CHAPTER 1

Introduction

1.1 Statement of the problems

Ovarian cancer is one of the foremost causes of death from gynecologic malignancies worldwide. The standard treatment for advanced ovarian cancer is combination chemotherapy of paclitaxel plus a platinum compound (carboplatin or cisplatin) [1]. The main cytotoxic activity of platinum compound is the adduction to DNA mainly formation of intra-stranded crosslinks, leading to triggering of several intracellular signaling pathways causing apoptosis [2]. Although platinum based chemotherapy is an effective treatment for majority of ovarian cancer patients, platinum drug resistance and side effects are the major limitation of current therapeutic concepts.

Platinum resistance can arise by various mechanisms related to increased DNA adduct repair due to overexpression of DNA repair proteins, alteration in intracellular signal transduction pathways, overexpression of anti-apoptotic proteins and interference in caspase activation [3, 4]. Exposure to platinum alters intracellular signal transduction with consequent changes in growth, survival and proliferation, leading to the development of platinum drug resistance. Recent studies proposed that the signal transducer and activator of transcription (STAT) pathway, the mitogen activated protein (MAP) kinase pathway and PI3K/Akt pathway become stimulated upon exposure to platinum drugs in various cancer including ovarian cancer. Cytokines also participate an important role in the natural history of numerous malignancies. Regarding to the biology of ovarian cancer, there is emerging proposal that cytokines such as IL-6 facilitate the situations relating to angiogenesis, cancer progression, invasion and resistance to chemotherapy, all of which are supportive to poor prognosis [5].

Since the survival of ovarian cancer patients have enhanced little with the usage of platinum-based chemotherapy, there is a renewed attention in the improvement of more

effective agents that could increment the conventional therapeutic approaches. Many investigators put emphasis on natural products as chemosensitizers, which augment drug sensitivity and improve anticarcinogenic effects. Among a wide variety of natural alkaloids, the herbal extract from the tubers of *Stephania venosa* have revealed the existence of aporphine alkaloids such as crebanine (CN) and *O*-methylbulbocapnine (OMBC) and protoberberine alkaloids such as tetrahydropalmatine (THP) and *N*-methyl tetrahydropalmatine (NMTHP) [6]. Recently, CN has attracted much consideration due to its wide biologic actions. Some studies on human cancer cell cultures point out that CN has anti-proliferative effects on cancer cell by induction of cellular apoptosis via intrinsic and extrinsic apoptotic pathways [7]. It had been reported that CN more selectively suppress the viability of cancer cell lines than it does in ordinary human fibroblasts [8]. Moreover, CN has shown to have anti-invasive effect via inhibition of constitutive NF-κB activity [9]. These recognized results have directed us to propose that aporphine alkaloids may probably improve the efficacy of the traditional anti-cancer drugs. However, the chemosensitizing effect of CN and its analogues on cancer cells as an adjuvant chemotherapy has not been elucidated yet.

Therefore, in the present study, the chemosensitizing effects and detail molecular mechanisms of CN and its analogues in human ovarian cancer cells when given together with platinum drugs were investigated. Moreover, the effect of CN on IL-6 induced ovarian cancer cells proliferation and invasion were also determined. Furthermore, we established the primary cell culture method from solid tumor tissues of ovarian cancer patients and determined the relationship between IL-6 production in *ex-vivo* studies and clinical response to chemotherapy. Additionally, the effect of CN on platinum sensitivity was confirmed in *ex-vivo* studies by giving combination treatment with CN and platinum treatment to primary culture cells with high IL-6 production induced by platinum drug.

Literature reviews

1.2.1 Ovarian cancer statistics and epidemiology

Ovarian cancer is a very heterogeneous condition and the term ‘ovarian cancer’ is often used to describe a group of tumors that arise from different tissues within the ovaries. Women have two ovaries, one on each side of the uterus (Figure 1.1). The functions of the ovaries are to produce eggs or ova as well as the hormones estrogen and progesterone. Ovarian cancer is the most lethal gynecologic malignancy among the gynecologic cancers and approximately 230,000 new cases were diagnosed each year globally. The median age at the time of diagnosis is about 63 years. White women are more likely to be diagnosed and death from ovarian cancer than other ethnic women. The five-year survival rates of ovarian cancer patients diagnosed at stage III and IV is 32% and 18%, respectively [10].

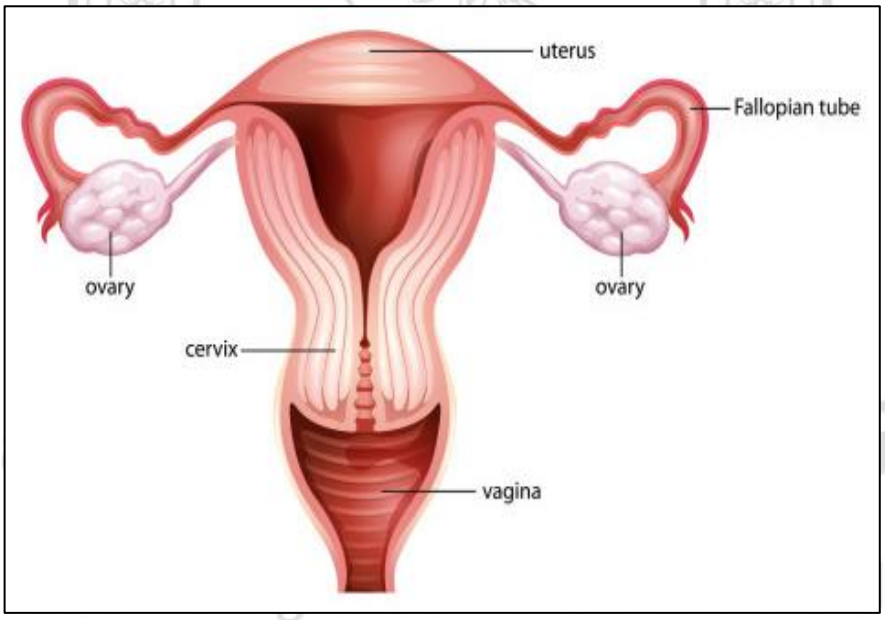

Figure 1.1 Anatomy of female genital tract showing uterus, fallopian tubes, ovaries, cervix and vagina [11]

In spite of the great advancement made in the ovarian cancer treatment in the past decades, the prognosis still remains poor. This poor prognosis is partly due to the asymptomatic nature of the disease, such that the primary tumor has often already spread at the time of diagnosis. Early signs and symptoms of ovarian cancer may be subtle or sometimes absent. The representative symptoms of ovarian cancer include abdominal or pelvic discomfort, bloating, feeling full, back pain, irregular menstruation, fatigue, loss of appetite and possibly urinary symptoms such as frequent or urgent urination [12].

Although most of ovarian cancers are sporadic (90-95%), there are numerous factors that influence the risk of ovarian cancer, with family history being the most important. The risk of ovarian cancer increases with age, nulliparity, obesity (BMI $\geq$ 30), estrogen hormone replacement therapy after menopause and exposure to talcum powder. On the other hand, a decreased risk has been detected with the use of oral contraceptives, fewer ovulatory events, multiparity, a low-fat diet, tubal ligation and hysterectomy. About 10% of cases are owing to inherited genetic profile, especially in women who have mutations in the BRCA1 or BRCA2 genes, have an increased risk of developing the disease [13].

