METASTATIC INHIBITORY EFFECT OF GALLIC ACID COMBINED WITH CISPLATIN ON HeLa CELLS PROLIFERATION

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by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

°C	degree Celsius
>	more than
<	less than
cm^2	square centimetre
mL	millilitre
mg	milligram
min	minute
g	gram
µg	microgram
µL	microlitre
µm	micrometre
rpm	revolutions per minute
$\Delta\psi m$	mitochondrial membrane potential
ATCC	american type culture collection
CDC	centers of disease control and prevention
CO_2	carbon dioxide
DMEM	dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide

DNA	deoxyribonucleic acid
EDTA	ethylenediamineteraacetic acid
FBS	fetal bovine serum
FDA	food and drug administration
GA	gallic acid
GSH	glutathione
HCC	human hepatocellular carcinoma
HeLa	human cervical cancer cell line
HER2	human epidermal growth factor receptor 2
HPV	human papillomavirus
H446	human lung cancer cell line
HepG2	human liver cancer cell line
IC50	inhibition concentration at 50% of growth population
MCF-7	human breast cancer cell line
MOA	mechanism of action
NCI	national cancer institute
PAP	papanicolaou
PBS	phosphate buffer saline
PD-1	programmed cell death protein 1

PenStrep	penicillin-streptomycin antibiotics
PS	phosphotidylserine
ROS	reactive oxygen species
SD	standard deviation
SMMC-7721	human liver cancer cell line
USA	united states of america
VIA	viral inspection with acetic acid
V/PI	annexin/propidium iodide
VEGF	vascular endothelial growth factor
WHO	world health organization

KESAN PERENCATAN PENYEBARAN OLEH KOMBINASI ASID GALIK DAN SISPLATIN TERHADAP PROLIFERASI SEL HELA.

ABSTRAK

Disebabkan pelbagai kesan sampingan dalam rawatan kemoterapi telah mendorong banyak penyelidikan berkaitan produk kimia hasilan semulajadi dijalankan untuk merawat pelbagai jenis kanser. Sejenis polifenol, asid galik telah dilaporkan dalam kajian terdahulu mampu untuk melawan pertumbuhan sel kanser. Justeru, kajian ini bertujuan untuk mengkaji kesan perencatan penyebaran sel kanser servik, HeLa yang dirawat dengan kombinasi asid galik dan sisplatin. Dalam kajian ini, asid galik digabungkan dengan agen antikanser, sisplatin. Migrasi sel, pencerobohan sel dan proliferasi sel diukur dengan asai penyembuhan luka gores, asai pencerobohan *Transwell* dan pembentukan koloni masing-masing. Selepas 24 jam asai penyembuhan luka gores, rawatan gabungan menunjukkan pengurangan kadar penyembuhan luka secara signifikan dimana 43.08 ± 6.21% luka telah diisi dengan sel HeLa dalam perbandingan dengan luka gores kumpulan rawatan kawalan. Kombinasi asid galik dan sisplatin juga menunjukkan pengurangan signifikan terbesar terhadap keupayaan pencerobohan sel HeLa pada 44.55 ± 6.04% dalam perbandingan dengan kumpulan rawatan kawalan diukur dengan asai pencerobohan *Transwell*. Kombinasi asid galik dan sisplatin menurunkan proliferasi sel HeLa secara signifikan melalui asai pembentukan koloni. Akan tetapi, kesan yang dihasilkan hasil daripada kombinasi kedua-duanya menunjukkan hasil yang lebih baik daripada rawatan tunggal asid galik dan sisplatin. Oleh itu, kajian lanjutan samada *in vivo* atau *in vitro* adalah diperlukan untuk menguatkan penemuan ini seperti penglibatan laluan isyarat PI3K/AKT/mTOR dalam menurunkan kapasiti migrasi dan pencerobohan sel HeLa.

METASTATIC INHIBITORY EFFECT OF GALLIC ACID COMBINED WITH CISPLATIN ON HELA CELLS PROLIFERATION.

ABSTRACT

Due to the multiple adverse effects of chemotherapy treatment, more researches with natural product discovery has been conducted to treat various types of cancer. A polyphenol derivative, gallic acid, was previously reported to combat the growth of cancer cells. Hence, this study aims to assess the metastatic inhibitory effect of gallic acid combined with cisplatin against HeLa cells. Cell migration, cell invasion and cell proliferation were measured by scratch-wound healing assay, Transwell invasion assay and colony formation assay, respectively. After 24-hour of scratch-wound healing assay, the combined treatment showed significant decrease in wound closure rate where 43.08 ± 6.21% of the wound was filled by the HeLa cells in comparison to control wound width. Combination of gallic acid and cisplatin also exerted a significant decrease in the number of invaded cells at 44.55 ± 6.04% as compared to the control group by Transwell invasion assay. Gallic acid and cisplatin were able to significantly reduce the proliferation HeLa cells by colony formation assay. However, the effect that was produced by their combination was greater than that of each agent alone. Hence further study either *in vitro* and *in vivo* is required to strengthen the current findings such as research regarding the involvement of PI3K/AKT/mTOR signaling pathway in decreasing migration and invasion capability of HeLa cells.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cervical cancer is a “woman’s disease” mostly caused by human papillomavirus (HPV) via sexually acquired infection. World Health Organization (WHO) reported cervical cancer as the fourth most frequent cancer in women worldwide with 7.5% mortality rate in 2018. In addition, cervical cancer appeared to be the third estimated prevalence of cancer in 2018 within 3 years’ time periods from 2015 to 2018. Asian women were the major contributor to these cases with 55.3% followed by Africa and Europe. Patients with cervical cancer commonly have irregular or abnormal vaginal bleeding, fatigue, weight loss due to decrease appetite and a single swollen leg (WHO, 2020). At present, prevention measures of cervical cancer include three different screening tests consisting of visual inspection of the cervix with acetic acid (VIA), Papanicolaou (Pap) smear, and HPV DNA test for precancerous lesions (Kadian *et al*., 2020).

