. Both FAK activity and Rho-family signaling promote cell migration and regulation of the actin cytoskeletal (27, 46).

Focal adhesion kinase (FAK)

Focal adhesion kinase, a 125 kDa protein, is a non-receptor tyrosine kinase which plays a major role in cell adhesion, proliferation, motility and promotes cell migration (47). As shown in figure 4, the structure of FAK contains three domains including the N-terminal FERM (protein 4.1, ezrin, radixin, and moesin homology) domain, a central kinase domain and a C-terminal focal adhesion targeting-domain (FAT) domain. Important tyrosine phosphorylation sites of FAK are shown in figure 4 (48, 49).

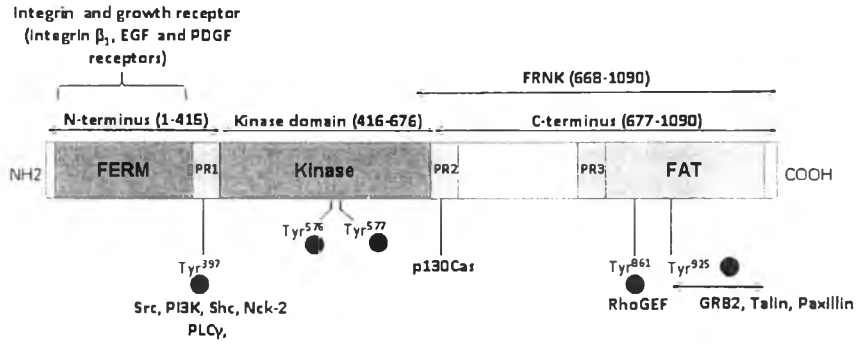


Figure 4 Structure of FAK and phosphorylation site (49).

The central catalytic domain is a kinase domain which flanked by non-catalytic domains, N-terminal and C-terminal domain. FAK protein has binding sites for many signaling proteins that regulate FAK function. The N-terminal domain of FAK directly interact with cytoplasmic domain of ß-integrin subunit and activate growth factor receptor while the C-terminal domain is a site of multiple protein-protein interaction and also responsible for Rho-GTPase activation (49-51).

Several important tyrosine phosphorylation sites of FAK protein are also shown in figure 4, including Tyrosine^{397}(Try^{397}), Tyrosine^{567}(Tyr^{567}), Tyrosine^{577}(Tyr^{577}), Tyrosine^{407}(Tyr^{407}), Tyrosine^{861}(Tyr^{862}) and Tyrosin^{925}(Tyr^{925}). The N-terminal domain has the Try^{397} site which appears to function in cell migration and only Try^{397} residue is known to be phosphorylated by FAK itself whereas other are phosphorylated by Src. Phosphorylated Try^{397} also interacts with Src, PI3K, Shc, Nck-2 and PLCgamma (51). Activation of FAK or phosphorylation of Try^{397} creates a Src-homology-2 domain (SH2-domain) binding site of cellular Src activation. Following that, the residues of Tyr^{567} / Tyr^{577} in kinase domain are phosphorylated by Src activation which promotes the maximal activation of FAK (52).

During cell migration, protein FAK is activated by integrin. Several studies suggested that the overexpression of FAK is widely observed in many types of cancer and correlates with increased migration of metastatic tumors such as breast tumors and ovarian tumor (53-55). The increased level of activated FAK (pFAK) has been found in the migrating cancer cells including lung cancer cells, Chinese hamster ovary (CHO) cells and primary mouse fibroblast (28, 29, 56) through the PI3K/Akt signaling pathway (29) and FAK/Src activation (56). In contrast, previous studies have reported that the suppression of cancer migration could inhibit cancer dissemination by suppression or decrease in pFAK level (24-26). Thus, FAK plays as a marker for cell metastasis and cell migration.

Rho-family protein

As described in the migration chapter, cell migration is a multistep process including lamellipodium extension, formation of the new adhesions, cell body contraction and tail detachment (Figure 2). Cell migration also involves dynamic changes in the cytoskeleton and cell-adhesion requiring for migratory behavior of the cells (57, 58). The Rho-family proteins controlled the dynamic changes in the cell migration (59). Several studies suggested the role of Rho-family in migratory behavior and the relationship between such activation FAK and Rho-family (27, 30, 31). The activated FAK was shown to promote cell migration through the PI3K/Akt-dependent pathway (30) that in turn promote the activations of Rho and Rac proteins (27). Both Rho and Rac induce actin remodeling, an important process for control the cell migration (31). Many studies suggested that the inhibition of Rac and Rho protein can be decreased the migration of the cells (31, 60)

The Rho-family (including Rho, Rac and cdc 42) is a subset of Ras superfamily of small GTPases (61). They act as molecular switch and generally cycle between an active GTP-bound form and inactive GDP-bound form as shown in figure 5 (59, 61).