1.2.2 Classification and histological subtypes

In general, ovarian carcinomas can be classified as epithelial tumors, germ cell or sex cord-stromal tumor, depending on the origin of the cell (Figure 1.2). Epithelial tumors start from the cells that line the outer surface of the ovary. Germ cell tumors originate from the cells that discharge the ova. Stromal tumors initiate from structural tissue cells that produce the female sex hormones [14].

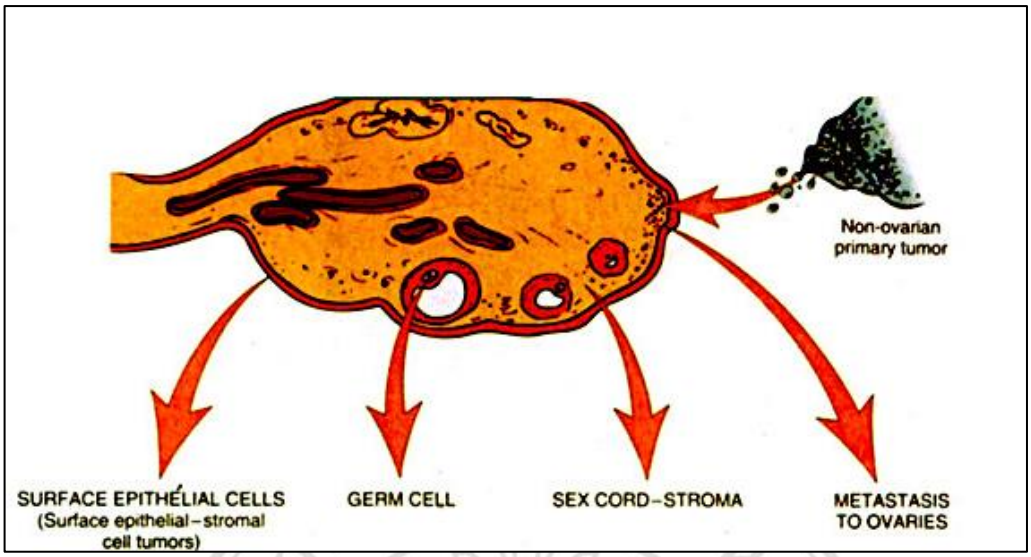

Figure 1.2 The picture illustrates the different types of ovarian tumors [15]

Of these, epithelial ovarian cancers are the most common type and they represent nearly 90% of all ovarian carcinomas. Epithelial ovarian cancer can be further classified into different histological subtypes: high-grade serous, low-grade serous, clear cell, endometrioid and mucinous type carcinomas [16].

Among them, high-grade serous carcinomas are found in nearly 60% of epithelial ovarian cancers and are characterized by mutation in TP53 or BRCA1/BRCA2 genes [17]. Low-grade serous carcinomas are considered to be less aggressive and harbor mutations in PI3KCA, BRAF and KRAS genes [18]. Endometrioid and clear cell carcinomas each account for 15-20% of epithelial ovarian cancers and are indeed related to endometriosis [19]. Mucinous type ovarian carcinomas are rare and it is about 3% of epithelial ovarian cancer [20]. Since there are many different molecular subtypes of ovarian cancer, many experts recommend that each subtype should be treated as distinct disease. As a result, treatments should be personalized for each patient in order to gain the most satisfactory clinical outcome.

1.2.3 Ovarian cancer diagnosis, staging and treatment

Ovarian cancer is often undetectable until it has scattered within the pelvis and abdomen. The absence of early signs and symptoms frequently results in the late detection of ovarian cancer, which has an important impact on staging and clinical consequences. Ovarian cancer at early stage is more likely to be treated successfully since the cancer cells are limited to the ovary. Advanced stage of ovarian cancer is more problematic to be treated and is frequently serious [21].

Once a pelvic mass is doubted through physical examination, serum concentrations of cancer antigen-125 (CA-125) and transvaginal ultrasound are the foremost screening methods used during preoperative assessment [22]. However, increased CA-125 has been reported in many other conditions such as cirrhosis of liver, endometriosis and pelvic inflammatory diseases. Moreover, CA-125 shows poor sensitivity for early stage of ovarian cancer diseases [23]. When a suspicious pelvic mass has been confirmed, a biopsy is usually performed in order to determine malignant potential, classify a histopathological typing, as well as determine the stage of the disease [24]. The International Federation of Gynecology and Obstetrics (FIGO) has established a classification system for staging ovarian cancers into four stages. These stages are principally concerned with the location of the cancer cells, as well as the tumor size for some of the later sub-stages. In general, stage I disease is confined to the one or both ovaries, stage II extends to the pelvis, stage III involves cancer spread beyond the pelvis into the abdomen and/or lymph nodes, and stage IV cancers comprise distant metastases to the lung, liver and extra-abdominal sites [25].

The standard therapy of ovarian cancer disease is cytoreductive surgery followed by combination of chemotherapy (a platinum-based drug and paclitaxel) [1]. Advanced stage of ovarian cancer may require further surgery to remove cancerous tissues or organs. Other surplus ovarian cancer treatments may include hormonal therapy since some ovarian cancers are estrogen hormone sensitive, radiation therapy and immunotherapy [26].

1.2.4 Chemotherapeutic drugs used in ovarian cancer

Chemotherapy remains the principal form of adjuvant treatment for ovarian cancer. For platinum-sensitive tumors, combination chemotherapy with paclitaxel plus a platinum compound (carboplatin or cisplatin) has been largely recognized as first-line chemotherapy [1]. Cisplatin (cis-diamine dichloride platinum (II)) and carboplatin (cis-diamine (1, 1-cyclobutanedicarboxylato) platinum (II)) belong to the group of platinum-based antineoplastic agents and their structures are shown in Figure 1.3. Although they have different molecular structures, their mechanism of actions are similar. Their important property is that they can rotate around the cis configuration of the two reactive groups of the platinum core of the molecule. This cis bond angle is relatively fixed and adducts to DNA with covalent bonds to form intra-strand crosslinks and adducts. This crosslinking leads to the conformational changes in DNA and affect DNA replication which ultimately triggers apoptosis [27].