Cervical cancer treatment comes with several modalities including surgery, chemotherapy and radiotherapy. Surgery might be the best choice of treatment as it removes infected tissue from the body (Schnipper *et al*., 2015). Not to mention, immunotherapy also one of the treatments where it stimulates patient's own immune response to recognize and kill the cancer cells without damaging normal cells (Atashzar *et al*., 2020). However, the late diagnosis of cervical cancer in the advanced stage leads to high mortality rates in low- and middle-income countries (Dunyo *et al*., 2018). Neoadjuvant chemotherapy had been used before surgery and radiation therapy. Neoadjuvant therapy is a treatment that being delivered and used as a first step to reduce tumor size before a primary treatment is given. However, neoadjuvant therapy can only be used before the tumor blood flow after surgery and radiation therapy is interrupted. A large tumor might be difficult to treat using neoadjuvant chemotherapy and have a high chance to develop micro-metastatic disease in cancer patient (Park *et al*., 2004). The ability of tumor cell to detach from its primary tumor, migrate, invade and form a new secondary tumor is truly a challenge for scientist and pharmacist to develop a drug. Advanced stage of cervical cancer treatment focused more on stopping and killing malignant cells from dividing and growing. At this stage, radiation and chemotherapy are the only option in combating the disease (NCI, 2020). However, those treatments come with several adverse side effects in the short and long term.

Cisplatin, a common chemotherapy agent approved by Food and Drug Administration (FDA), is prescribed intravenously to treat patients with advanced ovarian cancer, testicular cancer, and bladder cancer. Moreover, cisplatin also used off-label to treat advanced or recurrent cervical cancer and other type of cancers. However, there was

emerging evidence about the dark side of cisplatin therapy towards cancer patients. Being in the market for more than 38 years, cisplatin was said to cause major nephrotoxicity and may lead to drug resistance which is the top health worry (Manohar and Leung, 2018). Following the disadvantages of these conventional drugs, many researchers have been focusing their interest in natural compound as anti-cancer agent.

Gallic acid (GA; 3, 4, 5-trihydroxybenzoic acid) is a natural compound found in many types of plants. It is a phenolic organic compound and a part of tannin where it becomes a powerful antioxidants (Subramanian *et al*., 2015). The first curative property of GA was used as antimalarial in 1953 (Thompson *et al.,* 1953). On top of that, GA was proven to inhibit the growth of HeLa cervical cancer cells via apoptosis and necrosis (You *et al.,* 2010).

Nowadays, combination therapy using two or more therapeutic agents is becoming more popular among researchers. Combination therapy gives more efficacy as it can synergistically act on the same tumor cell with different approaches to kill the cells. Synergistic effect means that combination of two or more drugs exhibits a greater effect than their individual effect alone. It is important for two drugs to achieve the synergistic effect as synergistic interaction enables the use of lower drug concentrations thus reducing adverse side effect. Single therapy or known as monotherapy commonly leads to the development of drug resistance and several adverse effects. Hallmarks of the cancer were stupendous, and some characteristics were still in mystery. The development of drug resistance typically occurred because of their characteristics. Trastuzumab was the drug used as chemotherapy

agents to treat HER2-positive breast cancer tumors. However, to date, single used of trastuzumab have low efficacy as the cancer cells have developed resistance towards the drug (Housman *et al*., 2014). Thus, combination trastuzumab with doxorubicin plus cyclophosphamide followed by paclitaxel plus trastuzumab and total of 1 year of trastuzumab was used at present as research showed that both drugs gave a high efficacy effect in combating breast cancer tumor (Schnipper *et al*., 2015).

1.2 Problem statement

In this current study cisplatin with the combination of natural product, GA was selected to study the effect towards, cervical cancer cell proliferation. Combination of cisplatin and other natural plant sources have been studied to reveal the inhibitory effect on cancer cells. However, the use of cisplatin combined with GA as a treatment for cervical cancer is poorly understood. As both treatments have shown many successful effects on other cancer cells, thus it is beneficial to study and evaluate the effects of GA alone and in combination with cisplatin on cell proliferation and metastasis ability in human cervical cancer cell line (HeLa cells). Furthermore, cisplatin was reported to cause many side effects on cancer patient when taken as a sole medication. Thus, it is essential to find another concomitant drug used along with cisplatin to enhance treatment effectiveness.

1.3 Justification of the study

This study was conducted to provide evidence of a potential alternative combination chemotherapy that could be an option to treat cervical cancer patients. Cancer cells have numerous abilities (Hallmarks of cancer) to evade and change its approaches to escape from chemotherapy agents. On that account, researchers were searching for ways to overcome the problems. Neoadjuvant therapy can only be used before the tumor blood flow after surgery and radiation therapy is interrupted. In consequence, combination of chemotherapy agents is necessary to reduce the development of drug resistance in cancer patients. Multimodality treatments increase the magnitude of therapeutic efficacy even at low and optimum dose. These allow different agents to work on different targets with different mechanisms but achieving the same anti-cancer effect. Hence, it is critical to find the right alternative combination for future use of cancer therapy.

