Pretreating with Cannabidiol (CBD) enhances chemotherapy: evidence for mitochondrial "priming"

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Dedicated to

My Grandfather,

Mr. Andrew St. Finbarr Henley FRCS, FRCSI

Abstract

The current treatment for cancer is focused on chemotherapeutic treatments which can lead to many side effects including nausea, vomiting and chemoresistance. Combating chemoresistance is a key target in cancer therapy, along with enhancing the effects of current chemotherapy treatments. One progressive novel approach being investigated is pre-treating ("priming") cells prior to chemotherapeutic treatment. Evidence has shown that "priming" of cancer cells can sensitizes the mitochondria to respond better to chemotherapy and hence reduce severity of treatment and side effects.

In this thesis, I aim to define the mechanism by which "priming" enhances cell death through the mitochondria. Firstly, cannabidiol (CBD), a phytocannabinoid that has shown to have beneficial properties in inflammation, pain relief and in reducing cancer cell progression was examined for a potential "priming" agent. Secondly, the effect of the chemotherapy agent, cisplatin, was fully defined for its role in mitochondrial function along with a combination therapy of CBD and cisplatin. And finally, "priming" with CBD was assessed for any potential benefit to cancer treatment.

I have demonstrated here that "priming" with CBD can enhance the killing effect of cisplatin in cancer cells and not in control cells. More specifically, the underlying mechanism for "priming" by CBD was shown to be through the modulation of mitochondrial function by means of the high stress two-phase system p53/Nrf2 together with voltage dependant anion channel 1 (VDAC1) receptor activation. From this body of work, "priming" has been shown to have a potential in enhancing current chemotherapeutic treatment.

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"I am the master of my fate,
I am the captain of my soul"

William E. Henley (1849-1903)

Declaration of Contributors

The majority of the work contained in this thesis was performed by the author. Any assistance and collaboration is detailed below:

AMPK activity

AMPK activity was measured in collaboration with Dr. Fiona Leiper from the Cellular Stress Group, MRC CSC, Hammersmith Hospital, White City. The samples were collected and prepared prior to radioactivity labelling that was carried out by Dr. Leiper. The measurements were carried out on the scintillator by Dr. Leiper and the analysis was carried out by the author.

Global DNA methylation

The Global DNA methylation levels were measured through mass spectrometry with the collaborative help of Dr. Rachel Amouroux from the DNA group, MRC CSC, Hammersmith Hospital, White City. The samples were harvested, prepared and quantified before being prepared for mass spectrometry by Dr. Amouroux. The analysis was carried out with the help of Dr. Amouroux.

Confocal microscopy

The confocal microscope images were completed by the author with the aid of the microscopy team at the MRC CSC, Hammersmith Hospital. Ms. Ki Hng quantified the images obtained by the author using Cell with a specifically designed pipeline. Further analysis was carried out by the author.

Abbreviations

11-OH-THC	11-hydroxy-Δ^9-tetrahydrocannabinol
2-AG	2-arachidonoyl glycerol
2-DG	2-deoxyglucose
5-FU	Fluorouracil
Acetyl-CoA	Acetyl coenzyme A
ADP	Adenosine diphosphate
AEA	*N*-arachidonoyl ethanolamine [anandamide]
AEBSF	4-(2-aminoethyl)-benzenesulfonyl fluoride
αKDH	α-ketogluteratedehydrogenase
AKT	Protein kinase B
ALL	Acute lymphoblastic leukaemia
AMP	Adenosine monophosphate
AMPK	5' AMP-activated protein kinase
AREs	Antioxidant-response elements
ATP	Adenosine triphosphate
A.U.	Arbitrary units
Bax	Bcl-2-associated X
Bcl-2	B-cell lymphoma 2
Bcl-xl	B-cell lymphoma- extra large
Bim	Bcl-2-like protein 11
BP	Before Present
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumin
Ca^{2+}	Calcium
CB	Cannabinoid receptors
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBD-BDS	CBD-botanical drug substance

cDNA	Complementary deoxyribonucleic acid
Cisplatin	Cis-Diammineplatinum(II) dichloride
CNS	Central nervous system
CO_2	Carbon dioxide
CoQ	Coenzyme Q
CYP	Cytochrome P450
DAGLs	*sn*-1-diacylglycerol lipases
DAPI	4',6-diamidino-2-phenylindole
DiOC	3,3'-diethyloxadicarbocyanine Iodide
DCF	2', 7'–dichlorofluorescin
DCFDA	2',7'–dichlorofluorescein diacetate
DMEM	Dulbecco's modified eagle's media
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ECAR	Extracellular acidification rate
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
ELISA	Enzyme-linked immunosorbent assay
EMEM	Essential minimum eagle's media
EnR	Endoplasmic reticulum
ER	Oestrogen receptor
ER-	Oestrogen receptor negative
ER+	Oestrogen receptor positive
ETC	Electron transport chain
EU	European Union
FAA	Fatty acid amide
FAAH	Fatty acid amide hydrolases
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FCCP	Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone
FDA	Food and drug administration

FH	Fumerate hydratase
FOXO	Forkheadbox
G_1	Growth 1
gDNA	genomic DNA
GLUT1	Glucose transporter 1
GPCR	G-protein coupled receptors
H_2O_2	Hydrogen peroxide
HDAC	Histone deacetylase
HER2	Human epidermal growth factor receptor 2
HIF	Hypoxia-inducible factors
HIV	Human immunodeficiency virus
HK	Hexokinase
HPLC	high-performance liquid chromatography
HPV	Human papilloma virus
IDH	Isocitrate dehydrogenase
IL6	Interleukin 6
IVC	Individually ventilated cages
Keap1	Kelch-like ECH (Erythroid cell-derived protein with can ‘n’ collar homology)-associated protein 1
LDH	Lactate dehydrogenase
LKB1	Liver kinase B1
MAG	Monoacylglycerol
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinases
MCU	Mitochondrial calcium uniporter
Mitophagy	Autophagy of mitochondria
MOMP	Mitochondrial outer membrane potential
MPU	Mitochondrial pyruvate carrier
mRNA	messenger RNA
MS	Multiple sclerosis
MTORC1	Mammalian target of rapomycin complex 1
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide

NADH	Nicotamide adenine dinucleotide hydride
NaF	Sodium fluoride
NaCl	Sodium chloride
NAFLD	Non-alcohol related fatty liver disease
NaPP	Sodium pyrophosphate
NArPE	*N*-arachidonoylphosphatidylethanolamine
NF-κB	Nuclear factor - κB
Nitrogen mustard	Tris β-chloroethylamine
NO	Nitric oxide
NQO1	NAD(P)H:quinone oxidoreductase 1
Nrf2	Nuclear factor (erythroid-derived 2) -like 2
$O_2^{\bullet -}$	Superoxide
OCR	Oxygen consumption rate
$ONOO^-$	Peroxiynitrite
OOPC	Olive oil phenolic compounds
OXPHOS	Oxidative phosphorylation
p53	Protein 53
PBS	phosphate buffered saline
PDH	Pyruvate dehydrogenase
PFK	Phosphofructokinase
PGI	Phosphoglucose isomerase
PGK	Phosphoglycerate kinase
PGM	Phosphoglycerate mutase
PI	Propidium Iodide
PI3K	Phosphoinositide 3-kinase
PK	Pyruvate kinase
PMF	Proton motive force
PMSF	Phenylmethly sulphonyl fluoride
PTP	Permeability transition pore
PUMA	p53 upregulated modulator of apoptosis
qRT-PCR	Quantitative real time-polymerase chain reaction
RB	Retina blastoma

R.I.	Relative intensity
RLS	Reactive lipid species
RNA	Ribonucleic acid
RNAi	Ribonucleic acid interference
ROS	Reactive oxygen species
RPM	Revolutions per minute
S	Synthesis
SCFA	Short chain fatty acid
SDH	Succinate dehydrogenase
SIHM	Stress induced mitochondrial hyperfusion
SirT1	Sirtuin 1
SOD	Superoxide dismutase
TCA	Tricarboxylic acid
TBS	Tris-buffered saline
TBST	Tris-buffered saline and Tween
THC	Δ^9-tetrahydrocannabinol
THC-COOH	11-nor-9- carboxy-Δ^9- tetrahydrocannabinol
THCV	Tetrahydrocannabivarin
TNFα	Tumour necrosis factor α
TPI	Trioesphosphate isomerase
TRP	Transient receptor potential
TrpV1	TRP cation channel subfamily V member
TRPV-1	TRP vanilloid type 1
UK	United Kingdom
US	United States
VDAC	Voltage-dependant anion channels
XTT	2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide

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Chapter 1

Introduction

1. Introduction

1.1. Cancer

1.1.1. Introduction to cancer

The leading cause of death and disease around the globe is cancer (WHO., 2014). In 2012, 14.1 million people were newly diagnosed with cancer and 8.2 million died from cancer worldwide (Ferlay *et al.* 2013). The highest occurrence of cancer in 2012 was lung cancer and breast cancer (Ferlay *et al.* 2013). Data shows that more than 30 % of cancer deaths could be prevented by altering or avoiding key risk factors such as being overweight, lack of physical activity, unhealthy diet and tobacco use.

The predicted outcome of new cases in the United States (US) for 2014 was 1.67 million new cancer cases and over 580,000 deaths from cancer (Siegel *et al.* 2014). In the European Union (EU) a predicted 1.31 million people will die from cancer in 2014. However, in the last 5 years, death rates have reduced; US, <1.8 % in males, <1.4 % in females; EU, <6 % for males, <4 % for females.

1.1.2. Breast cancer

Breast cancer is the second most common cancer in the world affecting 1/8 women in the United Kingdom (UK) (Travassoli and Devilee 2003, Breast Cancer Care. 2014, Siegel *et al.* 2014). In 2014 a predicted 235,030 new cases will be diagnosed and a further 40,430 resulted deaths from breast cancer in the US (Siegel *et al.* 2014). This accounts for 29 % of new cases diagnosed in females. In the EU 14.6/100,000 new cases will be breast cancer in 2013 (Malvezzi *et al.* 2013). Interestingly, though since 1992 the incidence of breast cancer in the US has reduced 0.7 % and the death from breast cancer had a further 6.7 % reduction (Siegel *et al.* 2014).

The better survival rate of breast cancer patients is due to several factors including early diagnose, better education, improved surgery practice, targeted therapies and development of systemic chemotherapy (Duffy *et al.* 2010, Malvezzi *et al.* 2013, Siegel *et al.* 2014). Early screening has been shown to save 2-2.5 lives for every over diagnosed patient and in the UK it has reduced mortality by 20 % (Duffy *et al.* 2010, Marmot *et al.* 2013).

1.1.3. Hallmarks of cancer

The hallmarks of cancer described by Hanahan and Weinberg provide a foundation for the understanding of cancer biology (Hanahan and Weinberg 2000, Hanahan and Weinberg 2011). Tumours are no longer seen as a homogenous growth but a multifaceted entity comprising of a collection of distinct cell types that function actively to the initiation, development and metastasis of tumour cells (Hanahan and Weinberg 2000, Chaffer and Weinberg 2011, Hanahan and Weinberg 2011). This advanced progress in our understanding allowed Hanahan and Weinberg to provide a documentation of the elaborate hallmarks of cancer. It was proposed that each of these changes represent the successful inhibition of an anti-cancer defence mechanism that is capable in any type of cancer (Hanahan and Weinberg 2000). Due to the robust advancement in understanding the mechanism underpinning cancer regulation Hanahan and Weinberg revisited their hallmarks and adjusted them accordingly (Figure 1.1.). Several of these hallmarks are now key targets for novel chemotherapeutic therapies, such as the reprogramming of energy metabolism, tumour microenvironment targets and tumour promoting inflammation (Vander Heiden *et al.* 2009, Hanahan and Weinberg 2011, Galluzzi *et al.* 2013). Specific inhibitors to these targets are being created to slow down or stop cancer cell growth.

Figure 1.1. Hallmarks of cancer.
An illustrated schematic of the revised ten hallmarks of cancer proposed by Hanahan and Weinberg (2011). Therapeutics that target these hallmarks are being developed to target each characteristic. Taken from Hanahan and Weinberg, 2011.