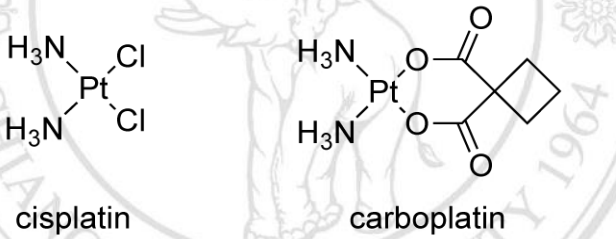

Figure 1.3 Structures of cisplatin and carboplatin [27]

Although platinum containing chemotherapy is the essential treatment option for the ovarian cancer patients, one of the major problems challenged by ovarian cancer patients is drug resistance and recurrence. This is because tumor cells attain a chemoresistant phenotype making them insusceptible to traditional chemotherapeutic agents. In such circumstances, patients with recurrent ovarian cancers are further categorized into either platinum-sensitive or platinum resistant, depending on the period since the last reappearance treated with platinum-based chemotherapy. Platinum resistance is defined as patients who have a progression of disease during the first-line platinum chemotherapy treatment or who have a relapse within 6 months after treatment. For platinum-resistant tumors, cyclophosphamide, vincristine or some combination of gemcitabine, paclitaxel and oxaliplatin can be used as a second-line therapy [28].

1.2.5 Platinum drug resistance mechanisms in ovarian cancer

Although platinum-based chemotherapy provokes an initial tumor response, cancerous cells ultimately develop resistance, which is the eventual reason for tumor recurrence over the course of treatment. The emergence of chemo-resistance is a time-dependent cellular process in which several mechanisms and pathways are involved. The mechanisms of platinum resistance is believed to be multifactorial in nature and can be caused by many cellular adaptations such as decreased influx of platinum drugs due to down regulation of copper transporter 1 protein [29], detoxification of platinum by intracellular thiols such as glutathione [30], increased efflux of drugs due to increased ATP-dependent efflux pumps [31], resisting drug-induced cell apoptosis due to disruptions in apoptotic signaling pathways [32], activation of transcription factors and gene induction such as NF-κB, increased cytokine and chemokine secretion such as IL-6, IL-8 and enhanced DNA damage repair [33] (Figure 1.4).

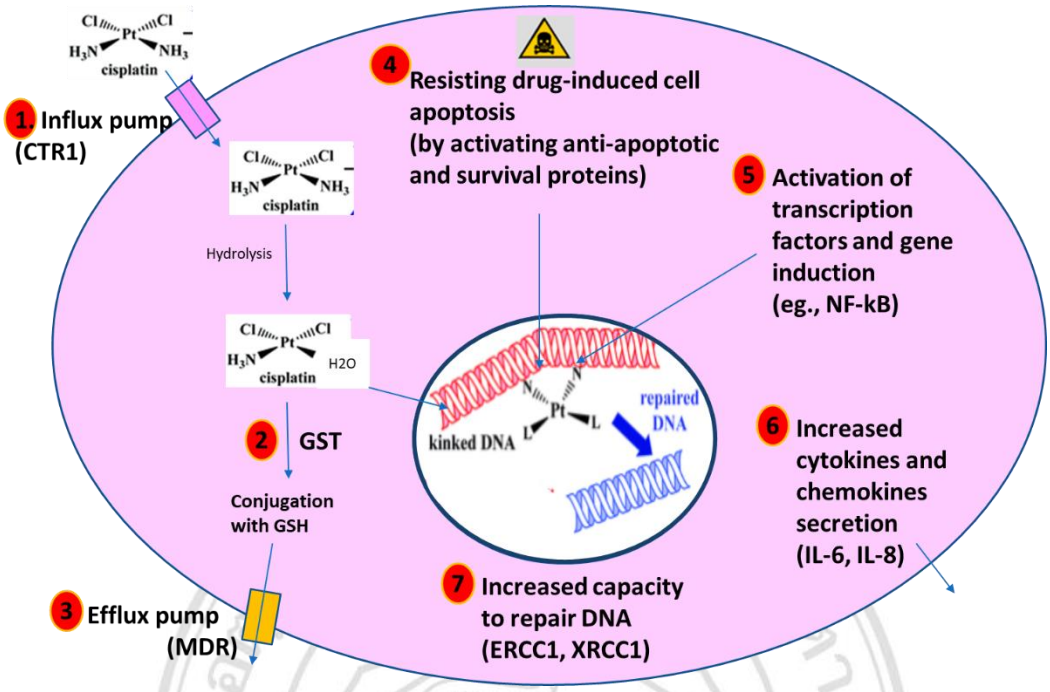

Figure 1.4 Schematic diagram shows the mechanisms of platinum resistance in ovarian cancer. 1) Decreased influx of drugs, 2) detoxification of platinum by intracellular thiols, 3) increased efflux of drugs due to ATP-dependent efflux pumps, 4) resisting drug-induced cell apoptosis, 5) activation of transcription factors and gene induction, 6) increased cytokine and chemokine secretion and 7) increased capacity to repair DNA damage.

1.2.5.1 Decreased intracellular drug accumulation and platinum resistance

The mechanism responsible for decreased intracellular drug accumulation in resistant cells may be attributed to either a decrease in drug influx or an increase in drug efflux, or both. Platinum drugs may enter into the cells by facilitated transport system or passive diffusion. The copper transporter-1 regulates the uptake of platinum drug into the cell, and down-regulation of these transporters results in reduced intracellular accumulation of platinum drug leading to platinum resistance in numerous cancer cell lines including ovarian cancer [29].

MRP-related transport proteins are responsible for platinum drugs efflux. MRP is a member of the ABC (adenosine triphosphate-binding cassette) family of transport

proteins that contributes to anticancer drugs efflux from cells. Many reports on deregulation of drug efflux pumps may influence platinum resistance and it becomes a rational strategy for the advancement of anticancer therapy [31].

1.2.5.2 Intracellular drug inactivation and platinum resistance

Glutathione is the most plentiful intracellular thiol which is responsible for detoxification of many cellular toxins, including platinum drug. Majority of intracellular cisplatin are conjugated with thiol by glutathione-S-transferase, and these cisplatin-thiol conjugates are eventually inactivated. Thus, reducing intracellular glutathione levels appear to be one of the targets to overcome platinum resistance [34].

1.2.5.3 Alteration in apoptotic pathways and platinum resistance

Apoptotic pathways are frequently dysregulated in cancerous cells. As a result, malignant cells proliferate uncontrollably despite the presence of apoptotic signal. Many anticancer agents induce apoptosis of malignant cells by re-sensitizing them to apoptotic signals. Induction of apoptosis can be mediated by multiple cellular pathways in response to various stimuli. Intracellular signaling molecules can induce apoptosis in response to cellular damage or stress. This route, called the intrinsic apoptotic pathway, mediates the apoptotic effects of many anticancer treatments, including platinum drugs and radiation therapy (Figure 1.5). Extracellular signaling molecules can also trigger apoptosis, via the extrinsic apoptotic pathway. There is increasing evidence that the failure of the cells to endure apoptosis may critically lead to carcinogenesis and cancer progression, which characterize the principal cause of tumor drug resistance [35].