1.4 Objectives of the study

1.4.1 General objective

To assess metastatic inhibitory effect of gallic acid combined with cisplatin on HeLa cells proliferation.

1.4.2 Specific objectives

1. To determine the migration capability of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid plus cisplatin.
2. To determine the invasion capability of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid plus cisplatin.
3. To determine the reproducibility of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid plus cisplatin.

1.5 Hypothesis

1. There is significant effect on migration capability of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid with cisplatin.
2. There is significant effect on invasion capability of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid with cisplatin.
3. There is significant effect on proliferation capability of HeLa cells treated with gallic acid, cisplatin and combination of gallic acid with cisplatin.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of cancer

Cancer is known as a disease that caused human cells to divide infinitely (National Institute of Health, 2015). Normal cells have the capability to divide. Its capabilities for cell division depends on its response towards certain stimuli. For example, cells that are damaged or dead will trigger the normal cells to divide in order to replace the damaged or dead cells. The underlying mechanism in which cells normally divide and grow depends on the presence of proto-oncogene. However, this gene can be mutated or altered to produce a gene which is termed by "oncogene" that will produce tumor cells or cancer cells. This genetic mutation can be passed from parents to offspring. This is the internal factor that caused cancer in an individual. Moreover, external factors that might cause cancer in a patient are exposure towards chemical, radiation and viruses from environment (Krishnan *et al*., 2020). Based on the statement, cancer can arise if there are factors that trigger initiation of the cancer.

Cancer is divided into four stages. Stage I indicates the early stage of cancer that is localize at one part of the body and does not spread to the other nearby lymph node or organs. Stage II is a bit advance than stage I where cancer has spread into the lymph node but not the other parts of the body. Cancer at this stage can be cured with the same treatment for stage I cancer which are surgery or radiation therapy. Stage III refers to a locally advance cancer where cancer has growth into specific size, and sometimes it can grow beyond its original location and metastasize to lymph node, other part of organs and tissues. Stage IV is the serious disease that need immediate expert care. At this stage, cancer has metastasized to the distant organ and usually found after one year of cancer surgery or therapy (Cancer Treatment Centre of America, 2020).

Cancer has its own mechanistic strategies to remain inside human body. Six hallmarks of cancer were proposed by Hanahan and Weinberg in the year 2000 (termed as Hallmarks I) which were self-sufficiency in growth signal, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis and evading apoptosis. Figure 2.1 shows the illustration used to represent Hallmarks of cancer. Later in 2011, they added another two hallmarks (termed as Hallmarks II): reprogramming energy metabolism and evading immune response, and two enabling traits: genome instability and mutation, and tumor-promoting inflammation (Yousef and Aanei, 2017). Mostly normal cells require a stimulus from growth factor to start proliferating. However, cancer cells owned their self-sufficiency in growth signals by mimicking growth signal from normal cells. Cancer cells also able to produce its own endogenous growth signal rather than depends on exogenous stimulus from neighboring cells.

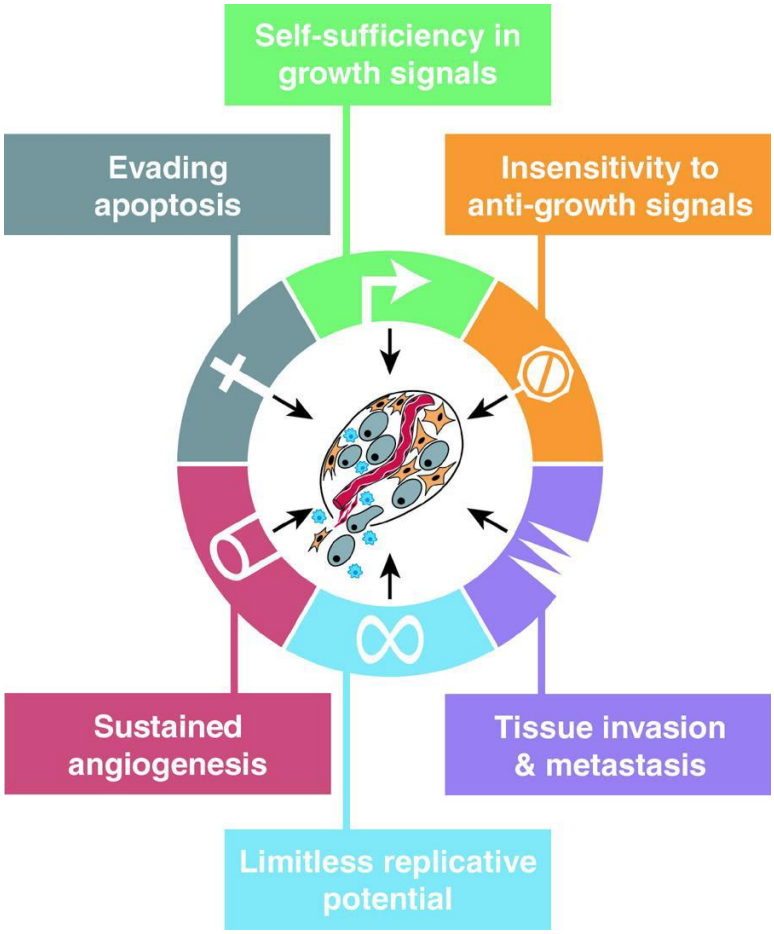

Figure 2. 1: Acquired capabilities of cancer - Hallmark I (Hanahan and Weinberg, 2000)**.**

2.2 Cervical cancer

Cervix is a cylinder-shape of tissue that connect vagina and uterus. Cervix is divided into two portions which are endocervix and ectocervix. Internal and external os are parts of uterus as shown in Figure 2.2. Endocervix extents from internal os (the opening into the uterus) inside of the uterus to external os. Ectocervix is a location at an external os that opens to allow passage from vagina to uterus (Bhatla *et al*., 2018). According to WHO, mortality rate of cervical cancer in Asia population contribute to 168 411 cases in 2018. Approximately 90% of deaths from cervical cancer occurred in low- and middle-income countries.