Tumour suppressors retina blastoma (RB) and protein 53 (p53) are important regulators in cell growth and control (Wade *et al.* 2013). p53 can trigger apoptosis and is regularly mutated in cancer cells (Hanahan and Weinberg 2011, Wade *et al.* 2013). Recent work has shown that p53 could be a prime target in cancer therapy in a novel restoration mechanism (Ventura *et al.* 2007) Liver kinase B1 (LKB1) is also a tumour suppressor that has been shown to be down regulated in cancer cells (Shaw 2009). LKB1 has been implicated in breast cancers and when down regulated decreases the capacity for 5' AMP-activated protein kinase (AMPK) in the cell (Shackelford and Shaw 2009, Shaw 2009). AMPK is key in regulating energy balance within the cell and can increase the generation of adenosine triphosphate (ATP) when levels are low (Hardie 2008, Shaw 2009). In cancer, loss of AMPK activity has been linked to enhanced cell growth and proliferation (Faubert *et al.* 2015). It has recently been suggested that AMPK has a major role in tumour suppression due to activation of the p53 pathway and as a negative regulator of aerobic glycolysis (Luo *et al.* 2010, Faubert *et al.* 2013).

Tumour cells have also been linked to resisting cell death which is a key target when examining novel chemotherapeutic targets. Chemoresistance is a common side effect when treating cancer cells which can lead to the use of a combination of anti-cancer agents or to fatal uncontrolled metastasis (Devita *et al.* 1970, Caley and Jones 2012). Cancer cells can circumvent apoptosis through controlling anti-apoptotic regulators (B-cell lymphoma 2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xl)) and down regulating pro-apoptotic factors (Bcl-2-associated X (Bax), Bcl-2-like protein 11 (Bim), p53 upregulated modulator of apoptosis (Puma)) (Sarosiek *et al.* 2013). It is through this abnormal control that tumours can continue to proliferate and grow.

1.1.3.1. Alteration of cancer metabolism

The metabolism of cancer cells has had renewed interest in the past thirty years due to the re-emerging research into the so called "Warburg Effect" and a better understanding of the altered metabolism in cancer cells (Vizán *et al.* 2008, Samudio *et al.* 2009, Vander Heiden *et al.* 2009, Hanahan and Weinberg 2011). In 1926, Otto Warburg showed that cancer cells carried out respiration through lactate acid fermentation even in the presence of oxygen (Warburg *et al.* 1927). This research had been based on Okamoto's work with hypoxia and glucose levels within the cell (Okamoto, 1925). However, Warburg managed to prove that lactic acid formation in tumours was not dependent on oxygen's presence (Figure 1.2.) (Warburg *et al.* 1927, Vander Heiden *et al.* 2009). Tumour cells show an increase in glucose uptake that leads to enhanced glycolytic rates (Schulze and Harris 2012). This in turn increases the cancer cells growth advantage as there is an activation of growth regulators; phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), mammalian target of rapamycin complex 1 (MTORC1) and mitogen-activated protein kinases (MAPK) (Cairns *et al.* 2011, Schulze and Harris 2012). AKT is activated by PI3K which increases cellular glucose uptake

through the increase in glucose transporter 1 (GLUT1) expression (Munoz-Pinedo *et al.* 2012, Schulze and Harris 2012). There is a reduction of ATP production within the cell through the "Warburg Effect" of about ~18 ATP molecules (Hanahan and Weinberg 2011). This reduction leads to an increase in GLUT1 (Hsu and Sabatini 2008, Hanahan and Weinberg 2011). AKT can stimulate the activation of MTORC1 and hypoxia-inducible factor (HIF) to increase glycolysis and cellular growth (Shackelford and Shaw 2009, Schulze and Harris 2012).

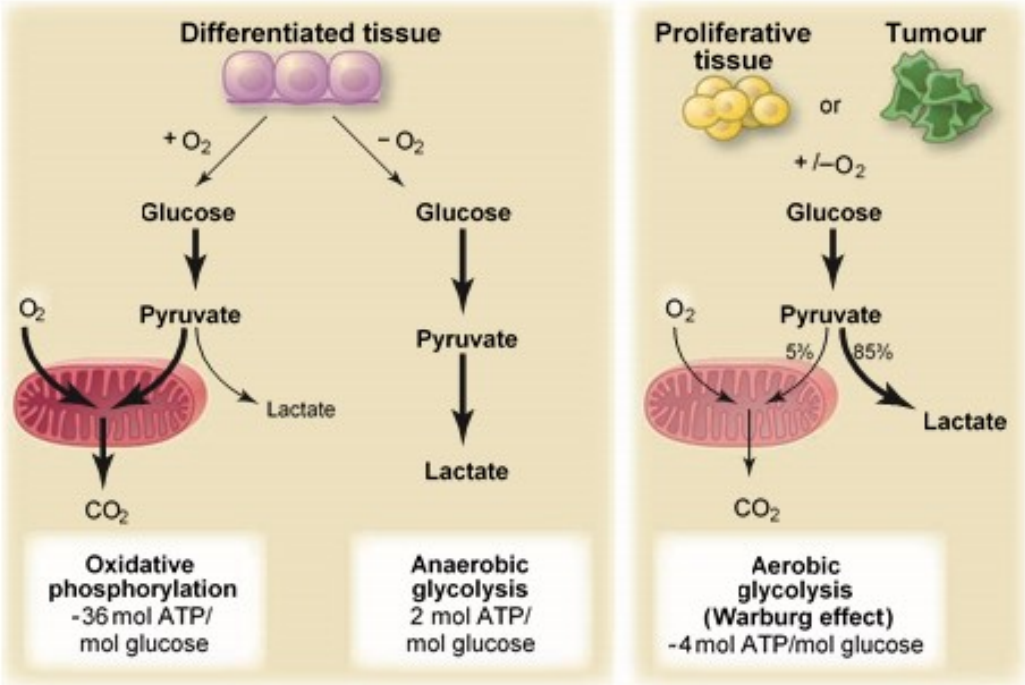

Figure 1.2. Illustrated schematic of the difference between anaerobic glycolysis and aerobic glycolysis ("Warburg Effect").
Normal cells undergo oxidative phosphorylation when oxygen is present through the mitochondria creating ATP and carbon dioxide (CO_2). In the absence of oxygen, normal cells carry out anaerobic glycolysis through lactate fermentation. However in tumour cells, whether in the presence or absence of oxygen they carry out aerobic glycolysis through lactate fermentation and by limiting the use of the mitochondria. Revised from Vander Heiden, 2011.

Interestingly, a metabolic switch has also been proposed whereby tumour cells may have the ability to work not only through the "Warburg Effect" but also through the

Crabtree effect (Diaz-Ruiz *et al.* 2011). The Crabtree effect is observed in fast growing cancer cells and is a glucose induced inhibition of cellular respiration and thus of oxidative phosphorylation (OXPHOS) (Dell' Antone , Diaz-Ruiz *et al.* 2011). Not much work has been carried out in cancer cells with regards the Crabtree Effect as it has been primarily pursued in yeast (Diaz-Ruiz *et al.* 2011).

1.1.3.2. The function of the mitochondria in the cell

Mitochondria are the main source of energy for the cell and their function and control are important for regulation of both cell proliferation and apoptotic control (Terada 1990, Antico Arciuch *et al.* 2012, Rehman *et al.* 2013, Song *et al.* 2013). The mitochondria were first identified by Benda in 1898 and were described as granular organelles though their function was yet to be identified (Benard *et al.* 2007). The mitochondria's central role was later formally identified to be in cellular energy production by the discovery of the respiratory chain and OXPHOS (Chance and Williams 1956). Morphologically the mitochondria exists in the cell as large tubular assemblies with close contact to the nucleus, endoplasmic reticulum (EnR) and golgi (Benard *et al.* 2007). This morphology is not rigid however and is constantly changing due to the double membrane that it is made up of (Nelson and Cox 2006, Benard *et al.* 2007). The outer membrane is protein and lipid based, while the inner membrane is more protein rich and less permeable (Nelson and Cox 2006). The mitochondrial genome encodes 13 proteins however the genes that code for the mitochondria's structure and function are found in the nucleus suggesting a tight link between the cell and its mitochondrial organelle for control of cell proliferation and cell death (Benard *et al.* 2007, Ferber *et al.* 2012, Kroon and Taanman 2014).

Energy is mainly produced in the mitochondria through regulated process' which when dysfunctional or altered can lead to mitochondrial death (mitophagy) and cellular

death through apoptosis (Vander Heiden *et al.* 2009, Hanahan and Weinberg 2011, Frank *et al.* 2012, Rehman *et al.* 2013, Wang *et al.* 2013). One source of energy production is from glycolysis whereby glucose is converted to glyceraldehyde-3-phosphate and to pyruvate in a complex process (Figure 1.3.).

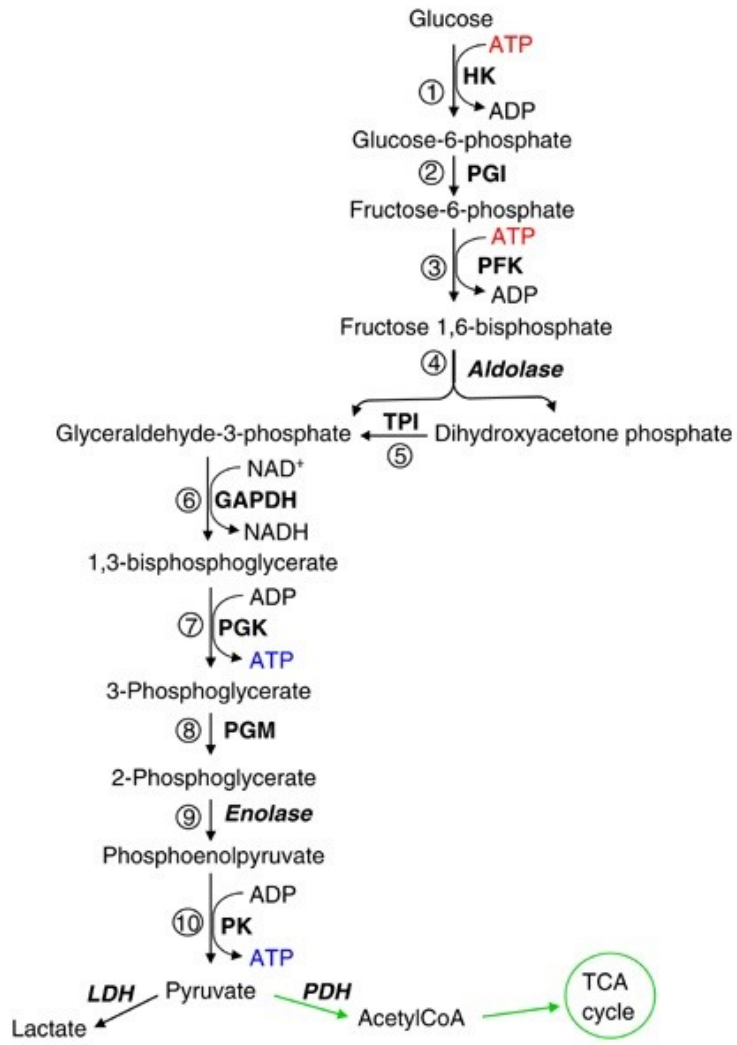

Figure 1.3. Glycolytic pathway.
Glycolysis is a cascade of reactions by which one molecule of glucose is catabolised to two molecules of pyruvate and two ATP. Pyruvate can be converted to lactate or acetyl CoA. The green arrow indicates further metabolism in the mitochondria. HK, hexokinase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; TPI, triosephosphate isomerase; PGK, phosphoglycerate kinase; PGM phosphoglycerate mutase; PK pyruvate kinase; PDH pyruvate dehydrogenase; LDH, lactate dehydroganse. Adapted from Pelicano *et al.* 2006.

The pyruvate can then be converted to acetyl coenzyme A (Acetyl-CoA) in the presence of oxygen in the mitochondria and converted to energy through the

tricarboxylic acid (TCA) cycle (Figure 1.4.) (Nelson and Cox 2006, Vander Heiden *et al.* 2009). The nicotamide adenine dinucleotide hydride (NADH) generated in the TCA cycle is further fed into another pathway that is coupled with the TCA cycle that is OXPHOS (Nelson and Cox 2006, Vander Heiden *et al.* 2009).

