THE CHEMO-SENSITISATION EFFECT OF CURCUMIN AGAINST LOW DOSE CISPLATIN ON NON-SMALL CELL LUNG CANCER (NSCLC) STEM CELLS

by

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LIST OF ABBREVIATIONS

MTS	[3-(4, 5-dimethylthiazol-2-yl)-2H-tetrazolium
CDF	3, 4-difluorobenzylidene curcumin
EF-24	3, 5-bis [(2-fluorophenyl) methylene]-4-piperidinone
NNK	4-(methylnitrosamine) -1- (3-pyridyl) - butanone
FOLFOX	5-FU plus oxaliplatin
AML	Acute myeloid leukaemia
AC	Adenocarcinoma
ALDH	Aldehyde dehydrogenase
ALDH1	Aldehyde dehydrogenase 1
ATCC	America type culture collection
ALK	Anaplastic lymphoma kinase
ABC	ATP- binding cassette
ABCG2	ATP- binding cassette superfamily G member 2
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CSC	Cancer stem cells
CO2	Carbon dioxide
CIAX	Carbonic anhydrase IX
CID	Collision induced dissociated
cDNA	Complementary DNA
CDk	Cyclin-dependent kinase
CYP	Cytochrome P450
DAVID	Database for annotation, visualization and integrated discovery
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
DEPC	Diethylpyrocarbonate
DMSO	Dimethyl sulfoxide
DMEM F12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
EGFR	Epidermal growth factor receptor
EGF	Epithelial cell growth factor
EPCAM	Epithelial cell adhesion molecule

EMT	Epithelial mesencymal transition
EDTA	Ethylenediaminetetraacetic acid
ECM	Extracellular matrix
FDR	False detection rate
FBS	Foetal bovine serum
bFGF	Fibroblast growth factor
FACS	Fluorescence activated cell sorting
FITC	Fluorescence isothiocyanate
FC	Fold change
FDA	Food and drug administration
GRPs	Gefitinib-resistant persisters
GO	Gene ontology
GAS6	Growth arrest specific 6
HNSCC	Head and neck squamous carcinoma cells
MB	Human medulloblastoma
HIF-1	Hypoxia inducible factor 1
SCID	Immune deficient mice
IC50	Inhibition Concentration
iPSCs	Induced pluoripotent stem cells
IPTA	Institut Pengajian Tinggi Awam
IPTS	Institut Pengajian Tinggi Swasta
IMR	Institute Medical Research
K1	K1 papillary thyroid
KEGG	Kyoto Encyclopedia of Genes and Genomes
LCC	Large Cell Carcinoma
Hep2	Laryngeal carcinoma cells
LTQ-orbitrap velos	Linear Trap Quadropole Orbitrap Velos
LC-MS/MS	Liquid chromatography mass spectrometry
IMR-90	Lung Fibroblast
LTQ-orbitrap velos	Linear Trap Quadropole Orbitrap spectometry
LRP	lung resistance protein
MAKNA	Majlis Kanser Kebangsaan
MNCR	Malaysian National Cancer Registry Report

mTOR	Mammalian target of rapamycin
MMP	Metalloproteinases
MTS	Methyl tetrazolium salts
MCACs	Monocarbonyl analogues of the curcumin
MRP-1	Multi resistant protein
MDR	Multi-drug resistant
NNN	N-nitrosonornicotine
NSCLC	Non-small cell lung cancer
NFKB	Nuclear factor-kB
PS	Penstrip
P-gp	P-glycoprotein
PBS	Phosphate buffer saline
PAH	Polycyclic hydrocarbon
PCR	Polymerase Chain Reaction
pH	Potentiol of hydrogen
PHBEC	Primary Human Bronchiol /Tracheal Epithelial Cells
PI	Propidium iodide
PC-3	Prostate cancer
RT-qPCR	Quantitative Real Time Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain reaction
Rc	Regenerated cellulose
rnase A	Ribonuclease A
RNA	Ribonucleic acid
rRNA	Ribosomal subunit RNA
RPMI 1640	Rosewell Park Memorial Institute medium
SP	Side Population
SCLC	Small Cell Lung Cancer
DM-1	sodium 4-[5-(4-hydroxy-3-methoxy-phenyl)-3-oxo-penta-1, 4-dienyl]-2-methoxy-phenolate
NACL	Sodium chloride
SCC	Squamous cell carcinoma
SD	Standard deviation
TSNA	Tobacco specific nitrosamines

TCA	Trichloroacetic acid
ULA	Ultra-Low Attachment
WHO	World Health Organization

LIST OF SYMBOLS

~	approximately
cm	centimeter
^{o}C	degree Celsius
g	g-force
gm	gram
hrs	hours
kV	kilovolt
m/z	mass to charge ratio
µg	microgram
uL	microliter
µm	micrometers
µM	micromolar
µ	micron
mm	milimetre
mM	milimolar
mg	milligram
mL	milliliter
mins	minutes
M	molar
ng	nanogram
nm	nanometre
nM	nanomolar
%	percentage
rcf	relative centrifuge force
rpm	revolution per minutes
s	seconds

KESAN KEMO-PEMEKAAN KURKUMIN TERHADAP DOS RENDAH SISPLATIN PADA SEL-SEL TUNJANG KANSER PARU-PARU SEL BUKAN KECIL

ABSTRAK

Kanser sel tunjang mewakili populasi kecil ketumbuhan dan memiliki ciri-ciri sel tunjang akan tetapi mempunyai daya rintangan terhadap ejen sitotoksik yang menjadi penyebab kepada kanser berulang. Bahan semulajadi seperti kurkumin yang tinggi kandungan polifenol mempunyai ciri kemo-pemekaan dan kemampuan untuk memeka kanser sel tunjang kepada agen sitotosik seperti sisplatin. Oleh itu, kajian ini telah dirancang untuk mengkaji keberkesanan kurkumin sebagai kemo-pemekaan terhadap kanser sel tunjang paru-paru dan juga menganalisa kadar perencatannya terhadap aktiviti kanser sel tunjang menggunakan garis sel A549 dan H2170 yang berasal daripada kanser paru-paru sel bukan kecil (*NSCLC*). Garis-garis sel *NSCLC* dikultur dan kemudian, dirawat dengan pelbagai kepekatan sisplatin dan kurkumin untuk mendapatkan separuh kepekatan merencat (IC_{50}) menggunakan teknik *3-(4, 5-dimethylthiazol-2-yl)-2H-tetrazolium, inner salt (MTS).* Kanser sel tunjang paru-paru dengan fenotip CD166+EpCAM+ telah dipencilkan menggunakan *fluorescence-activated cell sorting* (FACS). Keberkesanan kurkumin untuk memeka kanser sel tunjang diperhatikan melalui ujikaji apoptosis dan ciri-ciri sel tunjang seperti kemampuannya untuk bermigrasi, membentuk koloni dan sferoid sel. Ekspresi gen yang terlibat dalam apoptosis dan pembaharuan diri dinilai berdasarkan *mRNA* dengan menggunakan tindak balas rantaian polymerase masa nyata. Kesan kurkumin terhadap persekitaran kanser sel tunjang dianalisis dengan menggunakan spektrometri jisim kromatografi cecair (*LCMS*). Hasil kajian mendapati pendedahan garis-garis sel