Cervical cancer is the possible outcome of genital infection with high-risk human papillomavirus (HPV) and is preceded by a phase of persistent HPV infection during which the host immune system fails to eliminate the virus (De Jong *et al*., 2004). Cervical cancer is one of the common malignant tumors in women worldwide. Even though there are a lot of advance screening and preventive measure, the prognosis of cervical cancer is especially poor and the rate of advanced or recurrent patients is barely 10% to 20% with 1 year of survival (Wang *et al*., 2018). Cervical cancer comes with a few symptoms listed by WHO: irregular periods, abnormal vaginal bleeding after sexual intercourse, back, leg or pelvic pain, weight loss, odorous discharge and a single swollen leg (WHO, 2019).

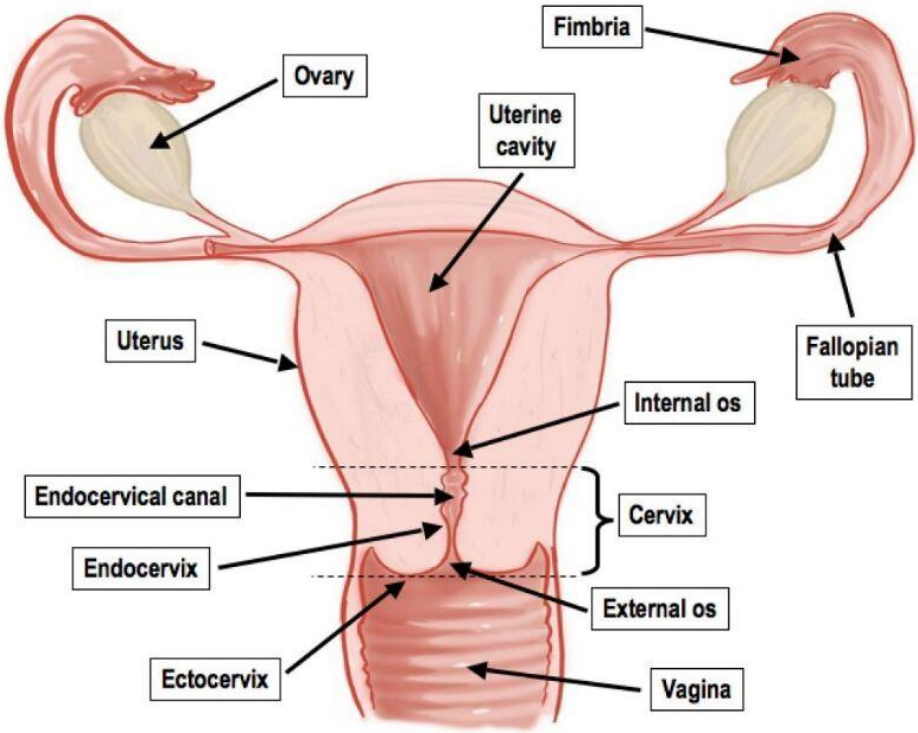

Figure 2. 2: Anatomy of uterus (Anatomy Note, 2019).

Cervical cancer is characterized into four stages and each stage is divided into several parts. Stage I is where the cancer was formed in the cervix. It is divided into IA and IB based on the size of the tumor and the deepest of the tumor invasion point. Stage II of cervical cancer has spread to the upper two-third of the vagina or to the tissue around uterus. It is divided into IIA and IIB based on the distant cancer has spread. Stage III of cervical cancer has spread to the lower third of the vagina and/or to the pelvic wall, and/or has caused kidney problems, and/or involves lymph nodes. Stage III is divided into IIIA, IIIB, and IIIC based on the distant cancer has spread. Stage IV of cervical cancer has spread to the other parts of organs other than pelvis . This stage is divided into IVA and IVB based on the location cancer

has spread (American Cancer Society, 2020). Figure 2.3 showed the illustration of cervical cancer stage IVA that has spread to the urinary bladder and rectum.