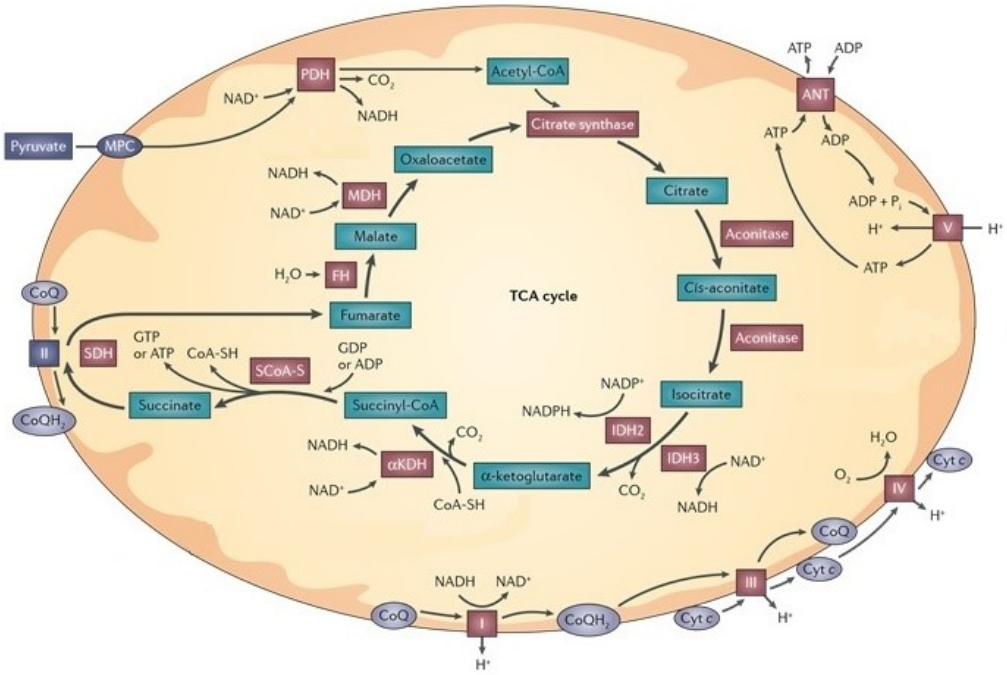

Figure 1.4. The TCA cycle in mitochondrial respiration.
Pyruvate enters the mitochondria through the mitochondrial pyruvate carrier (MPC) where it is oxidised to Acetyl CoA by PDH. Acetyl CoA enters the TCA cycle as citrate synthase. Citrate synthase converts Acetyl CoA to citrate which is then converted to *cis*-aconitate by aconitase. This is then converted further to isocitrate by aconitase. Isocitrate is converted to α-ketogluterate by isocitratedehydrogenase (IDH) creating NADPH and NADH. α-ketogluterate is converted to Succinyl CoA by α-ketogluteratedehydrogenase (αKDH) creating an NADH and CO_2 molecule. Succinyl CoA is converted to Succinate which is converted to fumerate through complex II of the electron transport chaing (ETC) by succinate dehydrogenase (SDH). Fumerate conversion by fumerate hydratase (FH) to malate consumes an H_2O molecule. Malate conversion to oxaloacetate by malate dehydrogenase creates an NAHD molecule. Adapted from Sabharwal and Schumacker 2014.

This process is carried out through a cascade of complexes in the inner mitochondrial membrane beginning with Complex I which converts NADH to Ubiquinone (Figure 1.5.). Complex II reduces succinate from the TCA cycle to Ubiquinone which is then converted to cytochrome *c* in complex III. Cytochrome *c* donates its electron to reduce molecular oxygen to H_2O in complex IV (Nelson and Cox 2006). The energy required to drive adenosine diphosphate (ADP) to ATP is supplied by the OXPHOS

cascade of events observed here along with the direct energy of the proton motive force (PMF) (Terada 1990). The PMF drives H^+ across the impermeable membrane by redox reactions to increase stored energy through this gradient difference (Terada 1990, Nelson and Cox 2006). ATP synthase releases tightly bound ATP and can be inhibited by oligomycin which stops ATP production (Boyer 1997, Symersky *et al.* 2012).

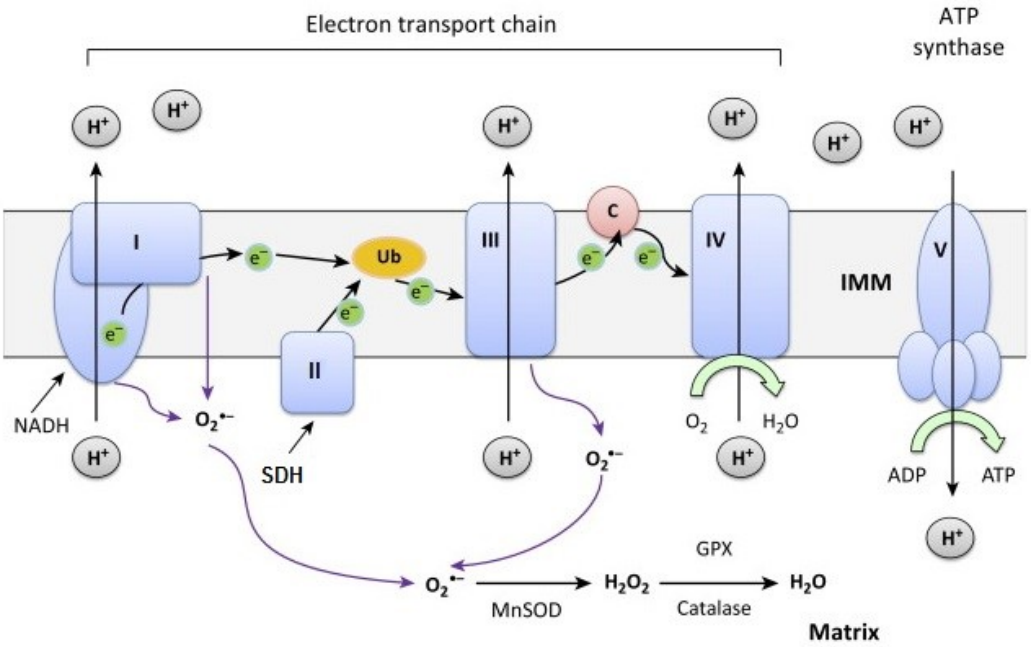

Figure 1.5. The mitochondrial electron transport chain.
The electron transport chain begins with electrons (e^-) being donated from NADH to complex I to form ubiquinone. Another ubiquinone molecule is created when SDH from the TCA cycle donates an electron through complex II during the conversion of succinate to fumerate. Ubiquinone mediates electron transfer from these complexes to complex III where it is converted to cytochrome *c* (C). At complex IV, electrons reduce molecular oxygen (O_2) to water (H_2O). As the electrons are transported through the electron transport chain, a proton (H^+) gradient called the PMF is created across the inner mitochondrial membrane (IMM). Reactive oxygen species (ROS) are created as a byproduct of the electron transport chain. Superoxide ($O_2^{\bullet-}$) is formed at complexes I and III. Under normal conditions this superoxide is dismutated to hydrogen peroxide (H_2O_2) by matrix manganese superoxide dismutase (MnSOD) and further to water by catalase or glutathione peroxidase (GPX). Adapted from Yu and Bennett 2014.

Furthermore, as previously discussed cancer cells rapidly proliferate and require a strong interaction between functioning mitochondria and proliferation (Hanahan and Weinberg 2011, Diers *et al.* 2012). This drive to proliferation leads cancer cells to

primarily carry out glycolysis even in the presence of oxygen as it leads to faster ATP production though much more inefficient (OXPHOS: 32 ATP/glucose, glycolysis: 2ATP/glucose) (Hanahan and Weinberg 2011, Diers *et al.* 2012). The mitochondria are important regulators not only in energy production but in the overall survival of normal and cancer cells. Their tight regulation can be beneficial to either state of the cell, however, due to this primary source of control it can be detrimental when it is damaged or dysfunctional.

1.1.3.3. Cellular calcium (Ca^{2+}) regulation

The importance of calcium (Ca^{2+}) in the body and cell is evident from birth and is a tightly regulated and controlled function (Prevarskaya *et al.* 2004, Parkash and Asotra 2010). Ca^{2+} is toxic to the cell in high doses and hence the tight regulation and control for levels ~ 100 nM (Saidak *et al.* 2009, Parkash and Asotra 2010). This control of Ca^{2+} is regulated through many different factors with the prime signalling controller being the mitochondria (Prevarskaya *et al.* 2004). Many of the controls for Ca^{2+} signalling and concentration are localized on the membranes of specific intracellular organelles or on the plasma membrane such as the transient receptor potential (TRP), voltage-dependant anion channels (VDAC), mitochondrial calcium uniporter (MCU) as well as others (Parkash and Asotra 2010, Kondratskyi *et al.* 2014). These receptors have been shown to regulate Ca^{2+} entry both in and out of the cell and organelles (Kondratskyi *et al.* 2014). An increase in Ca^{2+} release from the mitochondria can increase the rate of apoptosis within the cell (Rizzuto *et al.* 2003). Furthermore, it has been shown that an increase in Ca^{2+} can lead to the permeability transition pore (PTP) opening and induce mitochondrial fragmentation and swelling (Pinton *et al.* 2001). This damage to the mitochondria can lead to both an increase in ATP production with the cell trying to compensate for the fragmentation of the mitochondria and loss of the mitochondrial outer membrane potential (MOMP) and to apoptosis (Pinton *et al.* 2001, Rapizzi *et al.* 2002). A sensitization of the mitochondria to apoptosis has been documented with an

increase in VDAC expression, leading to an increase in Ca^{2+} uptake in the mitochondria which can lead to release of cytochrome *c* through VDAC (Rapizzi *et al.* 2002, Pastorino *et al.* 2005). Moreover, some cancers show an increase in Ca^{2+} leads to a reduction in cancer cells and a promotion of apoptosis (Mathiasen *et al.* 2002, Rizzuto *et al.* 2003).

1.1.3.4. The role of reactive oxygen species (ROS) in cellular regulation

Reactive oxygen species (ROS) are important regulators in both cell survival and cell death and must be tightly maintained to ensure controlled pathways (Gupta *et al.* 2012, J. Lee *et al.* 2012, Sena and Chandel 2012, Mei *et al.* 2014). There are several different types of ROS molecules that are derived from oxygen, these are hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet -}$), nitric oxide (NO), peroxynitrite ($ONOO^-$) and reactive lipid species (RLS) (J. Lee *et al.* 2012, Sena and Chandel 2012). The most abundant of intracellular ROS is $O_2^{\bullet -}$ whereby there are eight sites in the mitochondria that have been shown to produce this ROS molecule (Sena and Chandel 2012). ROS are always present in the cell as they are required for normal regulatory functions at low concentrations (Kuznetsov *et al.* 2011a, Sena and Chandel 2012). At low levels ROS can regulate homeostatic functions within the cell such as protecting the cell from damage, protein folding and disulfide bond formation in the EnR (Figure 1.6.) (Frank *et al.* 2012, Gupta *et al.* 2012, Sena and Chandel 2012). However, as ROS levels increase this can lead to senescence or cell death due to mitochondrial dysfunction (Ferber *et al.* 2012, J. Lee *et al.* 2012, Sena and Chandel 2012, Mei *et al.* 2014). The mitochondria, while generators of ROS, can also be targets for excessive ROS which can alter their function at high levels (Hahm *et al.* 2011, West *et al.* 2011, J. Lee *et al.* 2012, Sena and Chandel 2012, Mei *et al.* 2014). A reduction in mitochondrial respiration, ATP depletion, Ca^{2+} increase, increased lipid rafts and activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) are just a few of the effects of increased ROS in the mitochondria (Gniadecki

et al. 2002, Bitar and Al-Mulla 2011, Hahm *et al.* 2011, Kuznetsov *et al.* 2011b, Mei *et al.* 2014). Furthermore, the lack of a repair mechanism within mitochondrial mean they are 10 to 20 times more likely to have mutations that lead to apoptosis (Lisanti *et al.* 2010, J. Lee *et al.* 2012). Thus, modulating ROS levels has become a prime target when developing cancer treatment, with the example of Cis-Diammineplatinum(II) dichloride (cisplatin) a chemotherapeutic drug that increases ROS levels and consequently destroys the electron transport chain (ETC) and increases apoptosis of the cell through mitochondrial damage (Donadelli *et al.* 2011, Marullo *et al.* 2013). Increased ROS have been shown to increase MAPK and reduce superoxide dismutase (SOD) when it comes to non-alcohol related fatty liver disease (NAFLD) (Wang *et al.* 1998, Liu and Han 2014). Natural compounds such as withaferin A, curcumin, cannabinoids and cannabinoids synthetic analogues can increase ROS levels and also increase sensitivity to cancer cell death (Donadelli *et al.* 2011, Hahm *et al.* 2011, Gupta *et al.* 2012, Park *et al.* 2014). Furthermore, increased ROS has also been linked to innate immune signalling within the cell whereby the mitochondria's capacity to kill bactericidal activity is increased (West *et al.* 2011).