NSCLC terhadap kurkumin (10 µM – 40 µM) mengurangkan peratusan sel yang hidup kepada purata ~51% dan ~54% terhadap A549 and H2170. Tambahan lagi, pemekaan *NSCLC* terhadap kurkumin menyebabkan pengaruhan apoptosis terhadap CD166+EpCAM+ kanser sel tunjang sebanyak 18% dan 20% masing-masing pada A549 dan H2170. Di samping itu, kurkumin juga merangsang kemampuan perencatan sisplatin terhadap migrasi CD166+EpCAM+ kanser sel tunjang dengan penurunan sebanyak 9% dan 21% pada A549 dan H2170. Ini membuktikan kurkumin dapat meningkatkan pemekaan kanser sel tunjang terhadap sisplatin sekaligus merencatkan migrasi sel. Kurkumin menunjukkan kesan yang signifikasi (p<0.001) dengan kemampuan menghalang pembentukan koloni sebanyak 50% - 57% dan kekecutan sferoid di dalam A549 CD166+EpCAM+ dan H2170 CD166+EpCAM+ kanser sel tunjang sekaligus membuktikan perencatan terhadap kemampuan pembaharuan sel seperti yang ditunjukkan pada pengawalaturan menurun *SOX2, NANOG* dan *KLF5* yang dikawal atur secara menurun. Ekspresi *APAF1, CASPASE-9* dan *CYTOCHROME-C* dikawal atur secara menaik manakala *CYCLIN D-1* dikawal atur secara menurun mencadangkan gabungan rawatan adalah pro apoptosis dan merangsang kitaran sel terhenti lalu menyebabkan perencatan kanser sel tunjang. Tambahan lagi, kurkumin mempamerkan keupayaan merencatkan protein kemo-rintangan seperti *aldehyde dehydrogenase (ALDH)* lantas menunjukkan potensi terapi menggunakan kurkumin untuk mensasar kanser sel tunjang *NSCLC*.

THE CHEMO-SENSITISATION EFFECT OF CURCUMIN AGAINST LOW DOSE CISPLATIN ON NON-SMALL CELL LUNG CANCER (NSCLC) STEM CELLS

ABSTRACT

Cancer stem cells (CSCs) represent a small subpopulation within a tumour that possesses the stem-like properties but are also initiating resistance towards cytotoxic agent which contribute to cancer relapse. A natural compound such as curcumin that contains high polyphenol has been found to possess chemo-sensitivity effect with an ability to sensitise the CSCs to the cytotoxic agents such as cisplatin. Therefore, the present study was designed to investigate the efficiency of curcumin as a chemo-sensitiser in lung CSCs and to analyse its inhibitory effect on CSCs activity using A549 and H2170 cell lines that belong to non-small cell lung cancer (NSCLC). The NSCLC cell lines cultures were treated with various concentrations of cisplatin and curcumin to obtain the inhibitory concentration (IC_{50}) using 3-(4, 5-dimethylthiazol-2-yl)-2H-tetrazolium, inner salt (MTS) assay. The lung CSCs with phenotype CD166+EpCAM+ was isolated using fluorescence-activated cell sorting (FACS). The efficiency of curcumin to sensitise lung CSCs was observed on apoptosis and stemness characteristics including migration ability, colonies and spheroid formation. The mRNA level was analysed for genes involved in apoptosis and stemness using quantitative real time-polymerase chain reaction (RT-qPCR). Liquid chromatography mass spectrometry (LCMS) was used to evaluate the effect of curcumin on CSC niche. The results discovered that exposure of NSCLC cell lines to curcumin (10 µM – 40 µM) reduced the percentage of cells viability to an average of ~51% and ~54% in both A549 and H2170. Furthermore, sensitisation of NSCLC to curcumin induced the apoptosis of CD166+EpCAM+ CSC subpopulation by 18% and 20% in A549 and

H2170 cells respectively. Moreover, curcumin also enhanced the inhibitory capability of cisplatin on CD166+EpCAM+ CSC subpopulation, as manifested by the reduction of cell migration to 9% and 21% in both A549 and H2170 respectively, representing that curcumin may increase the sensitivity of CSC to cisplatin inducing migratory inhibition. Curcumin significantly ($p<0.001$) suppressed the formation the of colonies by 50%- 57% and shrank the spheroid in A549 and H2170 CD166+EpCAM+ CSC subpopulations thus indicating the inhibition of self-renewal capability as manifested by down-regulated of *SOX2*, *NANOG* and *KLF5*. The *APAF1, CASPASE 9* and *CYTOCHROME-C* were up-regulated while substantial decreased in *CYCLIN D-1* suggested that the combined treatments were pro apoptosis, induced cell cycle arrest thus triggering inhibition of CSCs. Curcumin also has demonstrated its ability to inhibit the chemo-resistance protein, aldehyde dehydrogenase (ALDH) therefore demonstrated the potential therapeutic approach of using curcumin in targeting CSC sub-population in NSCLC.

CHAPTER 1: INTRODUCTION

PREFACE

Some findings and protocols presented in this thesis have been published in Oncology Report journal. The research article published in Oncology Report entitled "Curcumin improves the efficacy of cisplatin by targeting cancer stem-like cells through p21 and cyclin D1-mediated tumour cell inhibition in non-small cell lung cancer cell lines" (Baharuddin et al., 2016) covers all results presented in this thesis. Permission to republish the data included in the manuscript has been obtained from the respective journals (Appendix A).

1.1 Cancer

Cancer is uncontrollable dividing growth of abnormal cells. It can develop anywhere in the body and may eventually spread to other parts of the tissues and organs. Cancer begins when genetic changes impaired therefore acquires a series of gene mutations that leads to uncontrollable growth thus forming a tumour (Futreal et al., 2004). Typically, tumour can be malignant or benign. A malignant tumour is a cancerous tumour that is capable of spreading to other parts of the body while benign tumour is non-cancerous and will not spread. The common type of cancers are carcinomas, sarcomas, leukaemias and lymphomas (*www.cancer.net*). Carcinomas usually are from solid tumours which begin in the skin that cover the surface of internal organs such as prostate, breast, lung and colorectal cancer. While sarcomas develop in the tissue that connect the body such as blood, muscles, nerves, tendons, joints or bone. Whereas, leukaemias is a blood cancer and lymphomas begin in the lymphatic system that help to fight the infection. The hallmarks of cancer are based on six characteristics (Hanahan & Weinberg, 2011).; 1) able to sustain proliferative signalling; 2) able to evade growth suppressors; 3) able to resist cell death; 4) enabling replicative immortality; 5) able to induce angiogenesis and 6) capable of activating invasion and metastasis.