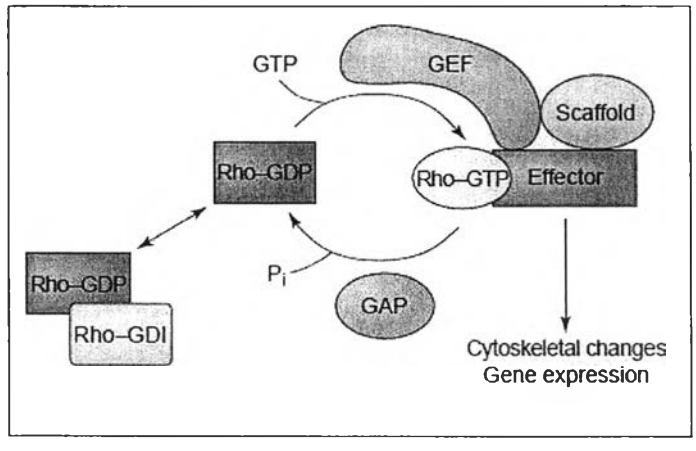


Figure 5 Regulation of Rho family proteins (58).

In cell, Rho-proteins are regulated by three groups of protein comprised of GTPase activating proteins (GAPs), guanine nucleotide exchange factors (GEFs) and guanine nucleotide dissociation inhibitors (GDIs). Activation is stimulated by GEFs which promote the Rho-GDP to Rho-GTP while GAPs switching the Rho-GDP form to Rho-GTP form. Moreover, Rho-family proteins also bind to GDIs in cytoplasm leading to an inactivation of the complex. Their active forms can bind to targeted proteins to induce downstream response including actin dynamic (57, 59-61).

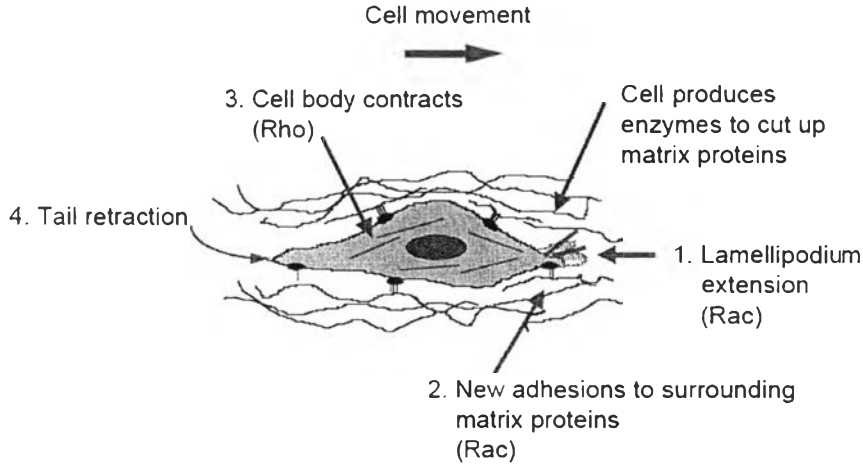


Figure 6 Step to move in cell migration (57).

Figure 6 shows the series of step to cellular movement. When a migrating cell needs to move, Rac-protein induces membrane protrusion and regulates actin polymerization at the front of the cell that leading edge-forming lamellipodium. This extension is stabilized via the formation of new adhesions to the ECM (58, 59). Activation of Rac is induced by growth factors, cytokines and extracellular matrix components (31, 60). Cell body is then moved forward by Rho activation, which regulates the cell contraction and cell retraction at the rear. Lastly, the tail of the cell detaches and retracts from the adhesion site (58, 59).

Anoikis

Anoikis, meaning 'homelessness' in Greek, refers to apoptotic process that induces cell death by loss of cell adhesion or inappropriate cell location. It is a self-defense mechanism after cells detach from their extracellular matrix (ECM) or neighboring cells (4, 62).

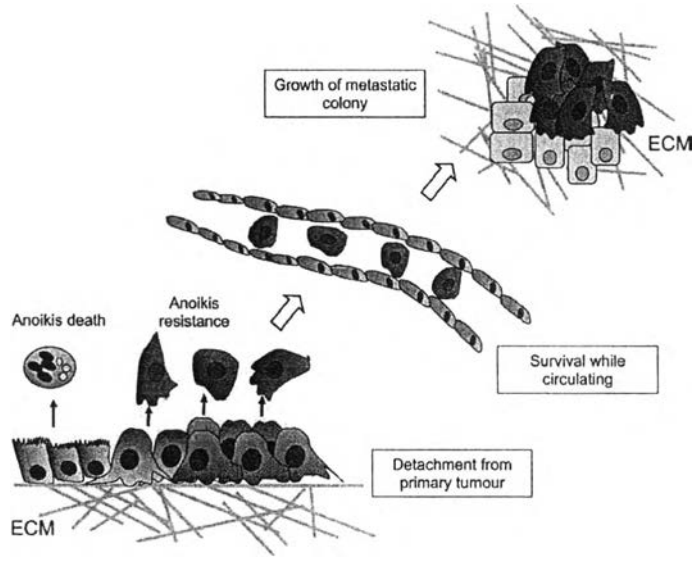


Figure 7 Anoikis and anoikis resistant (63).