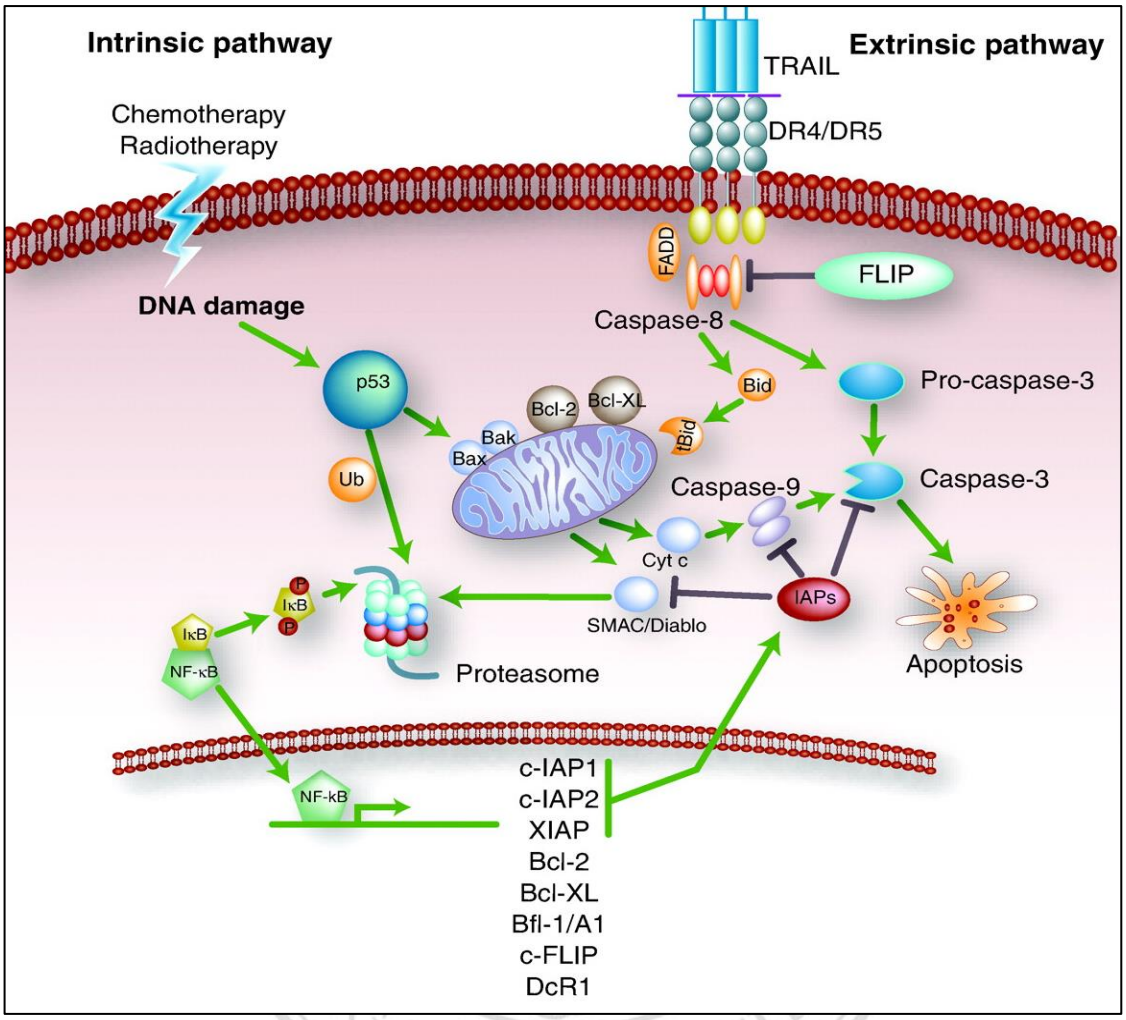

Figure 1.5 Schematic representation of the apoptotic pathways in which chemotherapy-induced apoptosis is accomplished through the intrinsic and extrinsic apoptotic pathways [36]

Platinum resistance is related to the apoptotic pathway defects including Bcl-2 family members and p53 [4, 37]. As shown in Figure 1.6, Akt and NF-κB signaling also play a role in drug resistance mechanisms of cancer cells. Most of the chemotherapeutic drugs including platinum drugs can encourage the stimulation of Akt and NF-κB signaling pathways. Akt activates IKK which hinders IκB and triggers NF-κB signaling pathway. Both Akt and NF-κB stimulate the anti-apoptotic proteins Bcl-2 and Bcl-xL which inhibit the release of cytochrome c from mitochondria. NF-κB and Akt also inhibit caspase cascade via survivin and XIAP respectively. All of these lead to inhibition of apoptosis, cancer cell survival and drug resistance [4].

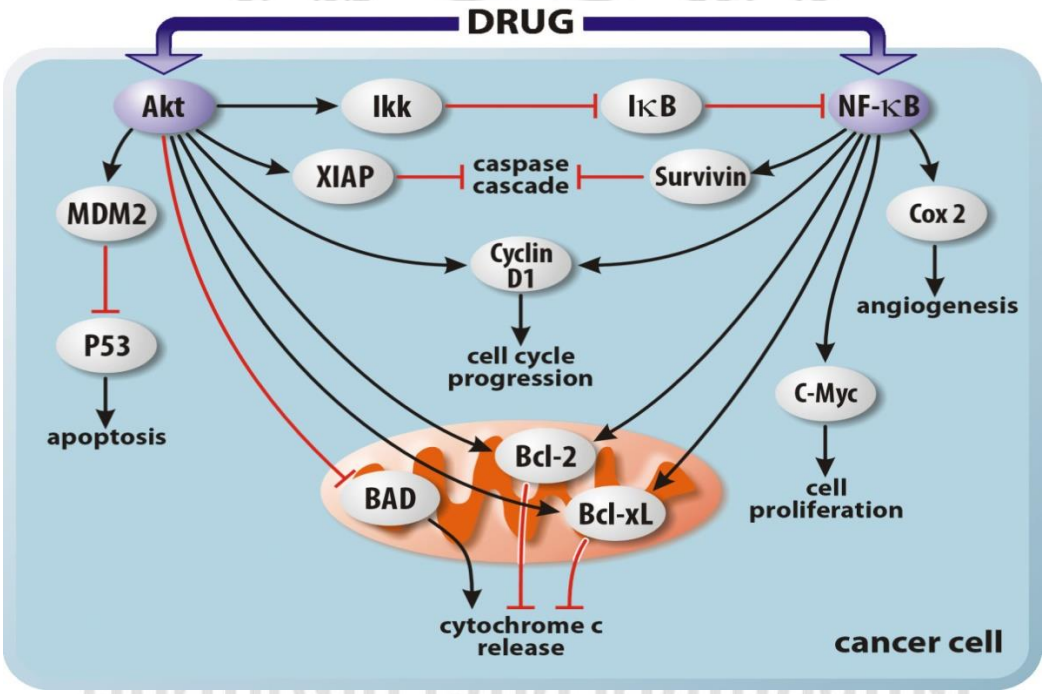

Figure 1.6 Role of Akt and NF-κB in drug resistance mechanism of cancer cell [4]

1.2.5.4 Alteration in intracellular signaling pathways and platinum resistance

Platinum resistance is also related to the abnormal activation of signaling pathways such as PI3K/Akt and PI3K/JNK signaling pathway [38]. Cisplatin-induced stimulation of the PI3K/Akt pathway causes the phosphorylation of IKKα and subsequent triggering of the transcription factor NF-κB, which blocks apoptosis by upregulating the expression of IAP family of proteins, in that way inhibiting the actions of caspase-3, -7 and -9 and promote cell survival [39]. Previous studies have validated that cisplatin exposure stimulates an AP-1-mediated increase in ERCC-1 expression in human ovarian tumor cells which would increase the ability to repair DNA and augment cancer cell survival [40, 41] (Figure 1.7). Inhibition of the PI3K/Akt pathway has been perceived to sensitize many cancer cell types to apoptotic cell death induced by platinum drugs [42] and thus, this pathway is a rational strategy for the advancement of novel anticancer therapy [43].