1.2 Global incidence of cancer

Cancer is a leading cause of death worldwide accounting for 8.8 million deaths in 2015. In the latest cancer statistic reported in 2016, the overall cancer incidence in Unites States was estimated 1,685,210 cases or equivalent to 4,600 new cancers was diagnosed each day. It was also indicated that cancer is expected to commonly occur in men with 42% than women with 38%. Based on recent cancer statistics (Siegel et al., 2016), in men, 44% of all cases covering the prostate, lung, bronchus and colorectal

cancer while in women, the 3 most common cancer types are breast, lung and bronchus that account for 29% of new cancer diagnosed. The recent trends in cancer incidence highlight 4 major types of cancer as having the leading incidence rates with lung cancer having the highest incidence rate followed by breast, prostate and colorectal cancer. Cancer occurrence also varies among the ethnic groups. It was revealed that black men have the highest overall cancer incidence and death rates than the Hispanic white men (Siegel et al., 2016). Factor contributing to racial disparities include access to high quality of health care such as cancer prevention and early detection facilities. According to US Census Bureau in 2014, 26% of black men are burden with poverty while 12% live without health insurance. Moreover, black men are unlikely to receive standard cancer therapies especially for lung, breast, colorectal and prostate cancer (Bach et al., 2002).

1.3 Cancer in Malaysia

The increase in trends of cancer incidence in Malaysia attracts attention of many government and non-government sectors. The establishment of *Majlis Kanser Kebangsaan* (MAKNA) or National Cancer Council, National Cancer Society Malaysia, *Institut Kanser Negara* and numerous research institutes in academic institutions, including *Institut Pengajian Tinggi Awam* (IPTA) and *Institut Pengajian Tinggi Swasta* (IPTS) or non-academic institution such as Institute Medical Research (IMR) shows the Malaysian government commitment towards eradicating cancer diseases. According to Malaysian National Cancer Registry Report (MNCR), during the period between 2007 and 2011, a total of 103,507 new cancer cases were diagnosed which included 45.2% reported cases in males and 54.8% reported cases in females. The five most common cancers among males were colorectum (16.3%), lung (15.8%),

nasopharynx (8.1%), lymphoma (6.8%) and prostate (6.7%) cancers. In the other hand, among females, the most common were breast (32.1%), colorectum (10.7%), cervix uteri (7.7%), ovary (6.1%) and lung (5.6%) cancers. MNCR also reported the incidence of 10 common cancers in Malaysia and the top three were breast, colorectal and lung cancers in descending order (Table 1.1).

Table 1.1 Ten most common cancer incidence in Malaysia, 2007-2011.
Data collected from MNCR.

Cancer type	Number of cases	%
Breast	18,343	17.7
Colorectal	13,693	132
Trachea, Bronchus, Lung	10,608	10.2
Lymphoma	5,374	5.2
Nasopharynx	5,090	4.9
Leukaemia	4,573	4.4
Cervix Uteri	4,352	4.2
Liver	4,128	4.0
Ovary	3,472	3.4
Stomach	3,461	3.3
Others	30,413	29.4
Total	103,507	100.0

1.4 Lung cancer

The most common death-causing cancer types as reported by World Health Organization in 2015 (*www.who.int/cancer*) are lung cancer with 1.69 million deaths, followed by liver cancer (788, 000 deaths), colorectal cancer (774, 000 deaths),

stomach cancer (754, 000 deaths) and breast cancer (571, 000 deaths). A recent cancer statistic published in 2016 revealed that lung and bronchus cancer has taken first place among others types of cancer in both males and females with 27% and 26%, respectively (Siegel et al., 2016). Meanwhile, in Malaysia, according to MNCR, lung cancer accounts for 10.2% of all cancers in Malaysia during the period of 2007-2011. The distribution of lung cancer in Malaysia has shown changes in pattern, in which squamous cell carcinoma (SCC) was the most common cell type in the past whilst adenocarcinoma (AC) is the most common cell type in recent years (Liam et al., 2006).

Lung cancer consists of heterogenous groups in pathological features and is commonly classified into the following two major types, small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC). SCLC comprises nearly 20% of lung cancer cases (Okudela et al., 2011, Freitas et al., 2014). In fact, SCLC is fast-growing and rapidly spreads compared to NSCLC which grows and spreads more slowly (Freitas et al., 2014). Approximately 80-85% of lung cancer is classified as NSCLC (Mou et al., 2011). NSCLC also is a group of heterogenous histological types, the majority of which are AC and SCC with roughly similar frequencies (30-40% each), and large cell carcinoma (LCC) with a lower frequency (<10%) (Okudela et al., 2011, Freitas et al., 2014)

Targeting NSCLC in lung tumours would be necessary as it comprises of approximately 85% cases with 5 years survival rates (Chen et al., 2014, Zheng, 2016). At least 70% of surgical cases come from AC that occupied almost 60% of the NSCLC cases (Lewis et al., 2014). In 2014, there is an intense effort on studying AC pathogenesis with the inclusive molecular profiling (Cancer Genome Atlas Research

Network, 2014). The study found 18 mutated genes such as Ras like without CAAX 1 (*RITI*), epidermal growth factor receptor (*EGFR*), RNA binding motif protein 10 (*RBM10*), neurofibromin 1 (*NF1*), MET proto-oncogene (*MET*), er-b2 receptor tyrosine kinase 2 (*ERBB2*) and others, some of which were commonly found in female and male patients. Meanwhile, SCC represent 20% of lung cancer cases (Lewis et al., 2014) and can be categorised into three subtypes; 1. Keratinizing SCC, 2. Non-keratinizing SCC and 3.basaloid SCC (Travis et al., 2015). Previously, treatment for SCC is difficult as no molecular target agents have been established. However, in 2012, a comprehensive genomic study (Cancer Genome Atlas Research Network, 2012) of SCC was conducted in Cancer Genome Atlas project. The information emanated from this project were used for subsequent beneficial studies that eventually led to new therapeutic approach such as using Nivolumab (Brahmer et al., 2015) or Pembrolizumab (Garon et al., 2015) for SCC treatments. Apart from that, LCC only comprises of 3% lung cancer cases but its diagnostic and clinical properties are yet to be properly understood. This is due to lack of evidence of morphologic differentiations and immunohistochemical characteristics (Rekhtman et al., 2013) thus its diagnosis required more attention under light microscope and validation through immonohistochemical staining.