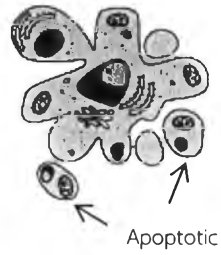
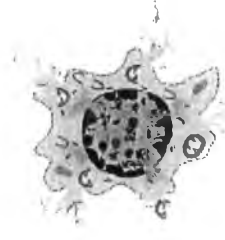
As shown in Figure 7, when cells suffer from a lack or inappropriate adhesion to the ECM, these cells normally die by apoptotic process whereas detached cancer cells are able to escape form apoptosis after loss of cell adhesion. Detached cancer cells are able to overcome anoikis and survive while circulating for a period of time. If suspended cancer cells can adapt to their new environment, they have probably become anchorage-independent and form metastases. This process allows cancer cell to resist anoikis giving rise to metastasis and leading to extremely aggressive cancer and rapid death in patients (3, 62, 64).

Apoptosis

Apoptosis is one of a characteristic of the mode of cell death. The morphological appearance of cell death can be classified in to two major group comprise of apoptosis and necrosis (65, 66). In case of anoikis, apoptosis could be occurs after the cell lack of adhesion (4).

Apoptosis is also known as a programmed cell death. The criteria of Apoptotic cells: cellular shrinkage, condensation of nuclear chromatin, DNA fragmentation and formation of apoptotic body (65) whereas necrosis has been considered as an accidental uncontrolled from cell death such as inflammation and injury. Characteristic of necrotic cell death based on morphology: cellular swelling, plasma membrane rupture and loss of intracellular contents (67). Table 1 presents distinct modalities of apoptosis and necrosis.

Table 1 Distinct modalities of apoptosis and necrosis

Features	Apoptosis	Necrosis
Cytoplasm	Minor modification of cytoplasmic organelles Plasma membrane blebbing	Cytoplasmic swelling
Nucleus	Reduction of cellular and nuclear volume	Reduction of cellular and nuclear volume
DNA fragmentation	Yes	Random digestion
Inflammatory response	No	Yes
Morphological change	Cell shrinkage and cell fragmentation into smaller bodies  Apoptotic body	Rupture of plasma membrane, Swelling of cytoplasmic organelles  Plasma membrane lysis

Anoikis pathway

Anoikis has been reported in several cell types although the initiation and execution of anoikis are activated from different pathways. The activation of caspases is a final step of all different pathways. The event of anoikis seems to suggest via disruption of mitochondria or the intrinsic apoptotic pathway, and triggering on cell surface death receptor is the extrinsic apoptotic pathways (62, 68, 69). Figure 9 shows both pathways.

The intrinsic apoptotic pathway

In this pathway, Bcl-2 protein family is a key protein and mediate cell apoptosis (63). The proteins of Bcl-2 family are divided into 3 subfamilies depending on the homology and function of each protein (Figure 8).

i). The anti-apoptotic proteins consist of Bcl-2, Bcl-XL and Mcl-1.

ii). The second subfamily is the multidomain pro-apoptotic proteins including Bax, Bak and Bok.

iii). The pro-apoptotic BH-3 only proteins which can be divided into 2 groups:

a. The activator BH-3 only proteins such as Bid, Bim.

b. The sensitizer BH-3 only proteins such as Bad, Bik, Bmf, Noxa, Puma and Hrk.

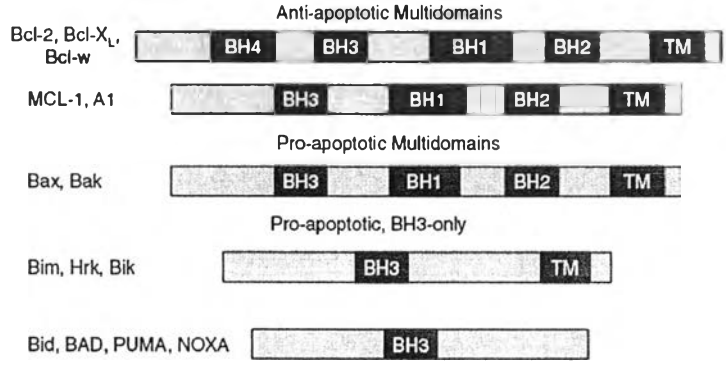


Figure 8 Bcl-2 familiy of pro- and anti-apoptosis protein (3).

In response to death signals, cells lose their adhesion to the extracellular matrix. The intrinsic death pathway is mainly initiated by pro-apoptotic BH-3 only protein, Bid and Bim, which rapidly promote the assembly of monomeric Bax and monomeric Bak. The Bax and Bak protein will then translocate from the cytosol to form Bax-Bak oligomer within the outer mitochondria membrane (OMM) where they create channels, causing mitochondrial permeabilization (3, 68). After the disruption of OMM, cytochrome c is released into cytosol to induce the formation of apoptosome which composed of caspase-9, the cofactor apoptosis protease activating factor (Apaf-1) and cytochrome c. Then, this formation activates caspase-3 and executes the apoptotic process (Figure 9) (3, 70) .