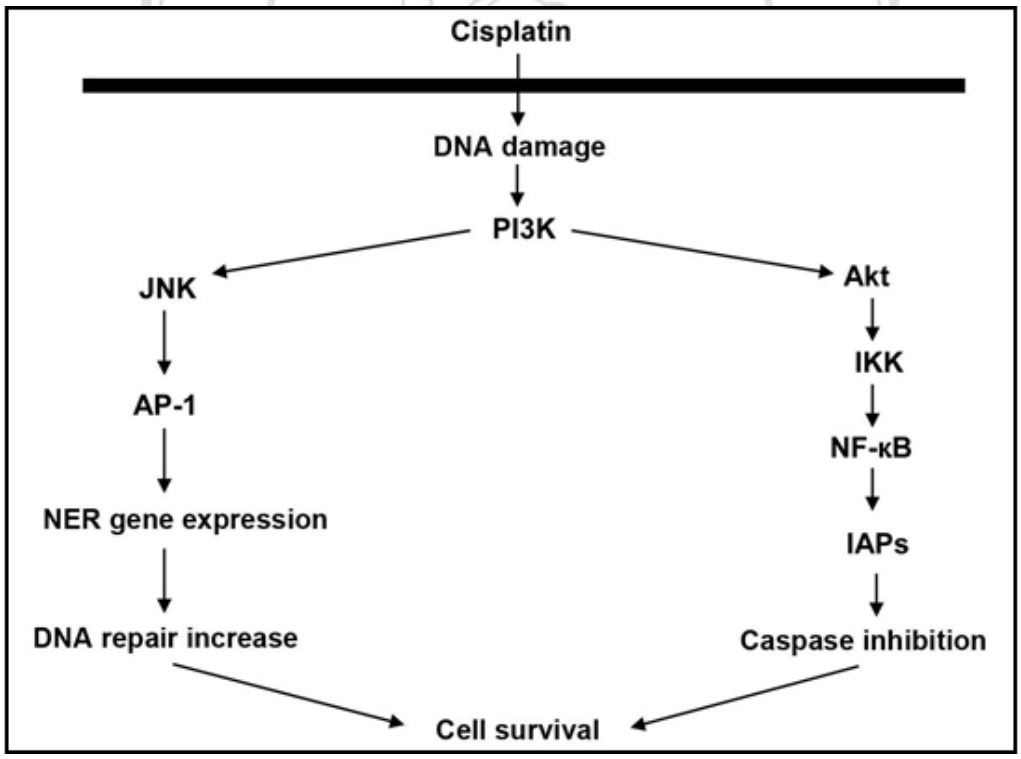

Figure 1.7 Activation of PI3K/Akt and PI3K/JNK signaling pathway upon exposure to cisplatin [40]

1.2.5.5 Inflammatory cytokine and chemokine networks and platinum resistance

Cytokines and chemokines also play an important role in the natural history of several malignancies. Emerging evidence has revealed that pro-inflammatory cytokines and chemokines form complex networks with each other, leading to tumor growth and metastasis (Figure 1.8) [44]. Increased secretion of several pro-inflammatory cytokines such as IL-6, IL-8, and macrophage inflammatory protein-1β, have been recognized in ascites fluid of ovarian cancer patients [45]. Lane et al. investigated the prognostic significance of IL-6 and IL-8 levels in ascites by using multivariate analyses and the authors drew a conclusion that IL-6 could predict the shorter progression-free survival [46].

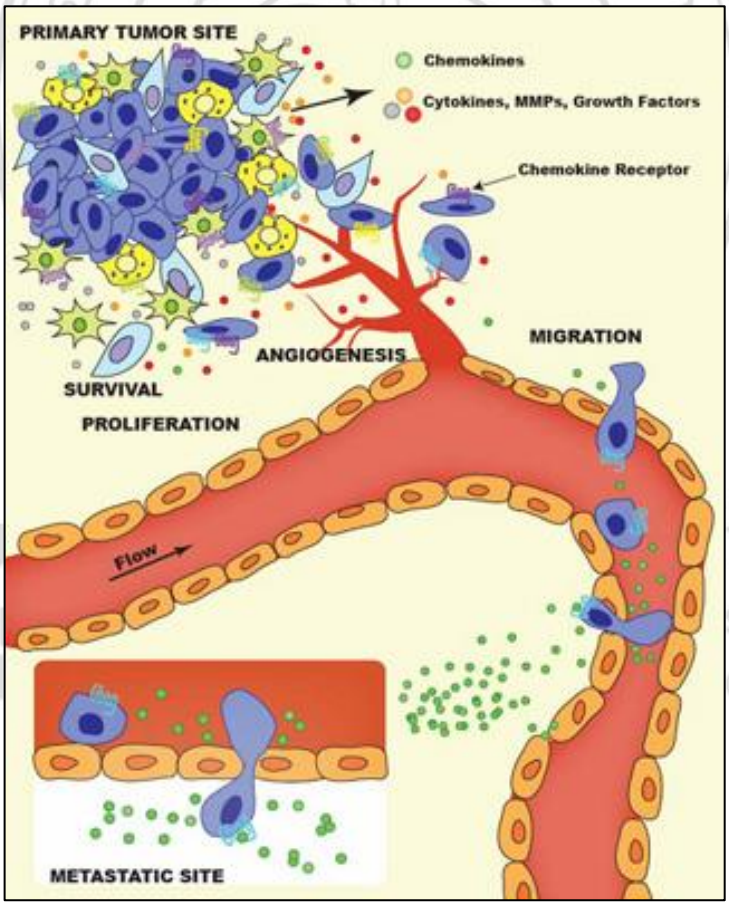

Figure 1.8 Role of chemokines/cytokines in cancer growth and progression [44]

Human IL-6 comprises of 184 amino acids and is produced by many host cells and tumor cells in the tumor microenvironment. It has a wide-ranging biological action relating to inflammation, cell differentiation and proliferation, immunomodulation, angiogenesis and oncogenesis [47] by activating downstream Janus kinase (JAK) signal transducer and activator of transcription-3 (STAT3) signaling (Figure 1.9). Activated nuclear STAT3 has been detected in various malignancies, including gastric, ovarian and head and neck cancers [48-50]. Enhanced activation of STAT3 has also been suggested as a major contributor to platinum resistance [50]. Conversely, conditional STAT3 disruption in gastric and other epithelial cell types was found to inhibit tumor development and progression [51].