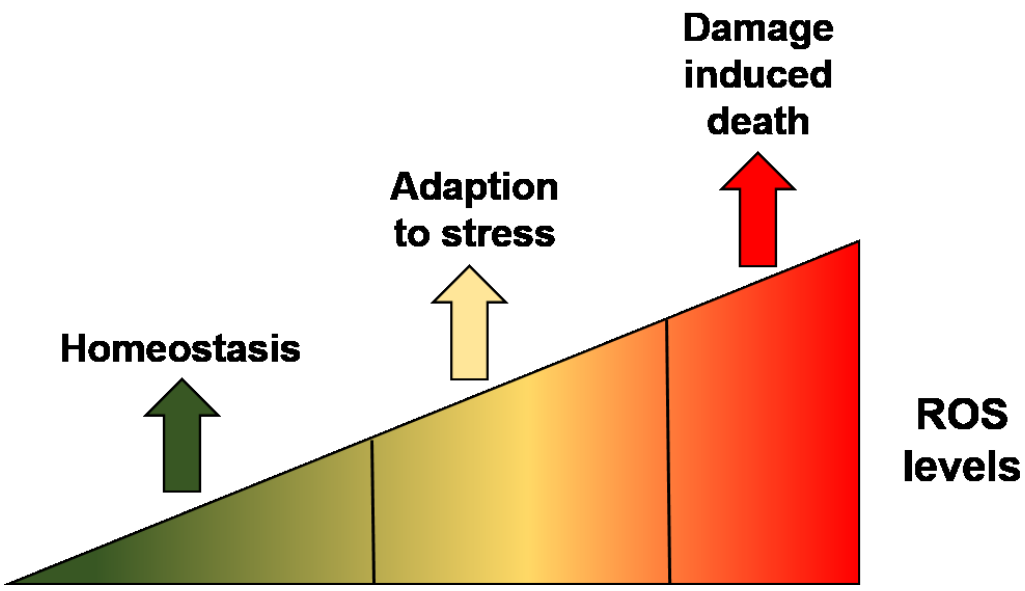

Figure 1.6. ROS levels determine physiological outcomes.
The level of ROS determines its function and the physiological outcome. Low levels of ROS are required for normal cell homeostasis. Whereas high levels directly damage proteins, lipids and nucleic acid leading to cell death. There is a fine balance of good and bad levels of ROS. Adapted from Sena and Chandel, 2012.

1.1.3.4.1. The critical role of the Nrf2 Pathway

A key regulator in controlling ROS levels within the cell is Nrf2 which is a transcription factor that is activated due to increased oxidative stress (Kensler *et al.* 2007, Bitar and Al-Mulla 2011). Nrf2 is a key regulator of cell survival by both external and internal stress stimuli (Kensler *et al.* 2007, Pi *et al.* 2007). It has been shown to counteract mitochondrial dysfunction in Parkinson's, suggesting a role in cancer prevention through inhibiting Nrf2 function (Tufekci *et al.* 2011). When Nrf2 is reduced or inhibited the toxicity of the cell increases leading to cell death (Kensler *et al.* 2007). The mechanism behind Nrf2's control of ROS levels is through the regulation of the antioxidant-response elements (AREs) which are the targets of Nrf2 (Figure 1.7.) (Ramos-Gomez *et al.* 2003, Kensler *et al.* 2007, Pi *et al.* 2007, Martin-Montalvo *et al.* 2011). When Nrf2 is inactive it is bound to Kelch-like ECH (Erythroid cell-derived protein

with can 'n' collar homology) -associated protein 1 (Keap1) and is retained in the cytoplasm, however, when ROS levels increase Nrf2 is liberated from Keap1 and translocates to the nucleus (Kensler *et al.* 2007). Nrf2 can then bind to the AREs and hence recruit for ARE-related genes for cell survival (Kensler *et al.* 2007, Pi *et al.* 2007). Nrf2 has also been shown to act as a tumour suppressor in inhibiting spontaneous and induced tumour growth (Martin-Montalvo *et al.* 2011). However, loss of Nrf2 has also been linked to deoxyribonucleic acid (DNA) damage, chemotherapy sensitivity and tumour death (Ramos-Gomez *et al.* 2003, Kensler *et al.* 2007, Martin-Montalvo *et al.* 2011, Tufekci *et al.* 2011).

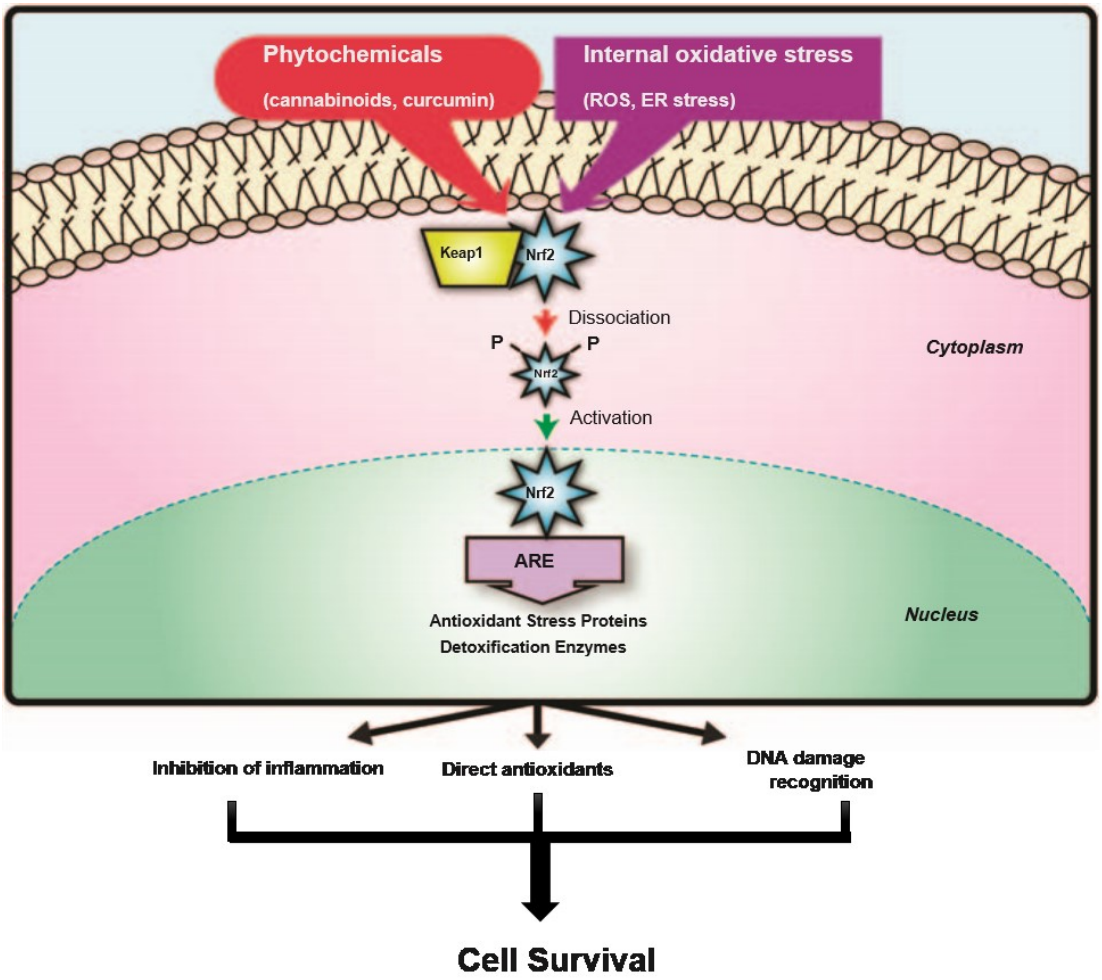

Figure 1.7. General activation of Nrf2.
External (phytochemicals) stress and internal oxidative stress can lead to the release of Nrf2 from Keap1 allowing it to become phosphorylated and active. Nrf2 then translocates to the nucleus where is interacts with the AREs which induce specific target gene functions. Hereby leading to an adaptive response that enhances the resistance of cells to environmental and intracellular stresses. Amalgamated from Kensler et al., 2007 and Martin-Montalvo et al., 2011.

1.1.4. The outlook for cancer in the future

The advancements seen in both the understanding and the development of cancer therapeutics have shown great potential in further cancer therapy. It is predicted that by 2030 there will be 23.6 million new cases of cancer worldwide each year (Bray *et al.* 2012). Furthermore, since 1971 over $300 billion has been spent on cancer research in the US (Brücher *et al.* 2014). The economical and personal strain that cancer has on our society needs to be reduced for a more substantial and sustainable future. The future of cancer research is ever evolving with many new targets that are focused on killing the cancer completely (Cairns *et al.* 2011, Munoz-Pinedo *et al.* 2012, Muñoz-Pinedo *et al.* 2013).

Some examples of these are through targeting the “Warburg Effect” and the cancer cells preference to not carry out OXPHOS (Vander Heiden *et al.* 2009, Munoz-Pinedo *et al.* 2012). By targeting this alteration in metabolism that is a metabolically preferred route in cancer cells could be both a targeted and successful method (Israelsen and Vander Heiden 2010, Diaz-Ruiz *et al.* 2011, Diers *et al.* 2012). Cancer cells also increase ROS levels that are abnormal which may also be targeted to induce cell death (Cairns *et al.* 2011, J. Lee *et al.* 2012, Sena and Chandel 2012). Recent work with natural compounds has shown that this could be a successful route for increasing cancer cell death (Donadelli *et al.* 2011, Gupta *et al.* 2012). Furthermore, the mitochondria itself can be targeted for inducing cancer cell death as it is the main regulator of both ROS and energy control (Vander Heiden *et al.* 2009, Hanahan and Weinberg 2011, Frank *et al.* 2012). Mitochondrial damage and death can lead to cell death which is also evident when certain tumour suppressing genes are altered such as p53 and Nrf2 (Kensler *et al.* 2007, Cairns *et al.* 2011, Martin-Montalvo *et al.* 2011, Munoz-Pinedo *et al.* 2012, Koczor *et al.* 2013, Muñoz-Pinedo *et al.* 2013). Finally, personalised medicine and epigenetic medicines are also emerging to combat cancer proliferation at an individual specific level. However, these medicines target specific genes and histone modifications

which could be expensive and may not be a rapid route for treatment (Muñoz-Pinedo *et al.* 2013).

The outlook for cancer is an expensive one that does not predict a reduction in cancer prevalence. However, the slowdown already observed with certain cancers as mentioned previously and the advances with novel methods in killing cancers this predicted cancer death number for the future may be much lower than perceived.