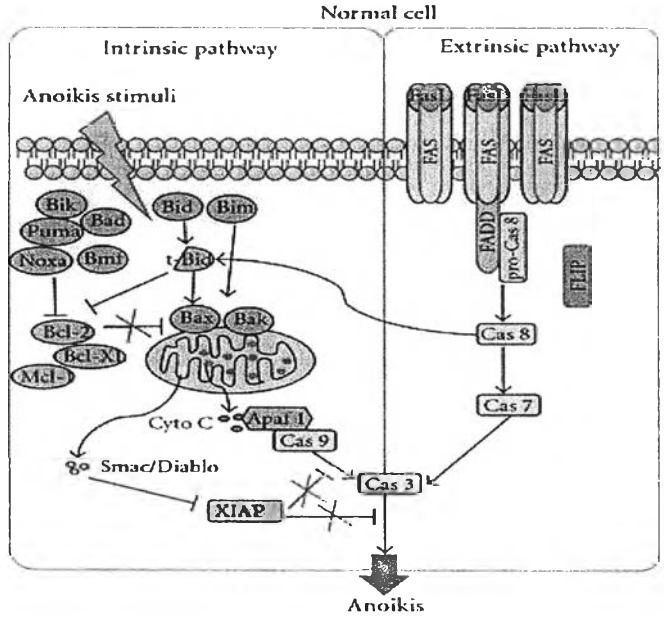


Figure 9 Intrinsic pathway and extrinsic pathway (70).

The key protein in anti-apoptotic Bcl-2 family is Bcl-2 (71) which relates to the Bax and BH-3 only proteins. The role of Bcl-2 protein is to prevent the apoptotic process including preserving mitochondrial integrity, escaping pore formation and the disruption of OMM. Bcl-2 inhibits apoptosis by sequestering Bim and Bid apoptotic activator which prevents the formation of Bax/Bak oligomer. As for the pro-apoptotic sensitizer BH-3 only proteins are unable to directly activate Bax and Bak oligomerization. However, these sensitizer proteins such as Bad and Bmf can induce apoptosis by competing for the binding to Bcl-2's BH-3 binding domain whereas Noxa exclusively counteracts Mcl-1 and deactivating the anti-apoptotic function (3, 63, 68, 70).

The extrinsic apoptotic pathway

Loss of ECM interaction is a result of an increase in of Fas expression and Fas-L expression. As shown in figure 9, this extrinsic cascade is initiated by the binding of extracellular death ligands, such as Fas Ligand (FasL) or tumor necrosis factor-alpha (TNF-α), to their transmembrane receptors called Fas and TNF-α receptor, respectively. The binding then leads to the formation of a death-inducing signaling complex or DISC, which in turn activates caspase-8. Finally, Activated caspase-8 can activate caspase-3 and -7 which triggers the death of cells. Cells that only require caspase-8 activation alone to induce cell death are called Type I cells. On the other hand, another type of cell called Type II cells, the caspase-8 activation itself can not recruit apoptosis as they crosstalk thru the intrinsic pathway. Caspase-8 can process the pro-apoptotic Bid into its active truncated form (tBid) which can release cytochrom c and promote the assembly of apoptosome leading to apoptosis of the cells (3, 63, 68, 70).

Bcl-2

The master of anti-apoptotic Bcl-2 family protein is Bcl-2 (B-cell lymphoma 2), a 26 kDa protein, which exerts anti-apoptosis activity (71). In the figure 9, Bcl-2 protein contains sequence homology with four regions, Bcl-2 homology (BH) 1 through 4. BH-4 domain is required for anti-apoptotic function. This protein is structurally related to the Bax and BH-3 only proteins (3).

In several researches, down-regulation of Bcl-2, was shown to be associated with anoikis sensitizing in lung cancer cell line (8, 9). In addition, the over-expression of Bcl-2 in human osteosarcoma cells also determines the prevention of suspended epithelial cell lines against anoikis (10). The role of Bcl-2 protein is preventing apoptotic process including preserving mitochondrial integrity, escaping pore formation and the disruption of OMM. Bcl-2 inhibits apoptosis by sequestering Bim and Bid apoptotic activator thus this prevents Bax/Bak oligomer. The sensitizer BH-3 only protein – Bcl-2 modifying factor (Bmf) – was investigated in detached condition. Normally, this protein interacts with the myosin V factor complex. After cell detachment, this protein is released from its interaction and accumulates in the mitochondria. Then Bmf protein neutralizes Bcl-2 protein which results in the release of cytochrome c and execution of cell death by apoptotic pathway (3, 63, 68, 70).