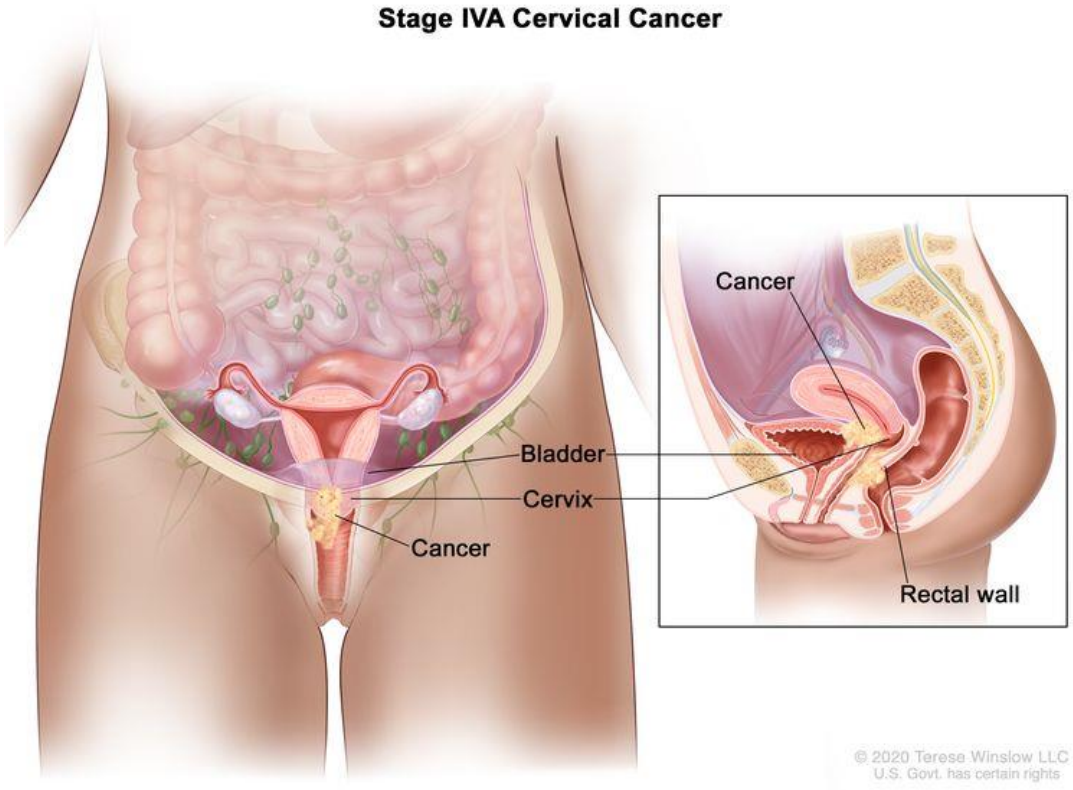

Figure 2. 3: Image of stage IVA of cervical cancer (NCI,2020).

2.3 Cancer metastasis

Tissue invasion and metastasis were one of the other six hallmarks that become a major problem to handle when happened. At this stage, cancer cells capable to invade tissue and organ in the body, move to another part of the body and caused secondary tumor. By that reason, to date, researchers were more focusing to search for a various method that can help to block and contained the cells from metastasize (Hanahan and Weinberg, 2000). Most of the cancer, metastatic cancer was named as stage IV (four) cancer. Furthermore, the metastatic cancer was named after the primary cancer. If cancer starts grew from liver cancer and metastasized to brain, the cancer is term as liver cancer not brain cancer. It is because when cancer cells are viewed under microscope, the feature of metastatic cells are similar with the primary cancer cell not the site where it was found. Cancer can spread to other parts of the body and the most common sites where cancer spreads are bone, liver and lung.

According to NCI, cancer metastasis spreads in a series of steps which could be summarized in Figure 2.0. Normally, metastatic cancer does not show symptoms. However, the symptoms can arise depend on the size and location of the metastatic cancer. Brain cancer patients may be having a headaches, seizures or dizziness; lung cancer patients may be having a shortness of breath; bone cancer patients may be having a pains or fractures; liver cancer patients may be having a jaundice or swelling in the belly. Metastatic cancer can be hard to treat. Even though some metastatic cancer can be cured with current treatment, but most of them cannot. In most cases where the cancer can no longer be controlled, palliative care is

the best to relieve the symptoms of cancer and side effect arise from the treatment (NCI, 2017).