In this study, the A549 and H2170 cell lines derived from NSCLC were used to represent the AC and SCC. According to cytogenetic information from ATCC, the A549 is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Unlike normal cells that have 46 chromosomes, both A549 and H2170 having an abnormality karyotype with 68 and 61 chromosome number respectively (Zakaria, 2017). The A549 cells are positive for keratin by

immunoperoxidase staining (*www.atcc.org*) and was able to synthesis lecithin with high percentage of desaturated fatty acids which essential for the maintenance of membrane phospholipids in cells (*www.a549.com*). The AC often expressing mutations in the *K-ras* oncogene and is characterised by peripheral location in the lung. The SCC are centrally located and usually carry *p53* gene mutations (Nacht et al., 2001). In the aspects of etiology, SCC is attributed to tobacco smoking while AC remains unclear (Bennett et al., 1999, Hainaut & Pfeifer, 2001). Briefly, both cells were originated from human lung tissue of male donor and showed epithelial morphology and are adherent type of cells. Precisely, A549 cell line was established in 1972 by D.J. Giard through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male while, H2170 was described to be squamous carcinoma disease which was established in April 1989 from non-smoker male donor. All the data were documented by the America Type Culture Collection (ATCC) (*http://www.atcc.org*). Human lung fibroblast (IMR-90) was a normal human lung cell used for the toxicity study. This cell was derived from W.W. Nichols and was associated from the lungs of a 16-week female foetus. The cells are epithelial morphology like and are adherent type of cells.

1.5 The risk factors associated with lung cancer

Tobacco smoking is the most factor contributing to many types of respiratory diseases including lung cancer (Hackshaw et al., 1997, Zhong et al., 2000). Sadly, the active smokers not only harm themselves, but they also harm the people surrounding them by second hand smoke. The second hand smoke is defined as exhalation of smoke by a smoker and the burning tip of cigarette (Law & Hackshaw, 1996) subsequently causes a contaminant in air , thus it is inhaled by everyone exposing both smokers and

non-smokers to its dangerous effect causing the risk to lung cancer. In fact, second hand smokers are responsible for almost 600, 000 premature deaths a year which involved women and children with 47% and 28% respectively (World Health Organization, 2014).

Unfortunately, there is no save level of exposure to tobacco smoke (Öberg et al., 2011). The force of tobacco smoke to induce cancer is due to large amount of toxic compounds that constitute in smoke where there are over 4,000 chemicals with at least 40 compound can cause cancer (World Health Organization, 2014). Mainstream cigarette smoke contains three potent carcinogenic compounds which are polycyclic hydrocarbon (PAH), N-nitrosamine and aromatic amines. Different forms of these three major compounds can be present in smoke. For instance, three forms of PAH which are benzo[*a*] pyrene, benzo [*a*] anthracene and chrysene are found in smoke (Rossini et al., 2008). While, eight different N-nitrosamine compounds are commonly present in cigarette smoke but the main ones were tobacco specific nitrosamines (TSNA), 4-(methylnitrosamine) -1- (3-pyridyl) - butanone (NNK) and N-nitrosonornicotine (NNN) (Rossini et al., 2008). All the carcinogenic compounds in smoke cigarette required a metabolic activation to utilise their effect as such cytochrome P450 (CYP) enzymes, where it will react with the DNA thus inducing tumour. Similarly, TSNA and NNN (Humans et al., 2007) as well nicotine (Nakajima et al., 1996, Messina et al., 1997) were metabolized by CYP2A enzyme, an enzyme that expressed 90% in lung microsomes of human (Zhang et al., 2007). It causes damage in DNA by forming DNA adducts through covalent binding and lung cancer risk (Wei et al., 2000).

Besides, exposure to polluted environmental gases which also contain carcinogens other than tobacco smoke are also contributing to the development of lung cancer, but the burden of these exposures is small as compared to cigarette smoking that account for ~10% of lung cancer (Alberg & Samet, 2003). These include asbestos, radon, tar and soot (sources of polycyclic aromatic hydrocarbons), arsenic, chromium, nickel, beryllium, and cadmium (Straif et al., 2009). In fact, cigarette smoke works synergistically with these carcinogens thus increase the risk of lung cancer (Saracci, 1987). Other than that, radiation exposure (Miglioretti et al., 2013) air pollution, (Katanoda et al., 2011), dietary factors (Marmot et al., 2007) and physical activity (Tardon et al., 2005) also contribute to the risk.

1.6 Treatment for lung cancer

Radiotherapy, surgery and chemotherapy are the common methods used in treating patients that are diagnosed with cancers. Among all these methods, chemotherapy is the most common practice in cancer treatment with the use of specific drug for specific cancers types. Chemotherapy drugs are usually used as the first line in treating lung cancer. Patients with NSCLC generally have epidermal growth factor receptor (EGFR) mutation (Cataldo et al., 2011, Rosell et al., 2012, Wu et al., 2014). The EGFR mutation appears on cell surface receptor and can be classified into four type receptor tyrosine kinases: 1. EGFR (ERBB1 or HER1), 2. ERBB2 (Her2), 3. ERBB3 (Her3) and 4. ERBB4 (Her4). Binding of specific ligand or secreted growth factor such as epidermal growth factor (EGF) to the receptor tyrosine kinase will result in phosphorylation of tyrosine residues thus triggering the intracellular signalling cascade which are involved in induction of cell proliferation, insensitivity to apoptosis, activation of angiogenesis and development of metastasis process (Salomon et al.,

1995, Yarden & Sliwkowski, 2001). Therefore, the knowledge on EGFR mutation has led to focusing research on development and discovery of EGFR tyrosine kinase inhibitors as target-specific therapeutic agents for cancer treatment. Erlotinib (Rosell et al., 2012) and gefitinib that possess specific small molecule tyrosine kinase inhibitors has shown to be effective in inhibiting the phosphorylation in tumours (Cataldo et al., 2011). A part from that, in 2014 (Wu et al., 2014), an improvement of drug selection has been made where afatinib, an irreversible ERBB family blocker was introduced. It was reported that afatinib showed greater anti-cancer activity than EGFR tyrosine kinase inhibitor. In addition, it was reported that patients taking afatinib had significant longer survival as compared to patients taking the standard or chemotherapy regimen (platinum-based chemotherapy).