Bax

Bax (Bcl-2 associated X), a 20 kDa protein, is a member of pro-apoptotic protein which promotes apoptosis of cell. As show in figure 8, the sequence of this protein – BH-1, BH-2 and BH-3, are closely related with Bcl-2 (71). Bax protein can form heterodimers with Bcl-2 was identified by co-immunoprecipitation method with

Bcl-2 protein. Bax can suppress the activity of Bcl-2 and block apoptosis (72). In addition, Bax normally forms antagonized interactions with anti-apoptosis protein, Bcl-2 and Mcl-1 (73).

During detachment condition, the up-regulation of pro-apoptotic proteins, Bax, has been shown to related to apoptosis (5, 7). As describe above, Bim and Bid act as apoptosis activators. After apoptotic stimuli, the loss of ECM adhesion, these proteins induce Bax and Bak to form oligomer in the OMM. This event leads to mitochondria permeabilization, releasing the pro-apoptotic factor, cytochrome c and execution of cell death by apoptotic pathway (68, 70). Translocation of Bax protein to OMM depends on the loss of survival signal from FAK and Bax protein's movement to OMM takes about 15 minutes but the cells do not die immediately because of cell death in suspended condition occurs after several hours (74).

Mcl-1

Another member of anti-apoptotic Bcl-2 family protein is myeloid cell leukemia sequence 1 (Mcl-1), a 41 kDa protein, which promote viability of the cells (68). This protein is a short-lived protein, 40-60 min. The rapid down-regulation of Mcl-1 protein is one of the responses of stress signals. In biology, the down-regulation of Mcl-1 occurs through the inhibition of Mcl-1 synthesis and the activation of ubiquitin-proteosomal Mcl-1 degradation pathway (75).

Under cell detachment condition, down-regulation of anti-apoptotic proteins, Mcl-1, was shown to be associated with anoikis sensitizing in lung cancer cell line (5-7). Mcl-1 protein has been implicated as a key regulator of cell anoikis (3). In B-RAF melanoma cells, the depletion of Mcl-1 protein can helps these cells sensitive to

anoikis (76). Moreover, the research of Chunchaha and his colleagues suggested that Cav-1 protein could interact with Mcl-1 and stabilize Mc1-1 protein in detached condition through the blocking ubiquitin-proteosomal pathway and inhibit the down-regulation of Mcl-1 (22).

Caveolin-1 (Cav-1)

Caveolin is a scaffold of essential protein of the caveolar coat structure. Figure 10 shows that the main structural protein of caveolae is Caveolin. The morphology of caveolae can be defined as spherical or flask-shaped invaginations of the plasma membrane. This structure comprises of cholesterol, glycolsphingolipid and caveolin (12, 77).

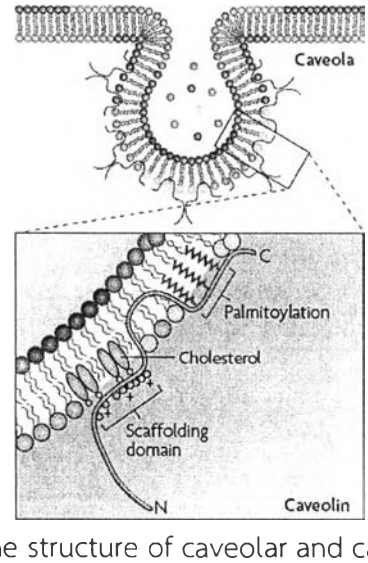


Figure 10 The structure of caveolar and caveolin (77).

Caveolin is composed of three members, namely, caveolin-1, -2 and -3. In most non-muscle cells, including adipocytes, endothelial cells and fibroblast cells, Cav-1 and Cav-2 are expressed. The expression of Cav-3 is high in specific muscles such as skeletal muscle cells, heart muscle cells and some smooth muscle cells.

Caveolin proteins can interact with themselve to form homo-oligomers and hetero-oligomers, which directly bind with cholesterol, allowing them to insert into lipid membrane (77, 78).

Caveolin-1, a 21 – 24 kDa protein, has two isoforms which are Cav-1α and Cav-1ß. Caveolin-1 is the most commonly investigated protein because it possesses a domain called caveolin scaffolding domain (CSD) which interacts with a variety of signaling molecules (78). Some studies concluded that Cav-1 acts as a tumor suppressor while the other studies suggested that Cav-1 is an oncogene. In breast cancer MCF-7 cells, Cav-1 can inhibit cell invasion, anchorage-independent growth and anoikis (17). Several studies suggested that the expression of Cav-1 in many cancer cells such as lung, breast and prostate cancers leads to the aggressiveness and progressiveness of cancer (16, 79). Rungtabnapa et al. indicated that the novel ability of H_2O_2 in cancer cells anoikis is to act as an endogenous suppressor by sustaining Cav-1 protein through its ubiquitination and proteosomal degradation (20). In clinicopathologic profiles of lung cancer, the up-regulation of Cav-1 protein is more associated with poor prognosis in patients than Cav-1 negative (14). The research of Sunaga and colleagues suggested that NSCLC is enhanced approximately 78% of this case whereas Cav-1 protein is not found in 95% of SCLC cases. In addition, in liquid colony formation assay showed that the expression of Cav-1 in NSCLC is strongly correlated with the increased in metastasis development and cell proliferation (80).