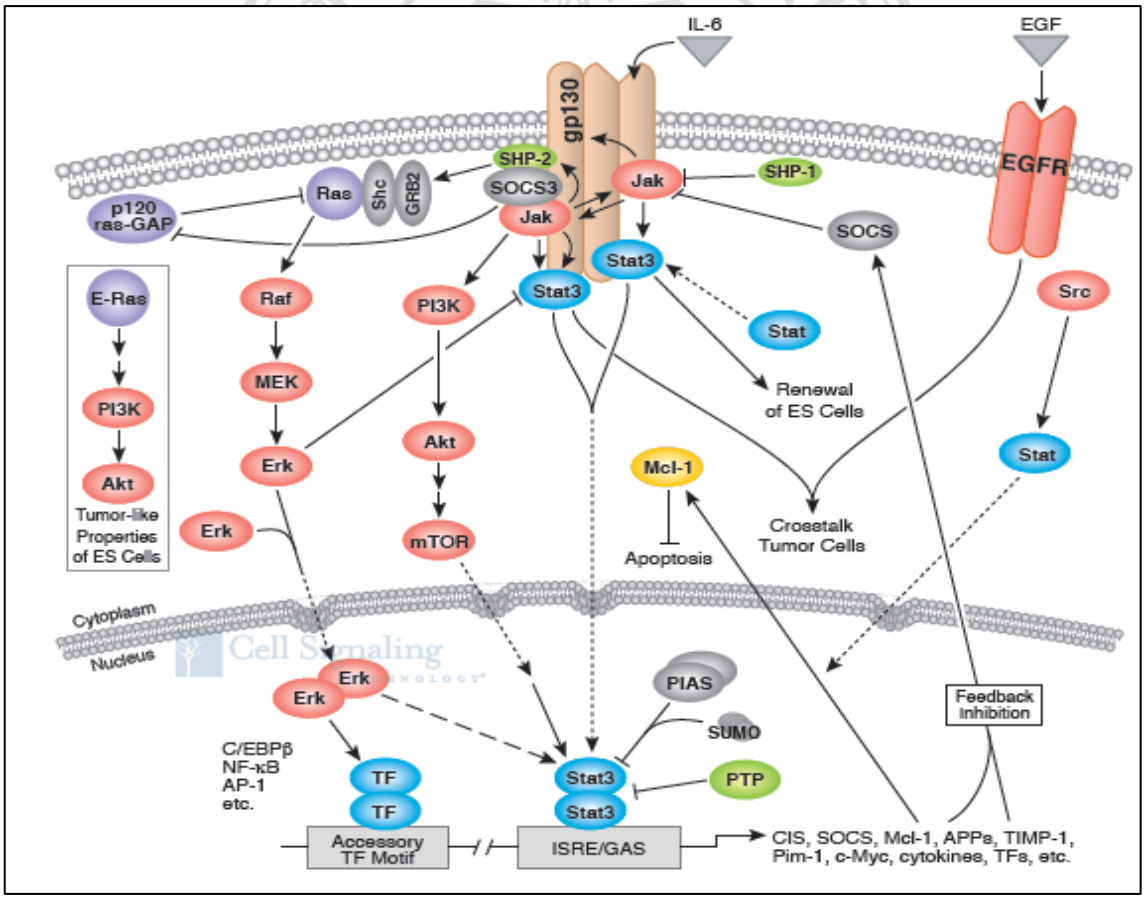

Figure 1.9 IL-6 signaling cascade [52]

One of the reasons for chemotherapy resistance is the ability of tumor cells to induce IL-6 production via the NF-kB pathway upon chemotherapy treatment (Figure 1.10). DNA damage caused by platinum drug leads to phosphorylation of Iκ-Bα, and subsequent ubiquination and proteasomal degradation, resulting in the translocation of NF-κB complex to the nucleus, thereby promoting the release of mediators, such as IL-6, which are concomitant with cancer-promoting inflammation [53].

It has been extensively stated that IL-6 are overexpressed in epithelial ovarian carcinoma and correlated with a poor clinical response to chemotherapy [54]. Anti-IL-6 antibody, Siltuximab, was displayed to abolish IL-6 signaling pathways by inhibiting STAT3 phosphorylation, leading to suppression of downstream antiapoptotic factors [55]. These data indicated that IL6 appears to be one of the important tumor promoting factors in ovarian carcinoma, particularly in the setting of tumor progression and drug resistance.

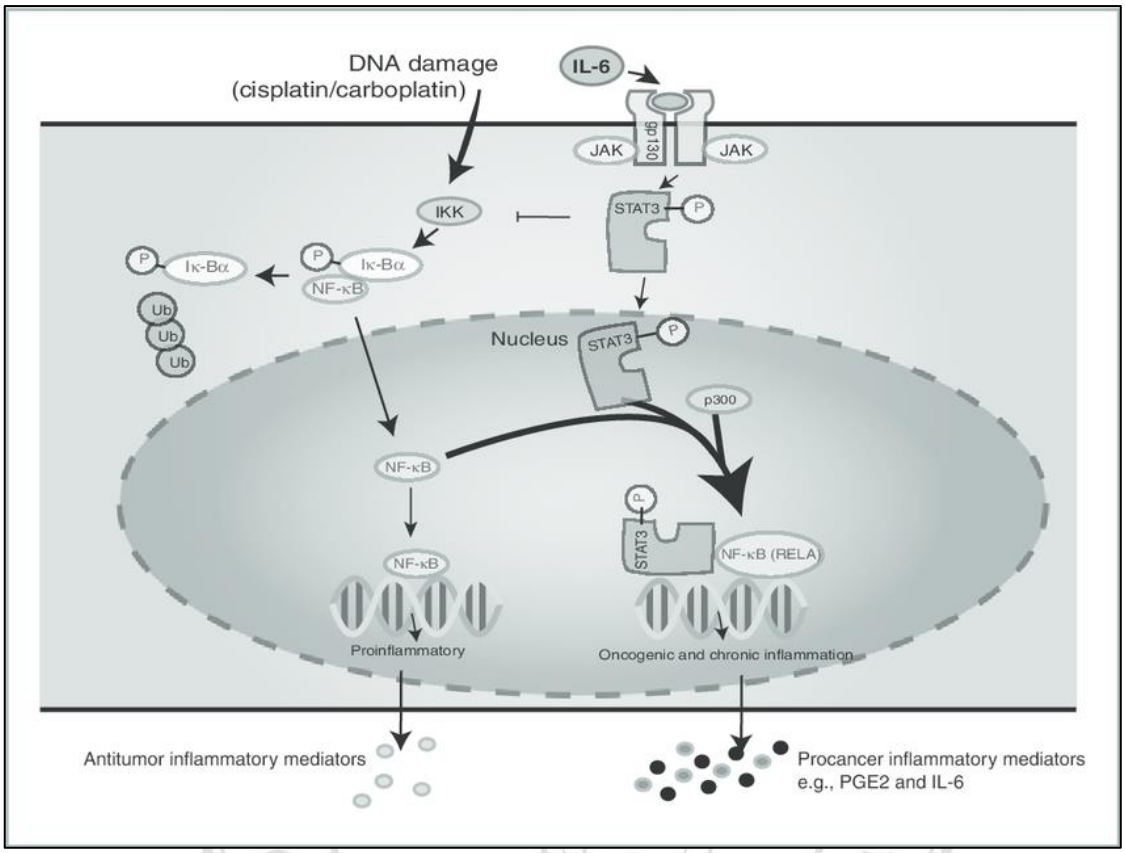

Figure 1.10 Schematic illustration of IL-6 production by cancer cells upon chemotherapy treatment [53]