At the time of diagnosis, more than half of patients with NSCLC are at advance stage of the disease. Generally, platinum-based is considered to be the current standard treatment in first line setting due to its efficiency and directly targeting DNA tumour. Cisplatin is one of platinum-based drugs, approved by FDA (Food and Drug administration) (Arnesano & Natile, 2009) in 1978 and it is the most potent anti-tumour agents widely used in clinical regimen. In brief, the cytotoxic mode of cisplatin in tumour is mediated by the binding of cisplatin with DNA in the nucleus thus disrupting the transcription or DNA replication process, therefore, triggering the death of cancer cells or induction of cell apoptosis (Fuertes et al., 2003). Cisplatin is usually used in combination with other drugs. Several studies have reported the combination of cisplatin with quite a few drugs including vinorelbine and dulanermin, a recombinant soluble human Apo2 ligand/tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) (Ouyang et al., 2017) and oral S-1 concurrent with

radiotherapy (Kaira et al., 2013, Yamaguchi et al., 2013). Recently, patients with advanced NSCLC were treated with cisplatin in combination either of two drugs, the nanoparticle albumin bound (nab) paclitaxel (nab-PC) (nab-PC/cisplatin) and docetaxel (docetaxel/cisplatin) (Chen et al., 2017). The objective of the study was to compare the efficiency and safety of the drugs and it was found that nab-PC/cisplatin is more preferable because it causes less severe neutropenia as compared to docetaxel/cisplatin. In the meantime, Torigoe and colleague (Torigoe et al., 2017) investigated the impact of a combination of three regiments therapy which include drugs chemotherapy, radiation and surgery for removing tumours in advance NSCLC. Initially, they administered docetoxel (40 mg/m^2) concurrent with cisplatin (40 mg/m^2) through intravenous injection on day 1 and 8 and this was repeated for 3-4 week intervals. Radiotherapy at doses between 40-46 Gy was employed on the first day of chemotherapy. Finally, after a certain period, patients will undergo surgery to remove the tumour. The evaluation is based on the viable cells in resection tumour showing if the tumour contained the viable cells, therefore, an adjuvant chemotherapy will be given, while if no viable tumour found, the patients has completed the treatment and will be follow up accordingly.

Although chemotherapy eradicates most of the tumorigenic cells but the tumour acquires resistance afterwards which is believed to be the main reason of cancer recurrences (Fong et al., 2010). As such, patients treated with kinase inhibitors frequently develop resistance within 9-12 months duration (Chen et al., 2014). Meanwhile, anaplastic lymphoma kinase (ALK) inhibitor, crizotinib that has 60% response efficiency (Shaw et al., 2014) also became ineffective after some period due to the resistance acquired by the tumour as it was observed that majority of patients

that took the drug had cancer recurrence within 12 months (Katayama et al., 2012). Other than that, 53% of patients with advanced NSCLC treated with combination of docetoxel and cisplatin as well as exposure to radiation before resection, still able to develop cancer relapse (Torigoe et al., 2017). Some tumours develop resistance after initial treatment such as ovarian cancer and SCLC. Whilst, NSCLC unfortunately develop an intrinsic resistance to cisplatin (Fuertes et al., 2003). The mechanism of cisplatin inducing resistance by the tumour is associated to multidrug resistance protein (MDR) that increase drug efflux (Siddik, 2003, Galluzzi et al., 2012). Hence, reducing cisplatin accumulation may subsequently lead to lower intracellular concentration of drug. The remaining cells that are resistance to therapy can repopulate causing cancer recurrence. A great deal of research has demonstrated about the existing of cancer stem cells (CSCs) based on specific characteristics (Li et al., 2011, Niu et al., 2013) and the CSCs are well known to be associated with highly resistance properties and responsible for cancer relapse.

1.7 Cancer stem cells (CSCs)

CSCs refer to a subset of tumour cells that has the ability to self-renewal and generate differentiated cells. These cells are referred to as CSCs to reflect their "stem like" properties and ability to continually sustain tumorigenesis. CSCs was first discovered in acute myeloid leukaemia (AML) in 1994 in which a small subset of leukemic cells typically similar to hematopoietic stem cells was able to develop AML after being transplanted into immune deficient mice (SCID) (Lapidot et al., 1994). This breakthrough finding has led to an intense effort made by many researchers to characterise and isolate CSCs from various tumour including breast (Al-Hajj et al., 2003), brain (Singh et al., 2003, Singh et al., 2004), prostate (Collins et al., 2005),

pancreatic, colon (Levina et al., 2008) and lung cancer cells (Zakaria et al., 2015). CSCs mirror to normal stem cells in which they shared similar characteristics including proliferation, reproduce themselves through the process called self-renewal and differentiation of multilineage cells types (Visvader & Lindeman, 2008). Self-renewal is the ability of cells to generate more population of stem cells (self-renewal) and to produce differentiated cells by undergoing both symmetrical and asymmetrical division. In brief, symmetrical division allows the CSCs to propagate either two differentiated daughter cells or two stem cells. In the other way, asymmetrical division only resulted in one differentiated cell and one stem cells (Kawasaki et al., 2008). CSCs possess the capacity to develop into any cell in the overall tumour population, have the ability to drive a continued expansion of the population of malignant cells, invaded and metastasise (Yu et al., 2009).

A diverse method is being used to identify CSCs including sphere forming assay, Hoechst dye exclusion and detection of cell surface markers. However, the most widely used method for identifying CSCs is based on specific surface markers (Zhang et al., 2013).Various type of markers have been used and validated for CSCs. For instance the CD133+ is a CSCs marker for laryngeal carcinoma cells (Hep2) (Zhang et al., 2013) as well as AC cells (A549) (Liu et al., 2014) while the CD24+ is a positive marker for head and neck squamous carcinoma cells (HNSCC) (Oh et al., 2013). There were also several other well-accepted stem cell surface markers including the CD44, CD24, CD133, CD166, EpCAM which were expressed in different tumours such as breast, lung, pancreas, prostate, colorectal, renal, and ovarian (Jaggupilli & Elkord, 2012). An example is the epithelial cell adhesion molecule known as the EpCAM, mostly expressed in several human carcinoma and in the majority of normal epithelial

cells (Laimer et al., 2008). Due to the strong association between high EpCAM expression and the progression of tumour cells in NSCLC, it is believed that this molecule would serve as a prominent marker that can distinguish CSCs from the non-CSCs in tumour specimen which are mostly consisted of squamous cell carcinoma (Litvinov et al., 1996, Munz et al., 2009, Pak et al., 2012). In addition, EpCAM was noticed to be an important biomarkers due to the ability to detect circulating tumour cells in blood of lung cancer patients (Skirecki et al., 2014). Another example is the leukocyte cell adhesion molecule (ALCAM) or CD166 that is also found to be a robust marker in lung CSC, based on the high expression detected in most lung cancer samples and the capacity of the isolated cells to exhibit self-renewal capacity and differentiation *in vivo* (Zhang et al., 2012a, Tachezy et al., 2014).