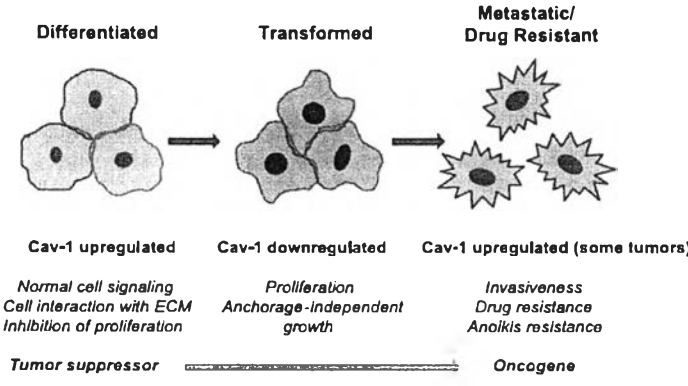


Figure 11 The level and role of Cav-1 in tumor progression (81).

As shown in figure 11, the level of Cav-1 is dynamic during tumor progression. The expression of Cav-1 is high in differentiated cells to control cellular signal transductions. In the early stage of tumor, Cav-1 is down-regulated as a result of the increase in cell proliferation and anchorage-independent growth. However, the level of Cav-1 in the later stages might be increased (in some tumors) to promote the development of drug-resistance, invasive properties, metastatic potential and anoikis resistance, allowing cancer cells to metastasize and survive in the new environment (81-83). Thus, the function of Cav-1 depends on the stage of oncogenic transformation and tumor progression.

Protein kinase B (Akt)

In biology, the ability of a cell to give an appropriate response to its environment depends on cell signaling. These signals are necessary to many processes such as cell growth, cell survival and differentiation. PI3K/Akt pathway is one of the signaling pathways of cell survival. Normally, cell survival signals are regulated by the binding of soluble growth factors to their cell surface receptors and by cell adhesion to ECM. Both signals are mediated through cell surface adhesion molecules (84). In case of cell-ECM interaction, the major mediator adhesion

molecule is integrin, the cell surface glycoprotein (46). Integrin-associated signaling molecules are related to cell survival protein are focal adhesion kinase (FAK) and integrin-link kinase (ILK). This formation stimulates the PI3K and its downstream target which is Akt (4, 69).

Akt, a 60 kDa protein, is a serine/threonine kinase and is one of the downstream targets of PI3K. Akt is expressed as three isoforms – Akt1, Akt2 and Akt3 (85, 86). Akt1 and Akt2 are expressed in all tissue whereas Akt3 is found abundantly in neuronal tissue. All isoforms of Akt are driven by PI3K signaling (87). Upon activation, Akt translocate to the plasma membrane and phosphorylates a number of downstream targets to regulate various cellular functions, such as cell survival, proliferation and metabolism (88).

Protein kinase B and apoptotic pathway

As described above, anoikis is a form of apoptosis after the loss of cell-ECM anchorage. Some evidence suggests the role of PI3K/Akt pathway in anoikis resistance. The activated Akt was found to be up-regulated in anoikis resistant in human osteosarcoma cells (89). Another study investigated this pathway and showed that the activated Akt can inhibit apoptotic process through inactivation of caspase-9 (90). On the other hand, several studies suggested that the inhibition of PI3K/Akt signaling pathway causes the translocation of Bad protein to the mitochondria. This results in pore formation in the mitochondrial outer membrane and activation of the mitochondrial pathway by cytochrome c release and subsequent activation of caspases (91, 92). All of these investigations promote anoikis process.

Protein kinas B and Cav-1

A few studies highlighted the role of Cav-1 and survival pathway. The inhibition of serine/threonine protein phosphatase PP1 and PP2A suggested the role of Cav-1 in sustaining Akt activation which reinforces that Cav-1 protein has a role in a survival signaling pathway (21).

Chemotherapeutic agents

The TNM classification divides NSCLC into 4 stages based on cancer progression. The treatment of NSCLC is also divided depending on histology and stages of tumor (37). Treatments of NSCL include surgery, radiation therapy or chemotherapy alone or in combination (38). Cisplatin, a platinum-based antineoplastic drug, becomes standard clinical regimen used in combination with other anti-cancer agents such as etoposide (39). These drugs act by different modes of action.

Cisplatin

Cisplatin [*cis*-diammine-dichloroplatinum (II)] (Figure 12) is a platinum-based antineoplastic drug commonly used in lung cancer therapy (39). Cisplatin acts as an alkylating agent that induces cancer cell damage by two main mechanisms that involve in the formation of DNA-adduct (93) and cisplatin-induced ROS process causing cellular oxidative stress (94).