1.2.5.6 Increased DNA repair and platinum resistance

The cellular response to platinum-DNA damage is a complex process relating to an array of DNA repair pathways, such as base excision repair (BER) and nucleotide excision repair (NER). Hence, malignant cells with higher DNA repair capacity or the ability to induce their DNA repair capacity upon exposure to cytotoxic drugs would be more resistant than those with lower DNA repair capacity [56]. DNA damage that affect a single strand without distorting the helical structure significantly are repaired by base excision repair, while DNA lesion obviously disrupting the DNA helix is repaired by nucleotide excision repair.

As shown in Figure 1.11, X-ray repair cross-complementing group 1 (XRCC1) and excision repair cross complementation group 1 (ERCC1) are important proteins in BER and NER, respectively. XRCC1 serves as strand-break sensor protein in base excision repair. It specifically interrelates with nicked and gapped DNA and promptly responds to DNA damage in the cells. The excision repair cross complementation group 1 protein (ERCC1) is 5′-3′ structure-specific endonuclease and plays a role in nucleotide excision repair. Its increased expression has been associated with resistance to platinum compounds in numerous types of cancers including gastric, ovarian and non-small-cell lung cancers [57, 58]. Recent clinical studies proposed that ovarian cancer patients with low XRCC1 and ERCC1 levels benefit preferentially from cisplatin-based chemotherapy and improved survival in patients with advanced ovarian cancer [59-61].

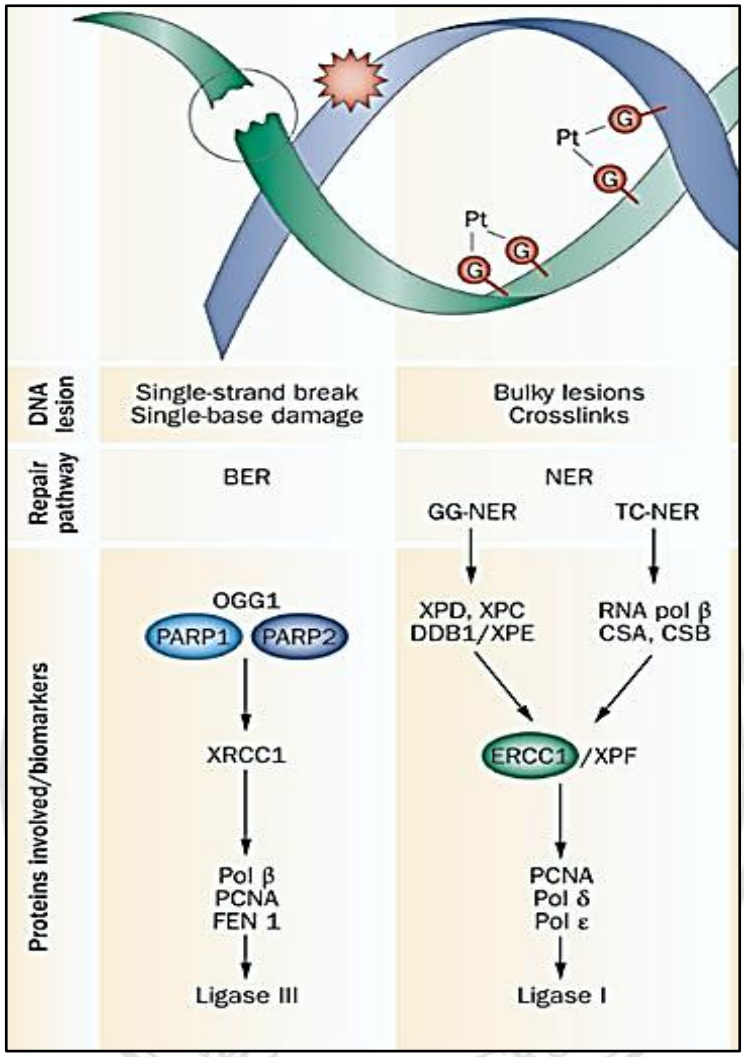

Figure 1.11 Role of XRCC1 and ERCC1 in DNA repair pathways [61]. XRCC1 is involved in the efficient repair of DNA single-strand breaks formed by exposure to ionizing radiation and alkylating agents. This protein interacts with DNA ligase III, polymerase-β and poly (ADP-ribose) polymerase to participate in the base excision repair pathway. DNA damaged due to chemicals which forms crosslinking between DNA strands are repaired by NER, in which the ERCC1 protein interacts with the XPF protein to coordinate DNA and protein binding.

1.2.6 Drug resistance mechanisms in human ovarian cancer cell lines (SKOV3, A2780 and A2780/cis)

SKOV3 cells are derived from the ascitic fluid from patient with ovarian tumor and these cells are intrinsic cisplatin resistance. This may be due to enhanced expression levels of anti-apoptotic proteins such as survivin and Bcl-xL [50], modulation of IL-6 expression and activation of MEK/ERK and PI3K/Akt signaling pathway. A2780 cells were derived from ovarian tumor tissue of untreated patient and they are sensitive to platinum drugs. Acquired cisplatin resistant ovarian carcinoma cell line (A2780/cis) has been established by chronic exposure of increasing concentrations of cisplatin to wild type cisplatin-sensitive A2780 cell line. Acquired cisplatin resistance in human ovarian carcinoma cells is associated with greater repair of cisplatin-DNA adducts by induction of ERCC1 mRNA expression [62], reduced drug accumulation as well as cytogenic abnormalities [63]. So as to maintain resistance, cisplatin has to be appended to the media every 2-3 passages.

1.2.7 Role of natural products in the treatment of cancer

Since the survival of ovarian cancer patients has been enhanced little with the usage of platinum-based chemotherapy, there is a renewed attention in the improvement of more effective agents that could increment the conventional therapeutic strategies. Over 60% of drugs are likely to be natural origin, and therefore, natural compounds have an important role over times in the progress of anti-carcinogenic and other drugs development [64]. In order to have better antitumor responses, the combination of natural products with anti-carcinogenic drugs is a new challenging strategy for anticancer chemotherapy [65].

Many scientists have studied on natural products since they are usually multi-targeted and suitable for chronic diseases like malignancy, which implicates in the abnormal regulation of multiple genes. Moreover, natural compounds are likely to display less toxicity for the reason, that they do not completely knock out or hinder a given protein. Natural products, especially phytochemicals have revealed anti-cancer actions by interfering with the initiation, growth and progression of cancer via the modulation of various cellular events including cellular proliferation, angiogenesis, differentiation, apoptosis and metastasis [65]. One auspicious medicinal plant is *Stephania venosa*, Family Menispermaceae,

which is known as Sabuleud in Thailand (Figure 1.12). The herbal extract from *S. venosa* has been widely used as traditional Thai medicine against Alzheimer's disease, microbial infection, hyperglycemia, malaria and cancer [66, 67]. Phytochemical investigations in many *Stephania* species have presented that there are various forms of aporphine and isoquinoline alkaloids with diverse chemical structures [67].