1.2. Chemotherapeutic treatments

1.2.1. Introduction to chemotherapy

The treatment of cancer with chemotherapeutic compounds only came to light in the 1940s when Gilman and his colleagues showed that treatment of non-Hodgkin's lymphoma patients with 0.1 mg/kg of nitrogen mustard (tris β-chloroethylamine) saw a remission in the patient's cancer (Gilman 1963, Chabner and Roberts 2005). This was the first case of tumour regression using a chemotherapeutic treatment to be shown. However, the remission observed only lasted a few weeks before recurrence of the tumour (Gilman 1963). Nevertheless, this treatment paved the way for further work into cancer treatment and prolonging patients' lives. Farber and colleagues showed that by inhibiting the function of folate-requiring enzymes which had been shown with 4-aminopteroyl-glumatic acid (a folic acid antagonist) remission was induced in children with acute lymphoblastic leukaemia (ALL) (Farber and Diamond 1948). The first solid tumour, choriocarcinoma, to be treated by a chemotherapy drug was carried out using methotrexate in 1958. They showed that by administering methotrexate at 15-25 mg for 5 days and 6-mercaptopurine at 600-800 mg for 5 days reduced the tumour size for 5 of the 6 females treated. Unfortunately none of the 6 males in this study survived treatment (Li *et al.* 1958). This work allowed many more advances to be pursued in cancer therapy work. Chemotherapy treatment began to be used in combination, with natural products,

as an adjuvant to surgery and further towards individual targeted-therapy (Chabner and Roberts 2005).

1.2.2. Current clinical chemotherapy treatments

1.2.2.1. Single-agent chemotherapy treatments

Single chemotherapy treatments were used from the 1940s as introduced in section 1.2.1. and much work has been carried out to advance this field. Though there is no direct evidence that using a single-agent chemotherapy can be more beneficial to the patient, it could aid in lower adverse side effects (Hudis 2002). Burris and his colleagues showed that gemcitabine had a beneficial response rate of 23.8% in pancreatic cancer compared to the fluorouracil treatment that had been previously used (4.8% beneficial) (Burris *et al.* 1997). Recent work has shown that the single agent trastuzumab has had beneficial responses with human epidermal growth factor receptor 2 (HER2)-overexpressing metastatic breast cancer in women. In this study 57% of patients showed an objective response and 51% had not experienced disease progression following treatment (Vogel *et al.* 2002). Furthermore, the single-agent Rituximab has been shown to be beneficial as a chemotherapeutic agent in chronic lymphocytic leukaemia or small lymphocytic lymphoma with 58% of patients responding to treatment with a further 62% having a progression-free survival one year later (Hainsworth *et al.* 2003). Research has shown that using single-agent treatment can be beneficial and can also lead to lower toxic treatments and better quality-of-life (Joensuu *et al.* 1998, Hudis 2002). It is these advances that must be remembered when looking into novel chemotherapeutic treatments.

1.2.2.2. Combination therapy's use in chemotherapy

Combination therapy is the effective use of more than one drug to enhance cell death (Chabner and Roberts 2005, Caley and Jones 2012). A strict selection process is followed as the generation of an effective combination regime can be complex and sometimes unsuccessful (Caley and Jones 2012, M. J. Lee *et al.* 2012). The single agent previously discussed, methotrexate, was also shown to prevent recurrence of osteosarcoma when used in combination with leucovorin post-removal of the tumour. They showed that when used in combination, as an adjuvant to the therapy, there was a delay in relapse and in some cases no relapse (Jaffe *et al.* 1974). Adjuvant therapy is the use of additional agents following the definitive primary treatment, either surgery or drug targets, to assist in eradicating the tumour and is used to prevent relapse (Caley and Jones 2012, Cognetti *et al.* 2012). It was further shown that methotrexate could be used in combination with vincristine (*Vinca* alkaloid), 6-mercapropurine (purine analogue) and prednisone (corticosteroid) to induce long-term remissions of ALL in children (Frei *et al.* 1965, Chabner and Roberts 2005). However, the combination that proved to be most beneficial in this study was when prednisone was used in combination with 6-mercapropurine (82% complete remission) and prednisone combined with vincristine (84% complete remission) (Frei *et al.* 1965). This combination therapy and the treatment's positive results drove more research into showing that combination therapy as an adjuvant could be more beneficial to patients (Frei *et al.* 1965, Chabner and Roberts 2005). In 1967, Moxley and his colleagues showed that by combining cyclophosphamide (nitrogen mustard), vincristine, methotrexate and prednisone could improve the remission occurrence of Hodgkin's lymphoma prior to radiotherapy treatment. They showed an 80% remission rate with a mean of 33 months with no reoccurrence of visible tumours (Moxley *et al.* 1967). Furthermore, Devita and colleagues showed that with a combination of cyclophosphamide, vincristine, procarbazine hydrochloride and prednisone for 6 months an 81% remission of advanced

Hodgkin's lymphoma was observed with a survival of over 42 months (Devita *et al.* 1970). When levamisole (antihelmintic) and fluorouracil (pyrimidine analogue) were combined in treating colon cancer a reduction of recurrence was 41% and a further 42 months later showed 63% of the patients remained recurrence-free (Moertel *et al.* 1990, Mutch and Hutson 1991).

1.2.2.3. Reaction to current chemotherapy

Many of these chemotherapy treatments mentioned in 1.2.2.1. and 1.2.2.2. only prolong remission stages and can induce both resistance and adverse side effects (Moxley *et al.* 1967, Devita *et al.* 1970, Caley and Jones 2012). Many of the studies mentioned above did not remove the cancer completely from the patients but only allowed a remission from the cancer before it reoccurring. Remission is recorded to last between 2 months and 42 months in the cases mentioned above (Farber and Diamond 1948, Moertel *et al.* 1990). Though the patients did overcome the moribund status they had, chemotherapy did not alone eradicate the cancers and further surgery was required (Farber and Diamond 1948, Moxley *et al.* 1967, Burris *et al.* 1997). Along with the remission rate adverse side effects are observed during chemotherapy treatment. These can range from loss of appetite, alopecia, renal problems, fatigue, low blood count, neurological deficit to chemotherapy resistance (Farber and Diamond 1948, Frei *et al.* 1965, Moxley *et al.* 1967, Devita *et al.* 1970, Jaffe *et al.* 1974, Burris *et al.* 1997, Caley and Jones 2012). The control of these side effects has been directed towards eastern medicine and western eastern medicine as they have been shown to alleviate pain, inflammation and appetite (Tramèr *et al.* 2001, Ye *et al.* 2002). Cannabinoids have been shown to be antiemetic, with a ~70% control of nausea and vomiting in several studies (Tramèr *et al.* 2001).

A major problem and cause of failure with chemotherapy is the resistance to treatment whereby there can be a reduced delivery of agent to the cell, decreased drug uptake and activation, inhibition of apoptosis and increased drug efflux (Figure 1.8.)

(Caley and Jones 2012). Platinum based drugs such as cisplatin have had a major impact on cancer therapy due to their toxic effects (Siddik 2003, Galluzzi *et al.* 2006, Santin *et al.* 2013). However, the 70% remission observed in cancer therapy does not remain as there is a high incidence of chemoresistance where 95% recurrence rate has been shown in small cell lung carcinoma and a further 15-20% survival after 5 years with ovarian cancer (Ozols 1991, Giaccone 2000, Siddik 2003, Galluzzi *et al.* 2006). This resistance has been shown to be 30–1000 fold increased in ovarian cancer cell which has been shown to coincide with glutathione levels, as well as resistance through mitochondrial apoptotic regulators Bcl-2 (Godwin *et al.* 1992, Hong *et al.* 2008, Santin *et al.* 2013). Cisplatin has been shown to work through DNA damage and induction of mitochondrial apoptosis (Siddik 2003, Galluzzi *et al.* 2006, Santin *et al.* 2013) and has more recently been shown to directly target the mitochondrial membrane through binding to VDAC receptors (Yang *et al.* 2006).

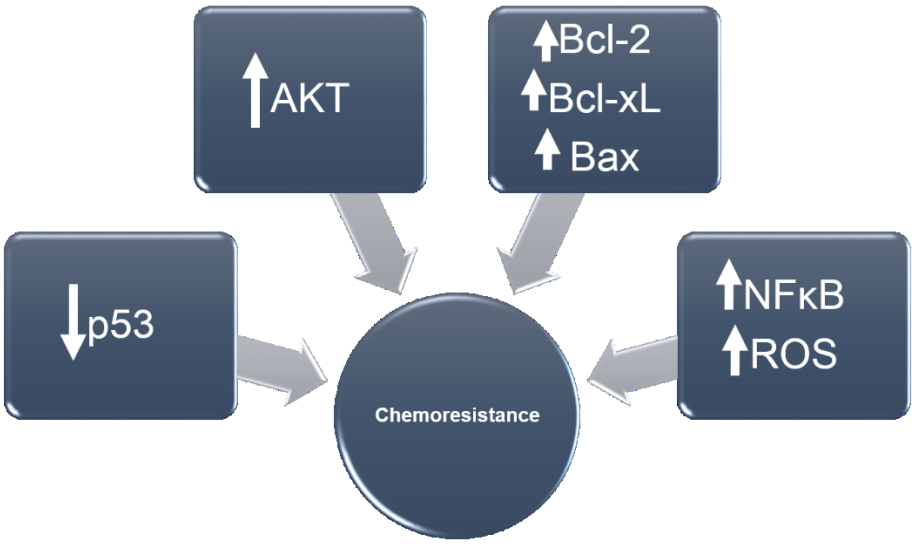

Figure 1.8. Drug resistance can be targeted by certain pathways.
There are several molecular regulators that can increase a cancer cells resistance to chemotherapy such as alterations with p53, AKT, the Bcl-2 family and NFκB pathway. Adapted from Vinod et al., 2013.

The adverse side effects observed with chemotherapy and the resistance described above in the example of cisplatin have shown that our understanding of

chemotherapy treatment is still quite basic. Cisplatin has shown to be advantageous at initially reducing cancer remission but a longer treatment and cure is required (Godwin *et al.* 1992, Giaccone 2000, Siddik 2003).

1.2.3. Restoration of mutated pathways – future therapy?

A prominent study has shown that restoring tumour suppressing pathways can lead to a regression in tumour advancement (Ventura *et al.* 2007). Mutations in the p53 pathway are the most commonly observed mutations in cancer and over 70% of tumours have been shown to have mutated p53 (Hui *et al.* 2006). However, of these mutations observed most are missense and not fully deleted or truncated mutants which can lead to the hypothesis that these genes could be restored (Hui *et al.* 2006, Ventura *et al.* 2007). In response to stress, p53 can aid in normalising ROS levels to ensure protection of the genome from ROS damage (W. M. Chen *et al.* 2012). It is this tumour control that is required for cancer cell prevention. Ventura and colleagues showed that the loss of p53 function can be restored in mice with autochthonous lymphomas and sarcomas without affecting normal tissues (Ventura *et al.* 2007). This restoration was suggested to be due to an increase in stress conditions to the cell which increased ROS levels. It has also recently been shown that in high stress conditions, p53 levels can increase sufficiently to increase ROS production in a chemopreventative way (W. M. Chen *et al.* 2012). As previously mentioned, Nrf2 is a cancer chemopreventative transcription factor which can control ROS levels within the cell (Martin-Montalvo *et al.* 2011, W. M. Chen *et al.* 2012, Namani *et al.* 2014). When p53 is increased due to increased ROS, a second phase of control is activated whereby p53 inhibits Nrf2 activity (Martin-Montalvo *et al.* 2011, W. M. Chen *et al.* 2012). Thus, there is no control of ROS levels in the cell resulting in increased cell stress and death (W. M. Chen *et al.* 2012, Sena and Chandel 2012). This restoration of p53 function has been shown to have a major role in cancer chemoprevention (Ventura *et al.* 2007, W. M. Chen *et al.* 2012).