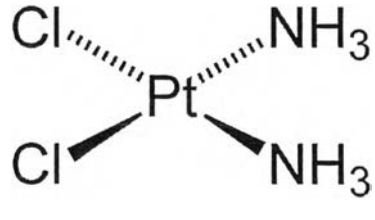


Figure 12 Structure of cisplatin (95).

In the figure 12, cisplatin is a small drug molecule that formed by a platinum ion surrounded by two amine and two chlorides atoms in the *cis* position (96). The chloride ions in cisplatin play an important role in the action of drug. In outside the cell, the chloride ions are stable because the chloride concentration is normally high. On the other site, cisplatin enters the cell through the plasma membrane by passive diffusion. Cisplatin loses all the chlorides and are substituted by the hydroxyl group or water due to the drop of chloride concentration inside the cell. Then, cisplatin creates reactive species allowing the platinum to attack to DNA base (96-98).

DNA-adduct formation

In the figure 13, cisplatin gets inside the cell which mainly through the passive diffusion. After that cisplatin undergoes aquation to form aquated molecule, $[Pt(NH_3)_2Cl(OH_2)]^+$ and $[Pt(NH_3)_2(OH_2)_2]^{2+}$, which are more readily to the targeted molecule. The low concentration of chloride in the cell simplifies cisplatin to form aquated structure. The primary biological target of the aquated form is DNA. Then, the N^7 positions of purine bases form covalent-bond with the platinum atoms of cisplatin to provide primarily intrastrand crosslinks and a lower number of interstrand crosslinks. This linkage causes various cellular response including replication arrest, transcription inhibition, cell-cycle arrest and cell death. Cisplatin-DNA adduct formation always occurs in the proliferated cell, thus, the effect of the adduct formation in cancer cells are more responsive and sensitive than non-cancer cells (95-98).

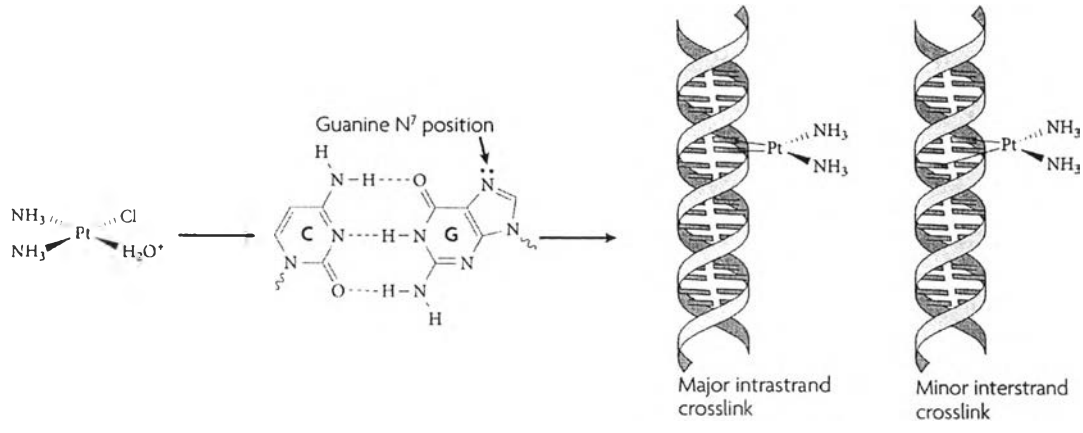


Figure 13 DNA-adduct formation of cisplatin (95).

ROS induction

The second main mechanism of cisplain is cisplatin-induced ROS causing cellular oxidative stress. ROS are known as mediators of intracellular signaling pathway in the cells. In general, the low or moderate ROS level is beneficial to biomolecular functions whereas the increase in expression of ROS can be harmful to the cells. Several studies suggested many kinds of ROS production, hydrogen peroxide, superoxide anion radical and hydroxyl radical, are increased in cultured cell and animal tissue by cisplatin induction, in which the relationship between such alteration of intracellular ROS level and activation of cell death signaling mechanisms such as apoptosis and necrosis is shown (99-103). Considerable evidence implicated that cisplatin-induces ROS plays a critical role on cisplatin-induce cell toxicity.

Etoposide

Etoposide is an inhibitor of the intranuclear enzyme topoisomerase II (Top-II) and has been widely accepted as an anti-cancer drug. As described above, etoposide

is usually used in combination therapy with cisplatin (39, 104). The structure of etoposide is shown in figure 14.

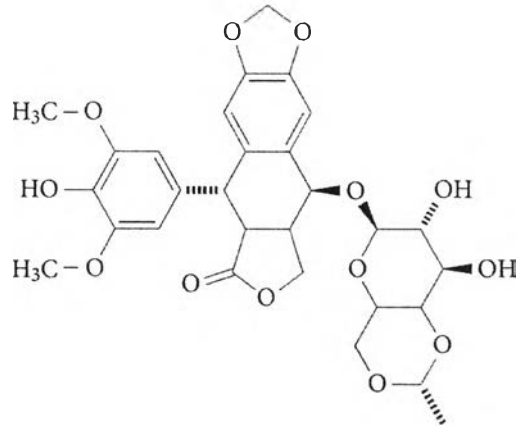


Figure 14 Structure of etoposide (105).