1.8 Targeting CSCs

The existence of CSCs plays a major role in chemotherapeutic resistance and cancer recurrence. Although CSCs have a minority population in the tumour subset, but they are known to be the driving force behind tumour growth besides preserving their stemness. A more strategic method is needed to discriminate CSCs population from other cell population. As mentioned earlier, CSCs are a unique population that have similarities to normal stem cells which relatively capable to initiate tumour growth, actively proliferating cells, possess self-renewal characteristics, have resistance to chemotherapeutic drugs and able to metastasise, thereby making them responsible for tumour recurrence or relapse. CSCs can be targeted through their cellular properties such as inhibiting the self-renewal capability, suppressing their migration, blocking the chemo-resistance and interrupting the CSCs niche. Hence, targeting CSCs may be a promising strategy for improved cancer treatment.

1.8.1 Self-renewal of CSCs

Many studies have discovered the role of *SOX2* in the regulation of self-renewal ability of CSCs of many tumours. A recent study (Lee et al., 2014) in HNSCC has proved that the overexpression of *SOX2* in tumour cells has exhibited a formation of sphere which is a definite hallmark of self-renewal properties. Furthermore, their study also showed that the overexpression of *SOX2* was associated with the enrichment of CD44 positive, a stemness marker used in HNSCC. Also, another study using side population (SP) derived from lung cancer (D121) which contain CSCs characteristic also demonstrated the up regulation of *SOX2* (Xiang et al., 2011). Therefore, *SOX2*, a transcription factor of CSCs, has been targeted in order to inhibit the CSCs as such using rapamycin, a mammalian mTOR (mammalian target of rapamycin) inhibitor since mTOR has been suggested to play a critical role specifically in stemness of CSCs (Xie et al., 2016). They found A549 spheres that highly expresses *OCT-4*, *TWIST*, *NANOG*, *SOX2*, CD133, *ABCG1*, *ABCG2* and *ABCG4* was significantly inhibited after treatment with rapamycin. In conjunction to this, the expression of *SOX2* was significantly reduced upon the treatment suggesting *SOX2* plays a crucial role in controlling the CSCs functions. Apart from self-renewal properties, *SOX2* also was correlated with other stemness features including cells proliferation, invasiveness, chemo-resistance and induction of tumour (Xiang et al., 2011, Zhu et al., 2012, Liu et al., 2013, Lee et al., 2014, Xie et al., 2016).

Besides *SOX2*, the *NANOG* also regulates the self-renewal in CSCs. In a study, the NANOG ^{Positive} CSCs were isolated from human hepatocellular carcinoma (HCC) and it was found that these isolated cells have enhanced ability of self-renewal and clonogenicity which are consistent with crucial CSCs hall mark. They proved NANOG

^{Positive} cells could form spheres efficiently as well as capable to induce more and larger colonies with more than 60% of colonies are identify as holoclones type comparing to NANOG ^{Negative} Cells (Shan et al., 2012). The NANOG ^{Positive} cells also demonstrate that they are more resistant to sorafenib and cisplatin drug as they are less sensitive to the therapy since NANOG ^{Positive} cells are known to overexpress multidrug resistance protein1 (MDR1), lung resistance protein (LRP) and multidrug resistance associate protein (MRP) as compare to NANOG ^{Negative} Cells. In addition, the NANOG ^{Positive} cells also display more migratory and invasive activities as evidence of decreasing expression of E-cadherin and increasing expression of vimentin and fibronectin, a molecular marker for epithelial mesenchymal transition (EMT).

OCT-4, also known as a member of the family of POU-domain transcription factors are known as key regulators in CSCs. Lung cancer derived CD133+ (LC-$CD133^{+}$) was suggested to associate with self-renewal (Ponti et al., 2005) and stemness properties due to up-regulation of *OCT-4* expression. Yu-Chih and colleagues (Chen et al., 2008) showed that the knockdown of *OCT-4* in LC-$CD133^{+}$ subsequently altered the morphology of the cells from floating spheres to epithelial-like cells. Perhaps, the effect of *OCT-4* knockdown continuously improves the chemo radiotherapeutic sensitivity in $CD133^{+}$ thus effectively inducing the apoptotic activity as annexin V and caspase 3 were expressed. *SOX2*, *NANOG*, *OCT3/4*, *Nestin* and *CD34* which are common specific molecular targets in CSCs have been extensively studied as each of these genes plays important role in the functioning of CSCs. Isolated CSCs from breast cancer patient with stage I, II, III and IV demonstrated that the expression of stemness transcription factor increased according to the stage of the diseases. Based on their data, they showed some of these transcription factors are

related to maintenance of the stemness while others might responsible for diseases progression (Apostolou et al., 2012). Similarly, Kruppel-like factor (*KLF4*) is also one of the factors contributing to self-renewal in CSCs. In fact, *KLF4* is one of the four transcription factors used in creating induced pluoripotent stem cells (iPSCs). In breast CSCs, consistent overexpression of *KLF4* led to the enrichment of CSCs while knockdown of *KLF4* has resulted in decreasing the mammosphere, colonies formation as well as inhibited the tumorigenesis in immunocompromised NOD/SCID mice (Yu et al., 2011). In addition, all these four transcription factors, *SOX2* (Lee et al., 2014, Xiang et al., 2011, Liu et al., 2013b), *NANOG* (Shan et al., 2012), *POU51F* (Chen et al., 2008) and *KLF4* (Yu et al., 2011, Yan et al., 2016) showed similar roles where they also responsible for migration of CSCs which is involved in the metastasis process in CSCs.

1.8.2 Metastasis of CSCs

Cancer begins as localise disease that if left untreated it will metastasise to other parts of organ in the body and be referred to as malignant tumour. It was proposed that CSCs is responsible in initiating the metastasis process and research regarding CSCs associated with metastasis has been extensively studied. The metastasis of cancer cells may result from the resistant of CSCs to conventional chemotherapy and this inherit resistant eventually leads to relapse in many cancer patients (Dean et al., 2005). Metastasise cells require several steps before migrating to other parts of organ tissue. The process involved EMT. The tumours cells must invade through the tissue and entering the blood circulation before establishing a new tumour. CSCs are believed to be the cells that metastasise (Berx et al., 2007) since it's the only cells that capable to initiate tumours. In fact, Bhagwandin et al. has demonstrated the SP isolated from

pancreatic carcinoma (Panc-1) which is enriched with CSCs showed aggressive metastatic effect and had produced primary tumours. This primary tumour from SP population also expresses *ABCG2*, an important source of drug resistance. Besides, another study showed the drug surviving cells (DSC) that own the properties of CSCs exhibit high metastatic capacity. Following inoculation of tumour cells in SCID mice, the DSCs had form numerous tumours in the lung with increase expression of adhesion molecules (VLA-5,ICAM-1 and VCAM-1) as well as *MMP2* and *MMP3* indicating the metastatic potential of CSCs (Levina et al., 2008). This is because metastasis is a selective process which requires tumour cells to survive during extravasation and invasion process through a distant part of the body. Therefore, only CSCs have the ability to survive during this process.