1.3. Cannabis and its role in medicine

1.3.1. Introduction to cannabis

The cannabis plant is the earliest domesticated plant and has been used as hemp from 6000 Before Present (BP) and as a medicine from nearly 5000 years ago where documentation shows it was recommended for malaria, constipation and childbirth pain among many other ailments (Mechoulam 1986, van Bakel *et al.* 2011). The first cannabinoid to be isolated was cannabinol in 1899 followed by cannabidiol (CBD) in 1934 and Δ^9-tetrahydrocannabinol (THC) in 1964 (Wood *et al.* 1899, Adams *et al.* 1940, Gaoni and Mechoulam 1964, Robson 2001). Furthermore, a specific receptor for cannabinoids (cannabinoid receptor 1, CB_1) was discovered in 1990 in rat nerve cells (Matsuda *et al.* 1990). From this, many advances in medicinal therapeutics have been observed through cannabis based medicines. Some of these include the development of Sativex® through the research carried out on multiple sclerosis (MS) (Robson 2001, Barnes 2006, Blake *et al.* 2006, Russo *et al.* 2007). Using cannabis as a treatment for MS, muscle pain was documented to be reduced 91–98% as well showing as a reduction in depression, sleeplessness, tremors and anxiety (Robson 2001, Blake *et al.* 2006, Russo *et al.* 2007). Moreover, much cancer work has shown that using cannabinoids can lead to a regression of tumours, a decrease in epileptic seizures and an increase in diet for human immunodeficiency virus (HIV) patients (Whalley *et al.* 2004, McAllister *et al.* 2011, Shrivastava *et al.* 2011, Riggs *et al.* 2012, Macpherson *et al.* 2014). The understanding of cannabis and its potential use in medicinal therapeutics is advancing rapidly due to the introduction of synthetic cannabinoids in treatment, the legalisation of marijuana use and the authorisation of Sativex® for use in Europe (GWPharma , Dalzell *et al.* 1986, Ryan-Ibarra *et al.* 2014).

1.3.2. Brief history of cannabis

The cannabis plant has been used in society as far back as 6000BP where the first documented evidence of its use as a medicinal treatment was observed as previously described in section 1.3.1. (Li 1973, Mechoulam 1986, Russo. 2004, van Bakel *et al.* 2011). There are subsequent records of its use from ancient Egypt with hieroglyphic data as a medicine and in every day house hold items such as fabric, paper, and food (Russo. 2004, van Bakel *et al.* 2011).

In the 19th century, an Irish physician, Dr. William B. O'Shaughnessy highlighted the medicinal use of Cannabis to the western world. He documented the treatment of rheumatism, hydrophobia, cholera, infantile convulsions and delirium through the use of Cannabis extracts (O'Shaughnessy 1843, Robson 2001). This led to the introduction of cannabis to Great Britain and the rest of Europe where it was rapidly used as a therapeutic treatment (Clendinning 1843, Robson 2001, Russo. 2004).

However, in 1928 Cannabis was outlawed by ratification of the 1925 Geneva Convention as a dangerous drug (Robson 2001). It was finally prohibited further through the 1971 Misuse of Drugs Act which was against the advice put forward by the Advisory Committee on Drug Dependence and is now a class B controlled drug (Robson 2001, UK Government 2014).

1.3.3. Cannabinoids and their derivation

The cannabis plant produces a unique set of compounds known as cannabinoids, which control its growth, development and survival (Guzman 2003, Russo 2004). Phytocannabinoids are not uniformly distributed in the plant with the highest levels of concentration found in the leaves and unfertilized flower heads (Russo. 2004). As previously mentioned, the first cannabinoid to be isolated from the Cannabis plant was Cannabinol in 1899 (Wood *et al.* 1899). Consequently, in the 1940s Adams and his colleagues discovered CBD, a cannabinoid with an abundance of ~40% in the cannabis

plant (Adams *et al.* 1940, Mechoulam and Hanus 2000). This abundance observed with CBD was shown to be as high as THC (Gaoni and Mechoulam 1964, Malfait *et al.* 2000). Furthermore, Mechoulam and his colleagues isolated and defined the structure of THC (Gaoni and Mechoulam 1964). Since this preliminary work on cannabinoids over 420 different compounds have been isolated, which include over 60 unique cannabinoids (Guzman 2003, Huestis 2007, Hosking and Zajicek 2008). Cannabinoids are classified based on their chemical structure and can be also further classified into phytocannabinoids (plant derived cannabinoids; THC, CBD), synthetic cannabinoids (chemically synthesised *in vitro*; Nabilone®, Dronabinol®) and endocannabinoids (endogenous compounds that are found in the human body, N-arachidonoyl ethanolamine [anandamide (AEA)] and 2-arachidonoyl glycerol (2-AG)) (Guzman 2003, Alexander *et al.* 2009).

The first endocannabinoid to be isolated and have its structure identified was AEA from porcine brain extracts (Devane *et al.* 1992). This was followed closely by 2-AG which was shown to have a high abundance in the central nervous system (CNS) compared to AEA (170 times greater) (Stella *et al.* 1997). These cannabinoids were members of the fatty acid amide (FAA) and monoacylglycerol (MAG) families and shown to also have an affinity to the CB_1 and cannabinoid receptor 2 (CB_2) (Silvestri and Di Marzo 2013). The endocannabinoids have been shown to regulate appetite, metabolic disorders, cardiovascular function and mood (Vazquez-Roque *et al.* 2011, Silvestri and Di Marzo 2013, Davis 2014).

1.3.3.1. Cannabinoid receptors and their role

A great deal of much research has been carried out identifying the receptors that interact with these cannabinoids. The CB_1 receptor was first discovered in cortical rat brain and was shown to only have an affinity to the CNS (Matsuda *et al.* 1990, Alexander *et al.* 2009). Consequently the CB_2 receptor was identified and shown to have little

affinity in the CNS but largely accumulated in macrophages and the immune system in humans (Munro *et al.* 1993). The cannabinoid receptors have many relevant homeostatic functions in the body and are tightly regulated to relieve from stress, control food deprivation and activate an immune response (Matias and Di Marzo 2007, Gasperi *et al.* 2013). These cannabinoid receptors are members of the 7-transmembrane-segment, G-protein coupled receptor (GPCRs) superfamily (Matsuda *et al.* 1990, Alexander *et al.* 2009, Davis 2014). The CB_1 receptor has been shown to be associated with feeding and in the promotion of liver fat (Guzman 2003, Bergamaschi *et al.* 2011, Cooper and Regnell 2014). Due to these observations, inhibiting and exciting the CB_1 receptor has been pursued for both weight loss e.g. Rimonabant, and weight gain e.g. in HIV patients where an increase in leptin and ghrelin activity has been noted to increase weight (Van Gaal *et al.* 2005, Riggs *et al.* 2012). The CB_2 receptor has been linked to increased apoptosis, decreased inflammation and reduced pain transmission (McKallip *et al.* 2002a, Quartilho *et al.* 2003, Whiteside *et al.* 2007). In normal breast tissue CB_2 receptors are not detected in large amounts but are found to be overexpressed in oestrogen receptor (ER) negative breast tumours and prostate cancer cells (Sarfaraz *et al.* 2005, Alexander *et al.* 2009, Shrivastava *et al.* 2011). Interestingly, cisplatin has been shown to increase AEA signalling at the CB_1 receptor reducing neurotoxicity of cisplatin (Khasabova *et al.* 2012). In addition to CB_1 and CB_2 receptors, TRP vanilloid type 1 (TRPV-1), GPR55, GPR119 and GPR18 have been identified as candidates for cannabinoid interaction (Costa *et al.* 2004, Fogli and Breschi 2008, Velasco *et al.* 2012).

1.3.3.2. Pharmacokinetics of endocannabinoids

Endocannabinoids are lipid based molecules that mimic the activity of phytocannabinoids by binding to CB_1 and CB_2 receptors (Tantimonaco *et al.* 2014). These cannabinoids are produced on demand and are rapidly degraded as they are

used immediately within the body and are tightly regulated (Bisogno *et al.* 2001, Maccarrone *et al.* 2010, Aviello *et al.* 2012, Fonseca *et al.* 2013). They are found in the brain, peripheral tissue, and in immune cells and have been known to regulate lipid synthases, glucose metabolism and inflammation (Izzo and Sharkey 2010, Vazquez-Roque *et al.* 2011, Silvestri and Di Marzo 2013, Tantimonaco *et al.* 2014). AEA is released from *N*-arachidonoylphosphatidylethanolamine (NArPE) and hydrolysed by fatty acid amide hydrolase (FAAH) (Vazquez-Roque *et al.* 2011, Tantimonaco *et al.* 2014). The *sn*-1-diacylglycerol lipases (DAGLs) which are abundant in the nervous tissue converts diacylglycerol to 2-AG which is then hydrolysed by monoacyelglycerol lipase (MAGL) (Fonseca *et al.* 2013, Tantimonaco *et al.* 2014). Both AEA and 2-AG when degraded release ethanolamine or glycerol and arachidonic acid which have no affinity for the cannabinoid receptors (Maccarrone *et al.* 2010, Roques *et al.* 2012). AEA has been shown to have a lower affinity for the CB_2 receptor than the CB_1 whereas 2-AG binds to both with no preference (Pertwee *et al.* 2010, Fonseca *et al.* 2013). However, endocannabinoids have been shown to bind to other cannabinoid associated receptors and also non-cannabinoid receptors (TRPV-1, GPR55) (Costa *et al.* 2004, Davis 2014). Furthermore, endocannabinoids mechanism within the cell has been linked to increased calcium levels and consequently linked increase in TRPV-1 channels (Di Marzo and De Petrocellis 2010).

1.3.3.3. Pharmacokinetics of phytocannabinoids

Phytocannabinoids are plant derived cannabinoids that can interact and bind to the cannabinoid receptors and regulate several key systemic regulations (Huestis 2007). As mentioned previously there are over 60 unique cannabinoids, however, only a handful of these have been shown to be beneficial (Guzman 2003, Huestis 2007, Hosking and Zajicek 2008). Cannabinoids are highly hydrophobic and distribution is based around its lipophilic state which allows cannabinoids to be taken up initially by

tissues that are highly perfused by blood vessels such as the heart, lung, brain, spleen, adipose tissue and liver (Huestis 2007, Sharma *et al.* 2012). The pharmacokinetics of phytocannabinoids are different to endocannabinoids however their final receptors and targets have been suggested to be similar (Russo 2004, Ligresti *et al.* 2006, De Petrocellis *et al.* 2012a).

1.3.3.3.1. The metabolism of Δ^9-tetrahydrocannabinol, THC

The highly lipophilic THC is distributed in many deposits within the body including adipose tissue, liver and lung (Huestis 2007, Sharma *et al.* 2012). Following inhalation, THC is detected in blood plasma in seconds and approximately 90% of the THC is circulated in the plasma (Sharma *et al.* 2012). Along with this THC is readily absorbed orally due to its high octanol/water partition coefficient (Huestis 2007). The metabolism of THC to the primary metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9- carboxy-Δ^9- tetrahydrocannabinol (THC-COOH) were first documented by Burstein and colleagues in 1972 (Burstein *et al.* 1972, Sharma *et al.* 2012). There are 2 phases of THC metabolism. The first is an initial oxidation step that modifies the structure of the cannabinoid to the active 11-OH-THC compound (Burstein *et al.* 1972, Huestis 2007, Sharma *et al.* 2012). In phase 2 metabolism of THC, the psychoactive 11-OH-THC is oxidised to the inactive THC-COOH (Lemberger *et al.* 1970, Huestis 2007, Sharma *et al.* 2012). Maximum THC plasma concentrations is reached 8 minutes after smoking, while 11-OH-THC peaked at 15 minutes and after 81 minutes THC-COOH peaked (Sharma *et al.* 2012). The final elimination of THC is through the liver by cytochrome P450 (CYP) (Burstein *et al.* 1972, Malfait *et al.* 2000, Huestis 2007, Sharma *et al.* 2012). However, in chronic cases THC can be stored in the body in fat stores though this is minimal as THC is eliminated completely in 5-13 days for chronic users (Huestis 2007, Sharma *et al.* 2012).

1.3.3.3.2. The metabolism of cannabidiol, CBD

As previously mentioned CBD has as high an abundancy in the cannabis plant as THC though is devoid of any hallucinogenic properties (Adams *et al.* 1940, Gaoni and Mechoulam 1964, Malfait *et al.* 2000, Mechoulam and Hanus 2000). Along with the non-psychoactive properties, it has recently been shown to partially counteract THC's effects (Malfait *et al.* 2000, Huestis 2007). CBD also has low toxicity and an affinity to bind to the CB_2 receptor (Malfait *et al.* 2000, Russo *et al.* 2005). The absorbance of CBD into the body is quite similar to THC due to its high hydrophilicity. Once absorbed into the body, it is detectable for up to 3-4 hours (Nadulski *et al.* 2005, Huestis 2007). There are over 34 metabolites from CBD with the main mechanism being the oxidation to carboxylic acid (Harvey and Mechoulam 1990). Within the plant CBD is produced through the non-enzymatic decarboxylation of cannabidiolic acid (CBDA) (Takeda *et al.* 2012). CBD has been shown to inhibit CYP-mediated conversion of THC to 11-OH-Δ^9-THC (Bornheim *et al.* 1993, Ligresti *et al.* 2006). CBD is detected within 30 minutes and remains in the body 3-4 hours. The final elimination of CBD is mainly unchanged through the faeces and the remaining metabolites are directed to the liver (Huestis 2007).