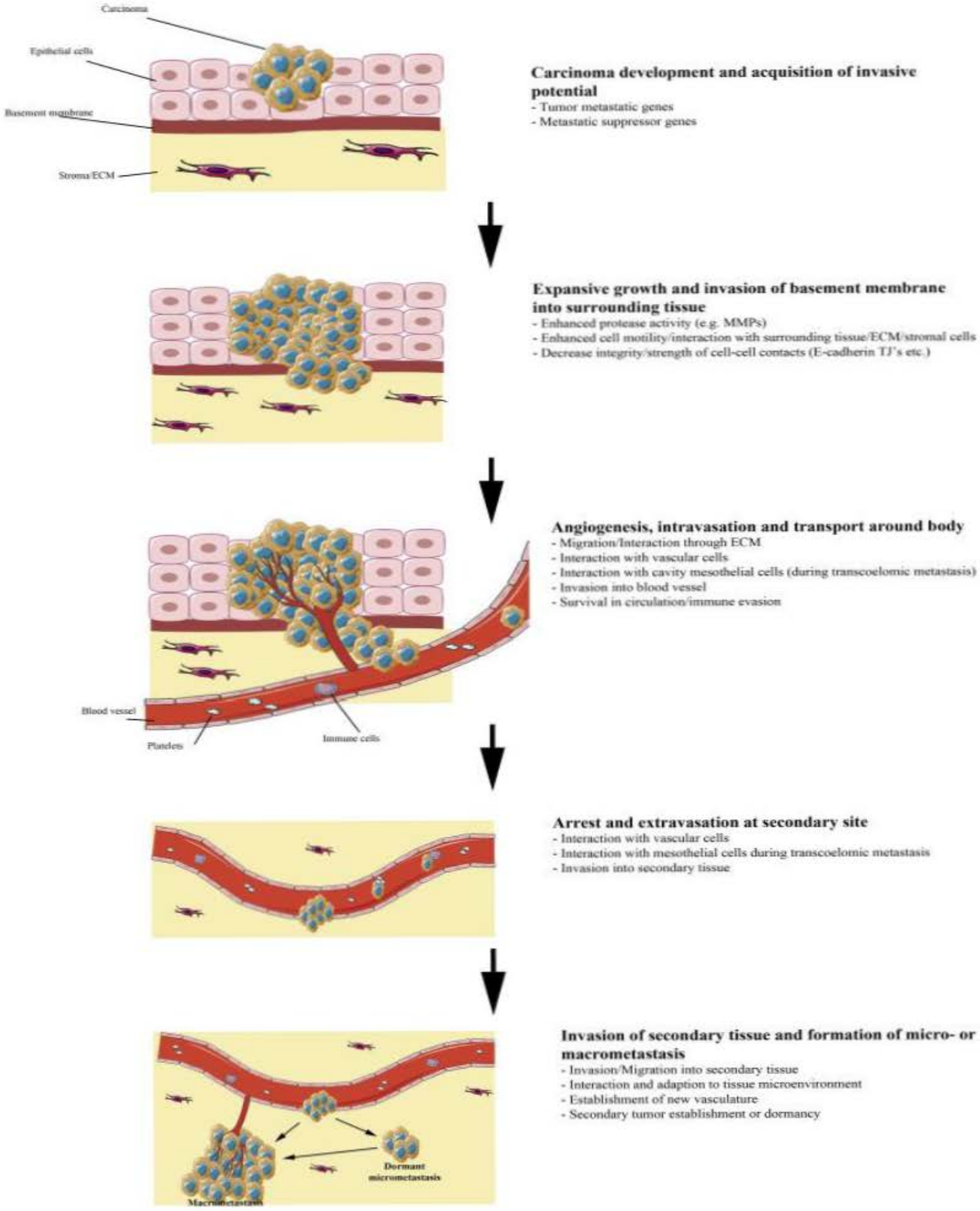

Figure 2. 4: Illustration of a series of steps in cancer metastasis (Jiang *et al.*, 2015)**.**

2.4 Anticancer drug for cervical cancer

According to National Cancer Institute (NCI), there is a list of approved drugs used to treat cervical cancer which are avastin (bevacizumab), bleomycin sulfate, hycamtin (topotecan hydrochloride), heytruda (pembrolizumab), mvasi (biosimilar to avastin), pembrolizumab, and topotecan hydrochloride (NCI, 2019). Pembrolizumab is a generic name for a drug name Keytruda®. It is a monoclonal antibody used to treat multiple type of diseases such as recurrent or metastatic cervical cancer, recurrent or metastatic head and recurrent or metastatic neck squamous cell carcinoma and non-small cell lung cancer. Pembrolizumab is inject intravenously through vein over 30 mins every 3 weeks (ChemoCare, 2002). Pembrolizumab specifically targets the immune checkpoint PD-1, blocking its interaction with its ligands and demonstrated durable antitumor activity and manageable safety in patients with advanced cervical cancer (Chung *et al.*, 2019).

Development of new blood vessels is termed as angiogenesis. Cancer tumors require oxygen and nutrient for cell function and survival. Hence, cancer cells will seize the way to develop new blood vessel for its own. Imbalance of angiogenic inhibitor and angiogenic activator will caused cancer. In cancer, angiogenic activator might be higher than angiogenic inhibitor. Bevacizumab is a monoclonal antibody and anti-angiogenesis drug given by intravenous injection. It is prescribed to patient with metastatic colon, breast, and glioblastoma cancer. According to Minion *et al*. (2015), bevacizumab was used to treat cervical carcinogenesis. Tumor-associated angiogenesis can be inhibited by targeting the

vascular endothelial growth factor (VEGF) pathway with the humanized monoclonal antibody bevacizumab.

Tigecycline is from the class of glycylcyclines. It is a FDA-approved antibiotic drug that can be used for cervical cancer and targeting Wnt (Wingless-related integration site)/β-catenin represents a potential therapeutic strategy in cervical cancer (Li *et al*., 2015). Cisplatin is a cytotoxic chemotherapy drug that is classified as an alkylating group. It is used as a treatment for advanced bladder cancer, metastatic ovarian cancer, and metastatic testicular cancer. Fuertes *et al.* (2012) reported that cisplatin induces its cytotoxic properties through binding to nuclear DNA and subsequent interference with normal transcription and DNA replication mechanisms.

2.5 Cisplatin

Cisplatin is a cytotoxic chemotherapy drug prescribed intravenously in cervical cancer patient. It is a platinum agent with chemical formula of $Cl_2H_6N_2Pt$ (diamminedichloroplatinum). To date, there is no cisplatin in the form of tablet or pill. According to Fuertes *et al*. (2012), biochemical mechanism of action (MOA) of cisplatin involves the binding of cisplatin to the DNA in cell nucleus. The binding will interfere with normal transcription and/or DNA replication. Thus, lead to the cytotoxic process (program cell death) of the cancer cells. The first step in MOA of cisplatin is cisplatin accumulation.

Intravenous injection of cisplatin diffused in tissue and transport throughout the blood vessel. In the blood, plasma protein act as a carrier where it binds to cisplatin and transport to its target. When reach the cell, most cisplatin enters the cell via passive diffusion, and other via facilitated and active transport. Upon arriving the cell, less than 1% of cisplatin will bind to DNA target while others bind with other cellular constituent inside the cytoplasm. N7 atom of guanine and adenine in the DNA are the most reactive site for metal binding. Thus, bind with the platinum agent, cisplatin and resulted in the changes of DNA structure (Fuertes *et al.*, 2012).