TOP-II is a nuclear enzyme, which is a cellular targeting molecule for anti-cancer drug such as etopside, topoisomerase II inhibitor (104). This enzyme plays an important role in DNA replication, transcription, recombination and chromosome condensation. TOP-II generates transient double-stranded breaks or unwinds in the DNA because in normal condition this enzyme keeps DNA in the proper shape before DNA replication (106, 107). As shown in figure 15, etoposide stabilizes topoisomerase II-cleavable complex leading to an increase in the level of the complex. This causes the DNA double-strand to be easily cleaved because etoposide prevents TOP-II from religating the cleaved DNA and converts TOP-II into a poison. This result shows that the strand breaks are difficult to repair and often lead to cell death (108, 109).

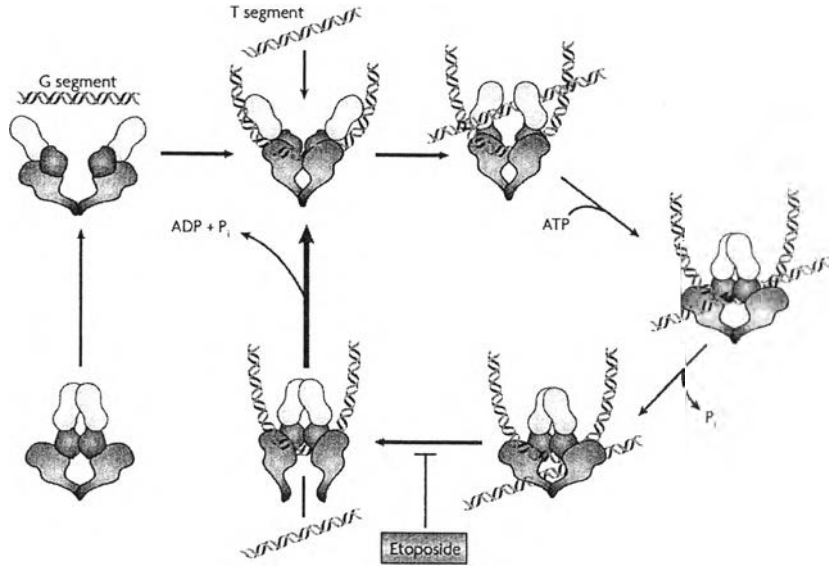
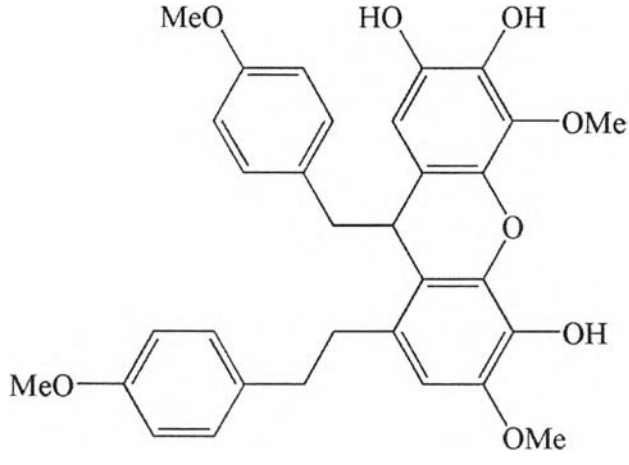


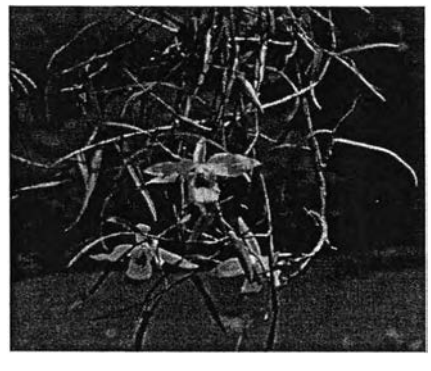
Figure 15 The mechanism of etoposide (110).

Dendrofalconerol A

Dendrofalconerol A (DF-A), a bis(bibenzyls), (Figure 16), was a methanol extract from the aerial of the stem from *Dendrobium falconeri* Hook. (Orchidaceae), which known as "Ueang Sai Wisut", is a plant growing in the northern region of Thailand (32).



A. Structure of Dendrofalconerol A



B. *Dendrobium falconeri*

Figure 16 Chemical structure of Dendrofalconerol A

Bioactivity of Dendrofalconerol A

The compound displays anti-herpetic activity of Herpes simplex type I (32). Several pharmacological studies have shown the activities of compounds isolated from some species of this genus such as anti-cancer activity in human esophageal cancer cell (111) and anti-motility and anti-invasion activity in lung cancer cell (26). However, there is no previous record of chemical examination of the effect of DF-A and the exact anti-tumorigenic mechanism of this agent in cancer cell anoikis is still unknown.