1.3.4. Therapeutic benefits of cannabis

Recent studies have shown the advantageous therapeutic effects of cannabis with endocannabinoids, phytocanabinoids and in synthetic cannabinoids (Robson 2001). Due to the beneficial properties first laid out by O'Shaughnassey and further researched by many groups, cannabinoids have proved beneficial in weight management, cancer regulation, pain relief, metabolic syndrome and depression (O'Shaughnessy 1843, Shrivastava *et al.* 2011, Vazquez-Roque *et al.* 2011, Riggs *et al.* 2012, Xiong *et al.* 2012, Lim *et al.* 2013).

1.3.4.1. The effect of cannabinoids on pain relief

The best known effects of cannabinoids include pain relief, anti-inflammatory response, anti-oxidant reactions, metabolic alteration, anti-tumourogenesis and mood effectors (Ashton 2001, Robson 2001, Guzman 2003, Musty 2004, Caffarel *et al.* 2006, Hosking and Zajicek 2008, Shrivastava *et al.* 2011, Caffarel *et al.* 2012, Riggs *et al.* 2012, Robson 2014). The main use of cannabis since the 19th century and currently in medicine is as a pain reliever (Iversen 2001, Robson 2001, Downer and Finn 2014). It was described by Walker that cannabis is as potent as morphine in pain relief for the body (Walker and Huang 2002, Musty 2004). Documentation on this analgesic effect and emerging evidence suggests that cannabis would be more useful in some pain relief treatments then the current therapies used (Holdcroft *et al.* 1997, Iversen 2001). The pain relief inhibition observed has been suggested to act through a reduction in inflammation and by inhibiting nociceptive neurotransmission (Guzman 2003, Lynch and Campbell 2011). This reduction in pain has been used in MS sufferers whereby a 1:1 ratio of THC: CBD (2.7 mg: 2.5 mg) has been used in Sativex® to alleviate muscle soreness and chronic pain (Joy *et al.* 1999, Zajicek *et al.* 2003, Hosking and Zajicek 2008, Pharmaceuticals 2011, Caffarel *et al.* 2012, Robson 2014). Preliminary work with cannabinoids and their anti-inflammatory effects showed a 98% improvement in muscle pain and spasms (Consroe *et al.* 1997). Furthermore, the reduction in pain also reduced inflammation which furthermore aided in improved muscle spasticity with cannabis extract showing a 15% improvement to patients and THC showed a 14% added improvement (Zajicek *et al.* 2003). Nabilone® a synthetic THC analogue has also been used for chronic inflammatory pain the US (Robson 2014).

1.3.4.2. The regulation of appetite by cannabinoids

The alteration of appetite with cannabinoid use has been well documented for weight loss and weight gain (Walsh *et al.* 2003, Wang *et al.* 2011, Cluny *et al.* 2012,

Riggs *et al.* 2012, Wargent *et al.* 2013). Historical documentation has shown the use of cannabis for the restoration of appetite for diseased patients and those with anorexia nervosa (Berry and Mechoulam 2002). However, recent efforts at weight loss through inhibiting the CB_1 receptor with a novel drug, Rimonabant, showed adverse psychiatric effects and had to be removed from the market even though there were promising weight reductions observed (Wargent *et al.* 2013, Cooper and Regnell 2014). However, it was not highlighted whether this was due to CB_1 receptor inhibition or due to Rimonabant itself. Further research with tetrahydrocannabivarin (THCV) has shown promising results as it is also a CB_1 antagonist though is a neutral THC analogue. The mode of action of THCV seems to be through increasing energy expenditure and insulin sensitivity (Wargent *et al.* 2013). On the other hand inducing weight gain through low doses of cannabinoids has been pursued for HIV and cancer patients that have disease-induced anorexia (Plasse *et al.* 1991, Robson 2001, Guzman 2003, Walsh *et al.* 2003, Caffarel *et al.* 2012, Riggs *et al.* 2012, Howard *et al.* 2013). The drug Marinol® (an oral capsule of dronabinol®) has been recently prescribed for HIV victims to help with weight management (Caffarel *et al.* 2012).

1.3.4.3. Epilepsy and arthritis control with cannabinoid treatment

Recently cannabinoids have also been shown to have potential beneficial properties in epilepsy and as anti-convulsant (Whalley *et al.* 2004, Jones *et al.* 2012, Devinsky *et al.* 2014, Iannotti *et al.* 2014). CBD has been shown to reduce nausea and vomiting in epileptic patients alone with not having any major detrimental effect on motor function (Whalley *et al.* 2004, Jones *et al.* 2012, Robson 2014). Moreover a patent has suggested that CBD used at 300 mg/day can be beneficial in treating epileptic fits and reduce pain and anxiety (Whalley *et al.* 2013). Moreover this reduction in pain and inflammation has been shown to also be advantageous in rheumatoid arthritis (Malfait *et al.* 2000, Fukuda *et al.* 2014, Robson 2014).

1.3.4.4. The regulation of cancer by cannabinoids

Another important effect of cannabis is its anti-inflammatory and anti-oxidant responses in cancer studies (Guzman *et al.* 2001, Guzman 2003, McAllister *et al.* 2011, Caffarel *et al.* 2012, Solinas *et al.* 2012, Massi *et al.* 2013, Downer and Finn 2014, McAllister and Desprez 2014). One of the mechanisms of the immunosuppression of cells may be from the induction of apoptosis. Both phytocannabinoids and synthetic cannainoids have shown pro-apoptotic activity in various cancer cell types including breast, colon, prostate, lung, liver cancer (McKallip *et al.* 2002b, Sarfaraz *et al.* 2005, McAllister *et al.* 2011, Shrivastava *et al.* 2011, Aviello *et al.* 2012, Ramer *et al.* 2012, Velasco *et al.* 2012). Much work has been carried out with cannabinoids, especially CBD with breast cancer (Guzman 2003, Caffarel *et al.* 2006, Ligresti *et al.* 2006, McAllister *et al.* 2011, Shrivastava *et al.* 2011, Caffarel *et al.* 2012, Takeda *et al.* 2012). In normal breast tissue CB_2 is not typically expressed, however in immune tissue and ER- breast tumours there is a 10-100 fold over-expression (Parolaro *et al.* 2002, Alexander *et al.* 2009). This expression is seen to directly correlate to the histological grade of the breast cancer (Caffarel *et al.* 2006, Alexander *et al.* 2009). MDA-MB231 breast cancer cells are an ER- tumour and studies have shown an inhibition of proliferation at the growth 1/synthesis (G_1/S) phase and tumour growth has been inhibited by CBD and CBDA (Sarnataro *et al.* 2006, Shrivastava *et al.* 2011, Takeda *et al.* 2012). Colon cancer has been reduced with CBD concentrations between 0.3 μM to 5 nM with an average of 5 μM to 10 μM CBD being optimum (Aviello *et al.* 2012, Macpherson *et al.* 2014, Romano *et al.* 2014). THC has been shown to reduce glioma tumours without being toxic to normal neuronal cells (Galve-Roperh *et al.* 2000, Aguado *et al.* 2007). Both synthetics (WIN-55,212-2) and natural cannabinoids have been shown to have beneficial effects on tumour cell progression in lung, prostate and liver cancers (Sarfaraz *et al.* 2005, De Petrocellis *et al.* 2012b, Ramer *et al.* 2012). Dronabinol®, Marinol® and Cesamet® (capsules of Nabilone®) have been permitted to be used in nausea and vomiting

resulting from chemotherapy for cancer (Guzman 2003, Caffarel *et al.* 2012, Howard *et al.* 2013). Finally, a recent patent has been published highlighting the use of CBD alone or in combination with THC for cancer treatment (McAllister and Desprez 2014).

1.3.4.5. Conclusion of cannabis in current medicine

The therapeutic benefits of cannabinoids are evident in many chronic conditions such as cancer, epilepsy, chronic pain (MS), HIV, arthritis (Guzman 2003, Xiong *et al.* 2012, Howard *et al.* 2013, Robson 2014). The reduction of inflammation observed following cannabinoid treatment in pain relief, cancer and arthritis to name a few show the broad range of beneficial effects through one of the many targeted pathways of cannabinoids (Malfait *et al.* 2000, Lastres-Becker *et al.* 2005, Cluny *et al.* 2012, Mecha *et al.* 2012, Downer and Finn 2014). The many effects that cannabinoids have within the cell that are still emerging give it great future potential for future clinical beneficial uses not yet realised.

1.4. "Priming" – Novel chemotherapy treatment

1.4.1. An introduction to "priming"

Novel chemotherapeutic treatments are always being sought after to either prolong remission or to completely ablate the tumour. One such novel mechanism is through "priming" whereby the tumour is pre-treated with an agent prior to chemotherapy (Figure 1.9.) (Sharma *et al.* 2005, Kuhar *et al.* 2007, Chonghaile *et al.* 2011, Reed 2011). "Priming" tumours with this treatment mechanism has been shown to chemosensitize the tumours to chemotherapy treatment, thus providing a better outcome for the patient (Sharma *et al.* 2005, Chonghaile *et al.* 2011, Vinod *et al.* 2013, Mu *et al.* 2014). However, the mechanism by which "priming" is regulated is still not fully elucidated. Though many different routes and cancers have been pursued with "priming" techniques such as

CD34+ in human hematopoietic recovery and with γ-rays prior to cisplatin treatment in human squamas carcinoma cells (Somlo *et al.* 1997, Caney *et al.* 1999). Furthermore, ribonucleic acid interference (RNAi) has also been targeted in human papilloma virus (HPV) where targeting the oncogenes mutated in HPV E6 and E7 led to an increase in cisplatin treatment sensitivity (Putral *et al.* 2005). Plant polyphenols have also been shown to have "priming" effects with thymoquinone, aspirin, curcumin and quercetin increasing chemotherapy sensitivity and increasing tumour cell death (Sharma *et al.* 2005, Kuhar *et al.* 2007, Kumar and Singh 2012, Mu *et al.* 2014). Recent research has shown that this sensitivity may be due to MOMP and that increasing its sensitivity can lead to a greater increase in chemotherapy sensitivity (Chonghaile *et al.* 2011, Reed 2011).

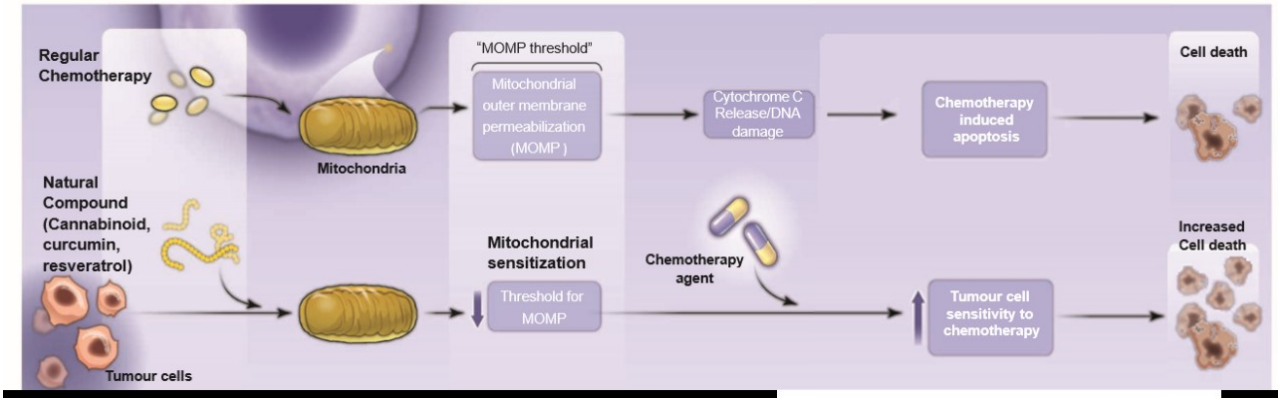

Figure 1.9. "Priming" increases cell death through mitochondrial sensitivity.
When treated with regular chemotherapy cancer cells mitochondria can be damaged and lead to cell death. However, with "priming" with either natural compounds of other the mitochondria can be sensitized prior to chemotherapy treatment which increases cell death. Adapted from Reed, 2011.