Chemotherapy with cisplatin was revealed to induce apoptosis in cancer cells as a mode of cell death (Kilic *et al*., 2015). In spite of that, resistant cancer cell lines showed necrotic cell death characteristic after treatment with cisplatin (Gonzalez *et al*., 2001). In the past, cisplatin had been documented as the most effective drug used as neoadjuvant chemotherapy to treat cervical cancer (Grigsby, 2001; Iwasaka *et al*., 1998). Cisplatin was practically and widely used as chemotherapy agent in cervical cancer (Kilic *et al*., 2015). However, there has also been information on other chemo-drug for cervical cancer such as pembrolizumab (Chung *et al*., 2019) and bevacizumab (Minion *et al.*, 2015). Cytotoxicity property of cisplatin exerts an important anticancer effect but causes several side effects such as nausea, vomiting, low blood count, kidney toxicity, and ototoxicity (ChemoCare, 2002). Initially, a positive response usually happened in patients treated with cisplatin. However, when cancer relapse cisplatin does not give the same curative effect. It is probable that cisplatin resistance has aroused. This resistance was said to result from epigenetic changes at

molecular and cellular levels (Shen *et al*., 2012). These pharmacological drawbacks have stimulated the exploration to find a better chemotherapy agent.

2.6 Role of natural product in cervical cancer treatment

Natural product has become more popular among researchers for their various curative potential of diseases. This is partly due to the presence of several undesired adverse side effects on current chemotherapy. Nowadays, scientists are more attracted to conduct research related to natural compound as an anti-cancer drug. Zaman *et al*., (2016) demonstrated that the usage of nanoformulation-curcumin in cervical cancer cells exert anti-cancer effect as it effectively inhibits cell growth, induces apoptosis and arrests the cell cycle at G1-S transition phase. Curcumin nanoformulation was found accumulate in high concentration in cervical cancer cells due to its smaller size. This study proved that nanotechnology can be used in future as an initiative approach to develop a better anti-tumor drug.

Another study about *tualang* honey (TH) was said able to reduce the mitochondrial membrane potential ($\Delta\psi m$) and induce mitochondrial apoptotic pathway in the breast and cervical cancer cell lines (Fauzi *et al*., 2011). Alabsi and his friends (2012) in their study indicated that goniothalamin, a natural occurring styryl-lactone able to induce apoptosis in HeLa cancer cells. Their flow cytometry analysis revealed accumulation of HeLa cells

arrested in S-phase when treated with gonithalamin and annexin V/PI double staining showed there is increase in the presence of phosphotidylserine (PS). PS translocate from the inner plasma membrane to the outer cellular activity indicates the process of apoptosis where the cancer cells undergo program cell death.

Another study proved that baicalein, active compound of *Scutellaria baicalensis* Georgi induces apoptosis of HeLa cells via mitochondrial and death receptor pathways (Peng *et al.*, 2015). Phenolic acid is divided into hydroxycinnamic acids and hydroxybenzoic acids. The most important compound from hydroxybenzoic acid class is gallic acid (GA). It was said that GA might be accountable in inhibiting angiogenesis in cancer cells (Moga *et al.*, 2016). Furthermore, GA induced apoptotic cell death in HeLa cells by annexin V-staining cells method accompanied by reactive oxygen species (ROS) increase and glutathione (GSH) depletion (You *et al*., 2010).

2.7 Gallic acid

Gallic acid (GA) is a trihydroxybenzoic acid in which the hydroxy groups are at positions 3, 4, and 5. Its molecular formula is $C_7H_6O_5$ or $C_6H_2(OH)_3COOH$ and its structure was shown in Figure 2.3. It has a role as an astringent, an antioxidant, antineoplastic agent, and apoptosis inducer. GA can be richly found in gallnuts, sumac, oak bark, green tea, grapes, strawberries, pineapples, bananas, lemons, witch hazel, red and white wines, apple peels and

orange juice (You *et al*., 2010; Arabi *et al*., 2017). Figure 2.4 illustrates the sources of GA and its derivatives that can be found. GA was reported to have pharmacological activities where it inhibits cell proliferation, cell viability, cell invasion, angiogenesis and induces apoptosis cell death (Nayeem *et al.*, 2016).

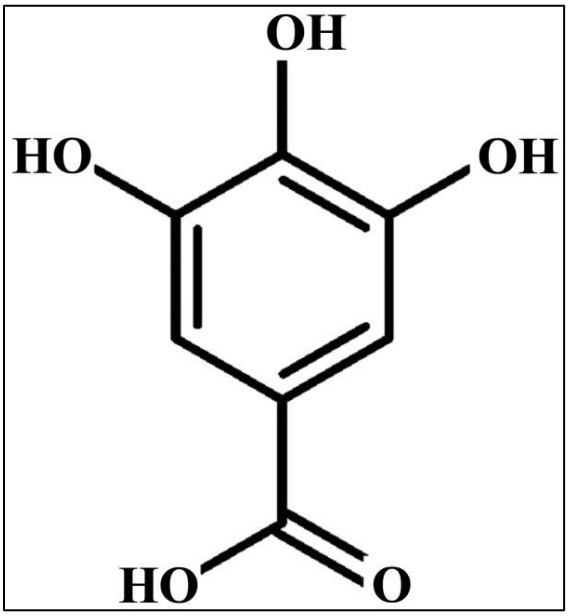

Figure 2. 5: Structure of GA (Sun *et al*., 2016).