1.8.3 Chemo-resistance in CSCs

Chemotherapy is designed to target rapidly dividing cells through interrupting the DNA replication process thus inducing cell apoptosis. One of the primary reasons for chemotherapy failure is due to drug resistance. Chemotherapy generally destroys drug sensitive cells but not the drug resistant cells. It is suggested that drug resistant cells are employed by CSCs population for progression and replication and this is the most challenging for cancer chemotherapy. In normal stem cells, to maintain the tissue and biological function of life, stem cells are protected from carcinogenic endogenous or exogenous agents in order to avoid any damage that disturb the function of the cells (Dekaney et al., 2009). Likewise, CSCs also possess similar features to protect them against chemotherapy drugs such as overexpression of MDR pumps. MDR pumps are a protective system that pumps out any chemicals or biotoxic against the CSCs. Also. ABC transporter family, including ABCA2, MDR1, and MRP1 are potential drug

pumps which are known to associate with drug resistance (Fong et al., 2010, Ho et al., 2007). Besides the aldehyde dehydrogenase (ALDH) family is also responsible for CSCs resistance to conventional chemotherapeutic drugs. ALDH could oxidize the aldehyde group of chemotherapy drugs to carboxylic acid, in that way detoxifying the cytotoxic chemotherapy drugs and metabolising them resulting into non-toxic forms. ALDH as well plays a vital role in ROS scavenging activity which protects tumour from oxidative stress injury that normally induced by radiation and chemotherapeutics (Xu et al., 2015). Chemo-resistant of CSCs may be explained by their state of quiescence or dormancy in order to preserve the self-renewal in stem like cell. The dormant CSCs are usually in undividing mode thus making them exhibit slow proliferation kinetics. Meanwhile, chemotherapeutic drugs are designated to attack the rapidly dividing cells, therefore making the dormant CSCs escape and survive from chemo drugs. Furthermore, the dormant CSCs are able to remain quiescence for years thus making CSCs an ideal candidate to explain the tumour recurrence (Maugeri-Saccà et al., 2011). Dormant CSCs is regulated by intrinsic and extrinsic signals from microenvironment interaction (Wilson & Trumpp, 2006). These interaction is critical for maintenance of CSCs dormancy (Spradling et al., 2001).

1.8.4 CSCs niche

Stem cells usually reside in a particular environment called the "stem cell niche" to maintain the stem cells and act as a homing for stem cells residues (Januschke & Näthke, 2014). The niche regulates the stemness, proliferation, metastasis and resistance of stem cells. The behaviour of stem cells is tightly controlled by the signals from the surrounding niche which eventually determined the cells fate (Kasai et al., 2014). Analogously, CSCs was surrounded by "cancerous niche" helping

them to retain their self-renew ability and propagate more differentiated progenitor cells while staying in undifferentiated state (Calabrese et al., 2007, Hill et al., 2009). The niche is enriched with nutrients, extracellular matrix, soluble factor, vascular network and metabolic process. The stroma cells that reside as a cellular part in niche recruit many others cell types including the immunological cells upon receiving signals from tumour cells, therefore, forming an additional inflammatory microenvironment (Fazilaty et al., 2013). The tumour microenvironment is a product of cell-cell interactions and cell-ECM (extracellular matrix) interactions. Tumours cells secreted a variety of proteins capable of providing signal that turn on the transcription factor thus regulating the CSCs activities. Proteins secreted by tumour cells in ECM microenvironment are commonly involved in cell adhesion, motility, intercellular communication and invasion. The niche contributes to pre-metastasis, an initial event of metastasis (Kaplan et al., 2005). For tumour cells to progress and develop themselves, they must have the ability to migrate, the capacity to degrade tissue matrix (ECM), capable to survive in blood and able to establish itself in new tissue environment (Mbeunkui & Johann, 2009). Numerous secreted proteins identified in tumour niche could be molecular therapeutic targets such as hypoxia-inducible factor 1, MMP-2, MMP-9 and MMP-12, nuclear factor of κB (NF κB), tumour necrosis factor (TNF), interleukin (IL-6) , vascular endothelial growth factor (VEGF), Integrin *αvβ3* and *αvβ5* (Mbeunkui & Johann, 2009).

The CSCs niche has been implicated with resistance and metastasis of CSCs as they survive conventional treatment. Perhaps, CSCs niche also play a protective role by sheltering the cells from diverse genotoxic agents that could harm the cells thus contributing to therapy resistance (Folkins et al., 2007, Hovinga et al., 2010). Hence,

targeting the CSCs niche may be a promising strategy for elimination of CSCs population. The niche of CSCs is mediated by hypoxia condition which often insufficient of oxygen supply (Lin & Yun, 2010). A study on breast cancer showed that carbonic anhydrase IX (CIAX), a metalloenzyme was up regulated in hypoxic tumours. CIAX regulated internal pH contributing to the acidification of the niche thereby enhancing cancer cells proliferation, invasion and metastasis by promoting EMT process (Lock et al., 2013). Therefore, inhibiting the CIAX in CSCs niche by specific small molecule inhibitor results in depletion of CSCs which in turns, leads to tumour growth and metastasis reduction. Similarly, a hypoxia condition was induced by high concentration of gefitinib under normoxic (hypoxic conditions) causing gefitinib-resistant persisters (GRPs) in NSCLC and is associated with high expression of CD133, *OCT4*, *SOX2*, *NANOG*, *CXCR4*, and *ALDH1A1* as well exhibiting tumorigenicity *in vivo*. Furthermore, the hypoxia condition in GRP significantly increased and activated insulin like growth factor 1 (IGF1) thereby enhancing resistance in chemotherapy. Consequently, inhibiting the IGF1 may assist in sensitivity of CSCs to chemotherapy drugs (Murakami et al., 2014). Apart from that, the CSCs niche is also responsible for regulating the conversion of CSCs from non-CSCs. Shiozawa et el. (Shiozawa et al., 2016) demonstrated that co-culture of prostate cancer and osteoblasts niche which is influenced by growth arrest specific 6 (GAS6) cause a significance shift of non-CSCs to CSCs. Thus, targeting only CSCs will limit the therapeutic efficiency because the CSCs niche also play a vital role in maintaining the CSCs by repopulating the non-CSCs to CSCs.

1.9 Curcumin as anti-cancer

Recent research has suggested that plant-based foods have health promoting effects (Meng et al., 2017) and prevention properties (Aggarwal & Shishodia, 2006). There are many phytochemicals that have been revealed to regulate multiple oncogenic targets involved in cancer growth, proliferation, chemo resistance, invasion, and metastasis. Moreover, certain phytochemicals have been identified as targets for CSC stemness (Singh et al., 2017). Phytochemical has been suggested to overcome cancer drug resistance (Fong et al., 2010). A number of phytochemicals, a naturally occurring substance, have been demonstrated to possess anti-tumour effects in various experimental systems. The use of phytochemicals derived from dietary components has attracted many public and scientific interests in order to combat human diseases, especially the cardiovascular disease and cancer, the two most common killers in developed countries. (Sharma et al., 2005). This is because they afford the opportunity to affect many different targets or portions of the signal transduction pathway that modulate gene expression, cell cycle progression, cell mortality, metabolism and apoptosis (HemaIswarya & Doble, 2006). Among natural products used in medicine, polyphenols have been found to possess chemo-preventive, chemo-protective, chemo-sensitising, radio-sensitising and radio-protective activities (Jagetia et al., 2008).