Figure 1.12 Photograph of *Stephania venosa* which is known as Sabuleud in Thailand

1.2.8 Alkaloids from *Stephania venosa*

The extraction methodology, the yield of extracts and chemical structure elucidation of hexane, ethyl acetate, acetone and methanol extract from the tuber portion of *S. venosa* has been characterized by Dr. Wilart Pompimon from Laboratory of Natural Products, Center for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Lampang Rajabhat University. According to the data from Dr. Wilart, purification of the active ethyl acetate fraction (126 g) after extraction and filtration of air-dried powder form of tuber (6.2 kg) of *S. venosa* yields crebanine (0.17 g) and tetrahydropalmatine (3.13 g) whereas, purification of the active acetone fraction (111.06 g) yields *N*-methyltetrahydropalmatine (0.73 g) and *O*-methylbulbocapnine (1.72 g) by using silica gel column chromatography method [68].

The major constituents obtained from the isolation and purification of the tubers of *S. venosa* are crebanine (CN), *O*-methylbulbocapnine (OMBC), tetrahydropalmatine (THP) and *N*-methyl tetrahydropalmatine (NMTHP). Out of four compounds, CN and OMBC

are isoquinoline derived aporphine alkaloids having similar molecular structure with different side chains. THP and NMTHP are isoquinoline derived protoberberine alkaloids. Their chemical structures are shown in Figure 1.13.

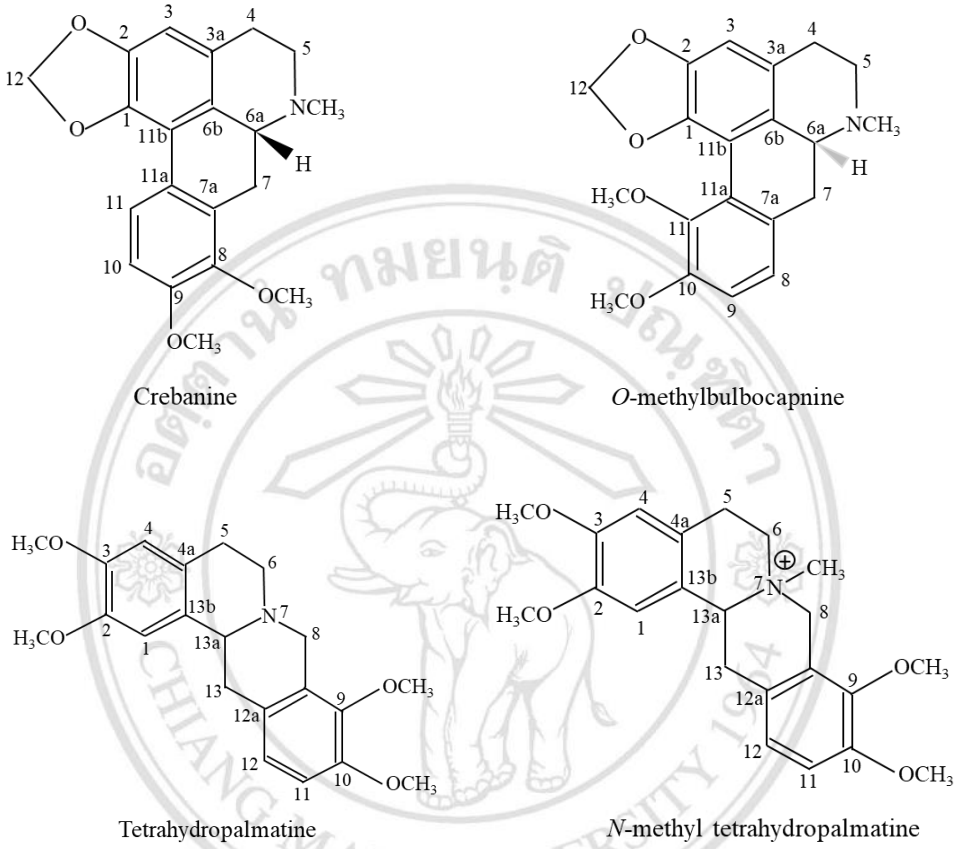

Figure 1.13 Chemical structures of CN, OMBC, THP and NMTHP isolated from *Stephania venosa* [68]

1.2.9 Effects of alkaloids from *S. venosa*

In recent years, CN has attracted much interest due to its many important functional activities such as antimicrobial, antiarrhythmic, relief of neurodegenerative disorder and anticarcinogenic properties [69, 70]. Some studies proposed that CN has an anti-proliferative effect on various cancer cells such as breast cancer cells, lung cancer cells, ovarian cancer cells and fibrosarcoma cells [7] via induction of cellular apoptosis through regulation of cell cycle proteins and activation of the intrinsic and extrinsic apoptotic pathways [7]. Additionally, CN has been displayed to have an anti-invasive effect by suppression of constitutive NF-κB activation [8]. It has also been revealed that both CN

and OMBC down-regulate the expression of genes such as urokinase plasminogen activator and matrix metalloproteinases [8]. Recent study by Sun et al. showed that THP involves with the multidrug resistant reversing properties in breast cancer cells [71]. These well-known results have directed us to hypothesize that aporphine alkaloids may probably improve the efficacy of the conventional chemotherapeutic drugs. However, the chemosensitizing effect of CN and its analogues on ovarian cancer cells as an adjuvant chemotherapy has not been explicated yet.

In the present study, the chemosensitizing effects of CN and its analogues when given together with platinum drugs in ovarian cancer cells were determined. Moreover, the molecular mechanisms of how CN and its analogues mediated their chemosensitizing effects in these cells were also examined. Moreover, the effect of CN on IL-6 induced ovarian cancer aggressiveness were also investigated. Furthermore, the primary cell culture method from solid tumor tissues of ovarian cancer patients was established in order to determine the relationship between IL-6 production in *ex vivo* studies and clinical response to chemotherapy. Additionally, the effect of CN on platinum sensitivity in *ex-vivo* studies was confirmed by giving combination treatment with CN and platinum treatment to primary culture cells with high IL-6 production induced by platinum drug. From this study, aporphine alkaloids might be used as adjuvant therapy in drug resistance cancer as chemosensitizers. Conventional chemotherapeutic drugs when combined with CN or its analogues may help to minimize the dose related toxicity of chemotherapeutic drugs. These findings may have implications for the clinical treatment of ovarian cancer patients.

1.3 Objectives of the study

1. To investigate the chemosensitizing effects and underlying molecular mechanisms of CN and its analogues in human ovarian cancer cells

2. To evaluate the effect of CN on IL-6 induced ovarian cancer aggressiveness