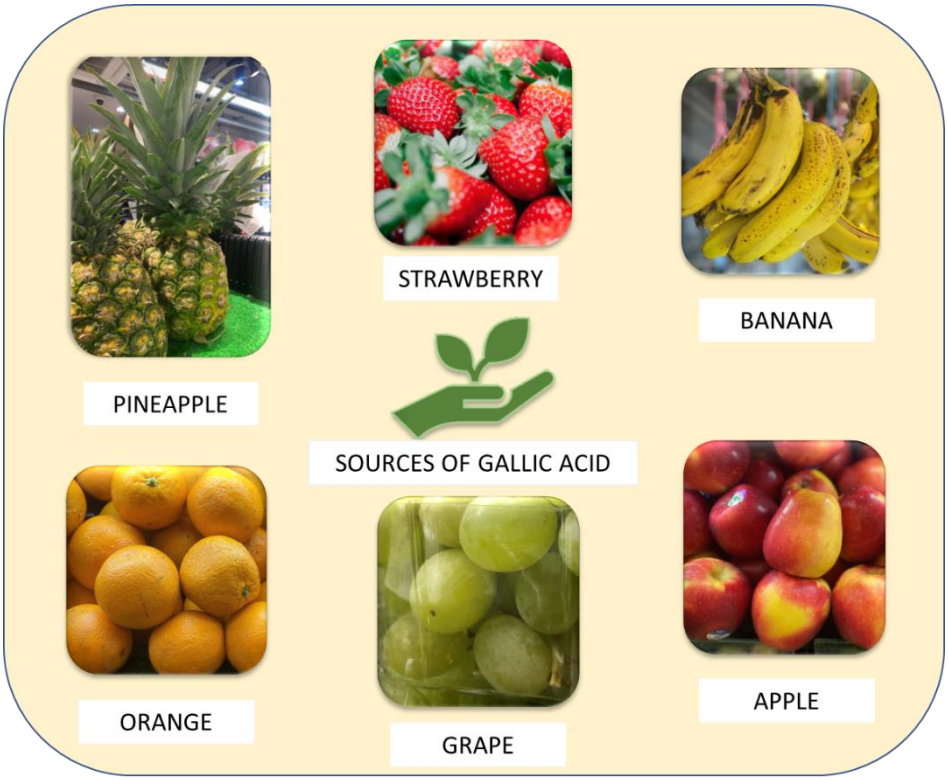

Figure 2. 6: Sources of gallic acid and its derivatives (personal collection).

Moreover, Zhao and Hu (2013) in their study demonstrated GA as an anti-cancer agent where it inhibits cell viability in a dose-dependent manner, cell proliferation, cell invasion and suppression of ADAM metallopeptidase domain 17 (ADAM17), estimated glomerular filtration rate (EGFR), phosphorylated Akt (p-Akt) and phosphorylated Erk (p-Erk) expression. Besides, GA was mentioned to have a high efficacy, lower dosage, and less side effect when combined with paclitaxel for cervical cancer cells treatment. Their combination showed a very promising future in pharmacological industry. However, a future studies need to be done to properly demonstrated the effect in animal models (Aborehab and Osama, 2019).

Liu *et al*. (2020) indicated that GA possess anti-inflammatory effect on rats by weakening lipopolysaccharide-induced neuroinflammation, oxidative stress and protein conjugation. In addition, GA also inhibited lipopolysaccharide (LPS)-induced programmed cell deaths of nigrostriatal dopaminergic neurons of the rat brain, demonstrated neuroprotective effect in central nervous system (CNS) neurogenerative diseases. As eloquently stated by Mahboob *et al*. (2020), GA was valuable for its biological and pharmacological activities. It shows many biological effects which include anti-bacterial, and anti-acanthamoebic. GA able to inhibit 83% of trophozoites and 69% of cysts in *Acanthamoeba triangularis* with no adverse side effects. Furthermore, encapsulated nanoparticles of GA shown to exhibit less cytotoxicity compared to normal GA in human fetal lung fibroblasts MRC-5 cancer cells. This means that GA in encapsulated nanoparticles form could play a major role delivering drugs in the future.

GA also is a strong anti-oxidants and apoptotic agent and it was used by researchers in studies regarding many degenerative diseases like atherosclerosis, cardiovascular disease, aging, and inflammation (Shabani *et al*., 2020). Jin *et al*. (2018) in his study revealed that GA able to improve cardiac dysfunction and fibrosis in pressure overload-induced heart failure where it decreases left ventricular end-diastolic and end-systolic diameter also perivascular fibrosis by Trichrome II Blue staining.

2.8 Combination therapy

Combination therapy in cancer is not a new method used nowadays. In most cases, practitioners combine surgery, radiation therapy, immunotherapy and chemotherapy depends on the profile cases. Focusing on chemotherapy, researchers are now striving to search for a new combination chemotherapy or a better drugs that can reduce the multidrug resistance. Park and his collegues (2004) reported that 39 out of 43 cervical cancer patients showed 90.7% response rate towards neoadjuvant chemotherapy of paclitaxel-cisplatin in phase II trial. In his study, toxicity of chemotherapy was reduced by optimizing the drug dosage. Besides, treatment effectiveness was increased in a shorter time by combining paclitaxel with cisplatin and modifying treatment interval.

In this study, searching for alternative treatment agent to treat advanced and metastatic cervical cancer were priorities. The combination of high doses of vitamin C and cisplatin could decrease the adverse effect of chemotherapy (Reddy *et al*., 2001). Combination therapy of cisplatin and radiotherapy increases survival rate of patient with locally advanced cervical cancer, large stage IB tumors (prior to surgery) and high-risk early-stage disease (following surgery) (Lukka *et al.*, 2002). The combination of paclitaxel with cisplatin to be used in neoadjuvant chemotherapy appears to be tolerated and very energetic in cervical cancer (Park *et al*., 2004). Mitochondria is a place for apoptosis intrinsic pathway. According to Wang and his friends (2016), combination treatment of GA and cisplatin exhibit