Curcumin is a well-known dietary polyphenol derived from the rhizomes of turmeric (*curcuma longa*), which is also a member of ginger family (*Zingiberaceae*) and Indian spice, usually used in preparation of mustard and curry (Sharma et al., 2005). The bright yellow of turmeric came from fat-soluble, polyphenolic pigments known as curcuminoids. Curcuminoids can be sub classified into three groups including diferuloylmethane which is also refer as curcumin, desmethoxycurcumin

and bis-desmethoxycurcumin (Akram et al., 2010). Commercially, curcuminoids contains approximately 77% diferuloylmethane, 17% desmethoxycurcumin, and 6% bisdemethoxycurcumin (Anand et al., 2007). Curcumin is the most active compound and can exist in at least two tautomeric forms, namely as keto and enol. The enol form is more energetically stable in the solid phase and in solution (Akram et al., 2010). Therefore, chemically, curcumin is a bis-R, -unsaturated -diketone (commonly called diferuloylmethane) as depicted in Figure 1.1, which exhibits keto–enol tautomerism having a predominant keto form in acidic and neutral solutions and stable enol form in alkaline medium. In pure turmeric powder, curcumin, the active compound contained 3.14% of concentration by weight (Tayyem et al., 2006). Traditionally, curcumin has been used as part of ancient Indians medical system known as Ayuverda to treat eye infections, to dress wounds, treat bites, burn, acne and many various skin diseases (Thakur et al., 1989). In Northern India, women after childbirth were given a hot glass of milk containing the mixture of turmeric paste with powder of dried ginger roots and honey to drink twice daily (Pandeya, 2005).

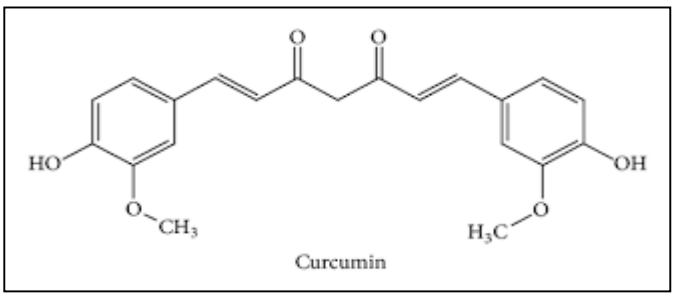

Figure 1. 1 Structure of curcumin

1.10 Pleiotropic role of curcumin

Curcumin is well known to possess the pleiotropic role such as anti-inflammatory, anti-oxidant, anti-microbial and anti-cancer which has been intensively

studied as a cancer model in breast, ovarian, prostate, colon, pancreatic, melanoma and head and neck cancers, over the decade (Li & Zhang, 2014). Exciting recent studies have shown that curcumin, either alone or in combination with other anti-cancer agents, has a pleiotropic therapeutic effect in cancer (Notarbartolo et al., 2005). Additionally, curcumin is also known to down-regulate the intracellular levels of three major ABC drug transporters; p-glycoprotein (P-gp), MRP-1 and ABCG2 that are important MDR (Ebert et al., 2007, Febbo & Ilio, 2008, Hou et al., 2008, Andjelkovic et al., 2008). Previous research demonstrated that curcumin in combination with doxorubicin could result in better treatment of human hepatic cancer cells (Notarbartolo et al., 2005). Amiji and Ganta (2009) have also demonstrated that simultaneous administration of curcumin and paclitaxel able to overcome drug resistances. Yu et al (2009) has reported that curcumin with no discernible toxicity synergises with 5-FU plus oxaliplatin (FOLFOX) to inhibit the growth of colon cancer cells (Yu et al., 2009). Their study also reveal that curcumin either alone or together with FOLFOX could be effective in eliminating CSCs (Yu et al., 2009). Another study by Zhang et al., (2013) indicated that curcumin has the ability to induce the sensitivity of CD133+ CSCs to cisplatin and therefore enhance the effectiveness of cisplatin on the Hep-2 cell line as manifested by the reduction expression of ABCG2 in CD133+ cells which suggested that it is responsible for the induction of sensitivity of CD133+ cells to cisplatin (Zhang et al., 2013). In addition, curcumin has been found to be safe, with no dose-limiting toxicity, when administered at doses up to 10 g/day in humans (Yum et al., 2001).

Apart from that, curcumin also has high anti-metastatic effect. Metastasis involve the process of spreading the cancer cells by migration ability from primary site

of tumour growth to the distant part of organ, causing the cancer morbidity and mortality. The effect of curcumin on cell migration have been reported in many other cancers included human medulloblastoma (MB) (Bangaru et al., 2010), K1 papillary thyroid (K1) (Zhang et al., 2013), prostate cancer (PC-3) metastasis (Killian et al., 2012) and lung metastasis of human breast cancer (Aggarwal et al., 2005). Curcumin is able to enhance the expression of anti-metastatic proteins, E-cadherin, which reduce the metastatic tendency of K1 papillary thyroid (Zhang et al., 2013). Curcumin was shown to induce marked reduction of MMPs, MMP-2 and MMP-9 activity leading to reduction of metastatic in tumour in mice (Teiten et al., 2010) as MMPs is closely related to the ability of tumour invasion and metastasis (Zhang et al., 2013). The anti-metastatic effect of curcumin had been used to test against DU145 prostate cancer cells both *in vitro* and *in vivo* and the study shows decreased in tumour volume and expression of MMP-2 and MMP-9 (London et al., 2003). In a human breast cancer xenograft model, administration of curcumin markedly decreased metastasis to lung and inhibited the expression of NF-KB, MMP-9, COX-2, VEGF, and intercellular adhesion molecule-1 which in turn altered the invasive and metastatic properties of the cells (Aggarwal et al., 2007).

1.11 Curcumin as chemo-sensitiser

Studies have shown that phytochemical-rich plants including curcumin exhibit chemo-sensitiser properties (Prasad et al., 2016). This property in important in order to increase the sensitivity of CSC to a chemotherapeutic drug. Curcumin is a promising chemo-sensitiser and was in advantage to overcome tumour resistance. Curcumin have shown to become the cisplatin sensitising agent in lung cancer (Chanvorachote et al., 2009). Curcumin was reported to reverse drug resistance in cancer cells overexpressing