1.4.2. Potential "priming" agents, natural plant compounds

Plant polyphenols are secondary metabolites that have been shown to have effective properties in the treatment of different clinical conditions (Asensi *et al*., 2011). Traditional Eastern medicines have used plants with specific flavenoids for various therapeutic treatments for thousands of years (Sies, 2010). A number of these plant

compounds have been revisited due to their historical beneficial properties (Sies 2010, Asensi *et al.* 2011, Vinod *et al.* 2013).

1.4.2.1. Curcumin's role in cancer prevention and "priming"

Curcumin a plant compound from *Curcuma longa* is the active component of turmeric. It has been shown to have anti-tumourogenic properties in breast and colon cancer (Chen *et al.* 2006, Watson *et al.* 2008). It was shown to upregulate the p21 pathway which increases cell cycle arrest and was shown to reduce chemoresistance in the cell (Watson *et al.* 2008). Curcumin recently showed antioxidative properties in liver disease, liver cancer, hepatitis B and hepatitis C by inhibiting nuclear factor κB (NF-kB) (Nabavi *et al.* 2014, F. Zhang *et al.* 2014). Further research showed that curcumin can also protect the liver from injury and fibrogenesis by increasing the PI3K/AKT/MTOR pathway (F. Zhang *et al.* 2014). Finally, curcumin enhances the cytotoxic effects of several chemotherapy agents such as fluorouracil (5-FU), gemcitabine and oxaliplatin in colon and pancreatic cancers and has been shown to chemosensitize various other chemotherapeutic agents (Vinod *et al.* 2013). Interestingly, curcumin has been shown to act as a "priming" agent in human laryngeal cancer. "Priming" was shown to increase cisplatin-induced cell death through the mitochondrial pathway (Kuhar *et al.* 2007).

1.4.2.2. "Priming" with quercetin

The natural plant compound quercetin is a flavonoid that is present in fruit and vegetables such as onions, and green tea (Kuhar *et al.* 2006, Moreira *et al.* 2013, Vinod *et al.* 2013). This flavonoid has shown to inhibit lactate production suggesting an abolishment of the "Warburg Effect" and decreases tumour cell viability (Moreira *et al.* 2013). Quercetin has shown beneficial properties in many other cancers and has recently shown to have chemopreventative effects on melanoma and laryngeal

carcinoma through mitochondrial sensitivity and in “priming” (Kuhar *et al.* 2006, Kuhar *et al.* 2007, Moreira *et al.* 2013). The “priming” effect of quercetin was shown in head, neck and laryngeal cancer and was due to the fact that quercetin and cisplatin acted through separate pathways (Sharma *et al.* 2005, Kuhar *et al.* 2007)

1.4.2.3. Anti-cancer role of resveratrol and its “priming” potential

Resveratrol is a widely used natural plant product that has many beneficial properties ranging from a decrease in heart disease susceptibility to decreasing tumour sizes and vasoprotective features (Pozo-Guisado *et al.* 2002, Ungvari and Csiszar 2011, Ungvari *et al.* 2011, Vinod *et al.* 2013). Resveratrol is found in grapes skin and cranberries, and hence red wine (Pozo-Guisado *et al.* 2002, Ungvari *et al.* 2011). Resveratrol can inhibit ROS production and increase mitochondrial biogenesis (Ungvari and Csiszar 2011, Ungvari *et al.* 2011). It has been shown to inhibit breast cancer cell growth through differing mechanisms depending on the type of cancer and has been shown to have an overall regulation in sirtuin 1 (SirT1) activity (Pozo-Guisado *et al.* 2002, D. H. Kim *et al.* 2011). Finally, resveratrol has also recently shown beneficial properties in obesity and metabolic syndrome (S. Kim *et al.* 2011). Since resveratrol has been shown to interact with the mitochondria and have a role in reducing tumour size the potential for its use as a “priming” agent is high.

1.4.2.4. Other polyphenol’s potential as “priming” agents

There are many more natural plant compounds that have recently been revived in research (Asensi *et al.* 2011, Prakash *et al.* 2013, Vinod *et al.* 2013). Carnosol found in culinary herbs such as rosemary and oregano have been shown to inhibit cancer through DNA damage and depolarise the mitochondrial membrane potential which could lead to chemosensitization (Al Dhaheri *et al.* 2014). The chili pepper plant *Capsicum*,

has a compound capsaicin that helped identify the TRP cation channel subfamily V member (TrpV1) channels and has also been shown to inhibit cancer, reduce the mitochondrial membrane potential and increase calcium concentration (Sharma *et al.* 2013). A short study on pomegranate juice showed it decreased oxidative stress in the placenta. However, this study also showed, though did not emphasis, that apple juice decreased labour time in pregnancy compared to pomegranate juice (B. Chen *et al.* 2012). Finally, olive oils have been shown to have many beneficial properties due to their high abundance of oleic acid. The beneficial properties of olive oil phenol compounds (OOPC) have been seen in their anti-oxidative and anti-inflammatory properties as well as in the protective effect on DNA oxidation and in inhibiting colon viability (Martín-Peláez *et al.* 2013).

The beneficial advantageous observed in natural plant compounds has recently been revived and has an exponential growth in the study and use of these therapeutics (Figure 1.10.) (Asensi *et al.* 2011, Shankar *et al.* 2012, Vinod *et al.* 2013).

PPs	Plant name	Molecular target
Resveratrol	Grapes (*Vitis vinifera*)	↓COX-2, ↓iNOS, ↓JNK, ↓MEK, ↓AP-1, ↓NF-κB, ↑P21 Cip1/WAF1, ↑p53, ↑Bax, ↑caspases, ↑TNF, ↓survivin, ↓cyclin D1, ↓cyclin E, ↓Bcl-2, ↓Bcl-xL, ↓CIAP, ↓Egr-1, ↓PKC, ↓PKD, ↓casein kinase II, ↓5-LOX, ↓VEGF, ↓IL-1, ↓IL-6, ↓IL-8, ↓AR, ↓PSA, ↓CYP1A1, ↓TypeII-Ptdlns-4kinase, ↓Cdc2-tyr15[a], ↑HO-1, ↑Nrf2, ↓endothelin-1
Ellagic acid	Pomegranate (*Punica granatum*)	↓NF-κB, ↓COX-2, ↓cyclin D1, ↓MMP-9, ↓PDGF, ↓VEGF, ↑GST, ↑p21/WAF1, ↑p53
Flavonoids, catechins	Tea (*Camellia sinensis*)	↓NF-κB, ↓AP-1, ↓JNK, ↓COX-2, ↓cyclin D1, ↓MMP-9, ↑HO-1, ↓IL-6, ↓VEGF, ↓IGF, ↑p53, ↓Bcl-2, ↑p21/WAF1
Quercetin	Citrus fruits, apple (*Citrus* sp.)	NF-κB, ↑Bax, ↓ Bcl-2, ↓cyclin D1, ↑caspase, ↑PARP, ↑Gadd 45

AR androgen receptor, *NF-κB* nuclear factor kappa B, *NO* nitric oxide, *PGE* prostaglandin, *iNOS* inducible nitric oxide synthase, *COX-2* cyclooxygenase-2, *IL* interleukin, *MAP* mitogen-activated protein, *TNF* tumor necrosis factor, *CYP7A1* cholesterol 7alpha-hydroxylase, *CYP* cytochrome p450, *HO* heme oxygenase, *IAP* inhibitor-of-apoptosis protein, *PKC* protein kinase C, *PKD* protein kinase D, *LOX* lipoxygenase, *VEGF* vascular endothelial growth factor, *PSA* prostate-specific antigen, molecules, *GST* glutathione *S*-transferase, *MMP* matrix metalloprotease

Figure 1.10. Molecular targets of polyphenols for prevention of cancer.
Resveratrol, ellagic acid, catechins and quercetin all have anti-tumourogenic properties that are controlled through many difference molecular targets. Adapted from Shankar et al., 2012.

1.4.3. "Priming" alters mitochondrial sensitivity

The mitochondria are double-membrane enclosed organelles that aid in controlling energy metabolism, cell growth, synthesis of amino acids, lipids, and are central in regulating apoptosis (Tu *et al.* 2011, Frank *et al.* 2012). When under stress mitochondria have been shown to fuse together in a mechanism known as stress induced mitochondrial hyperfusion (SIHM) (Frank *et al.* 2012, Shutt and McBride 2013). This autophagy of mitochondria (mitophagy) clears damaged mitochondria and non-functioning mitochondria from the cell in a controlled fashion so as the cell doesn't pursue apoptosis (Galluzzi *et al.* 2011, Ashrafi and Schwarz 2013). A link between mTOR and damaged mitochondria has recently been shown as well as an increase in

calcium concentration by the damaged mitochondria (Galluzzi *et al.* 2011, Ashrafi and Schwarz 2013, Yun and Finkel 2014). Furthermore, damaged mitochondria lead to an increase in ROS and hence Nrf2 levels which can lead to a sensitivity of the mitochondria to toxins and further damage (Yun and Finkel 2014). Therefore, by inhibiting mitochondrial function the cell can be directed towards apoptosis which has been seen with the diabetic drug Metformin (Xiaonan Zhang *et al.* 2014).

This increase in mitochondrial sensitivity to other toxins due to damage, has been observed with several natural plant products including resveratrol and CBD (Ryan *et al.* 2009, Luo *et al.* 2013). CBD treatment targets the mitochondria, leading to a decrease in the MOMP, an increase in calcium flux to the cytosol and consequently a decrease in ATP production which leads to apoptosis (Sarafian *et al.* 2003, Ligresti *et al.* 2006, Athanasiou *et al.* 2007, Ryan *et al.* 2009). Increases in p53 have also been shown to enhance mitochondrial sensitivity to oxidative stress and increase mitochondrial dysfunction (Koczor *et al.* 2013). This is what has been documented with resveratrol, whereby an increase in ROS and p53 was linked to senescence and apoptosis of lung cancer cells (Luo *et al.* 2013). Furthermore, when lung cancer cells were treated with curcumin, a reduction in anti-apoptotic AKT levels was observed leading to an increase in ROS production and further lung cell damage (Martindale and Holbrook 2002, F. Zhang *et al.* 2014).

1.4.4. VDAC1 receptors role in mitochondrial control

Cancer cells have been documented to suppress MOMP, reducing its activity and regulation (Galluzzi *et al.* 2006, Kuznetsov *et al.* 2011b). The most abundant membrane protein, VDAC1 has been shown to be required for apoptosis (Figure 1.11.) (Galluzzi *et al.* 2006, Simamura *et al.* 2008). The blocking of VDAC1 can reduce the potential for apoptosis in the cell (Galluzzi *et al.* 2006, Simamura *et al.* 2008, Shoshan-

Barmatz *et al.* 2010). As previously mentioned, VDAC1 is located in the mitochondrial outer membrane and is responsible for regulating cellular energy and metabolism in the cell (Madesh and Hajnóczky 2001, Simamura *et al.* 2008, Rimmerman *et al.* 2011, Rimmerman *et al.* 2013, Shoshan-Barmatz *et al.* 2014, Tewari *et al.* 2015). The VDAC1 receptor can control the mitochondria's function as well as its integrity (Madesh and Hajnóczky 2001). This multifunctional regulator has a key role in controlling mitochondria-mediated apoptosis as well as calcium and ROS levels within the cell (Shoshan-Barmatz *et al.* 2010, Rimmerman *et al.* 2013, Shoshan-Barmatz *et al.* 2014). CBD, cisplatin and curcumin have been shown to have a high affinity of binding and regulating VDAC1 (Yang *et al.* 2006, Tajeddine *et al.* 2008, Rimmerman *et al.* 2013, Tewari *et al.* 2015).

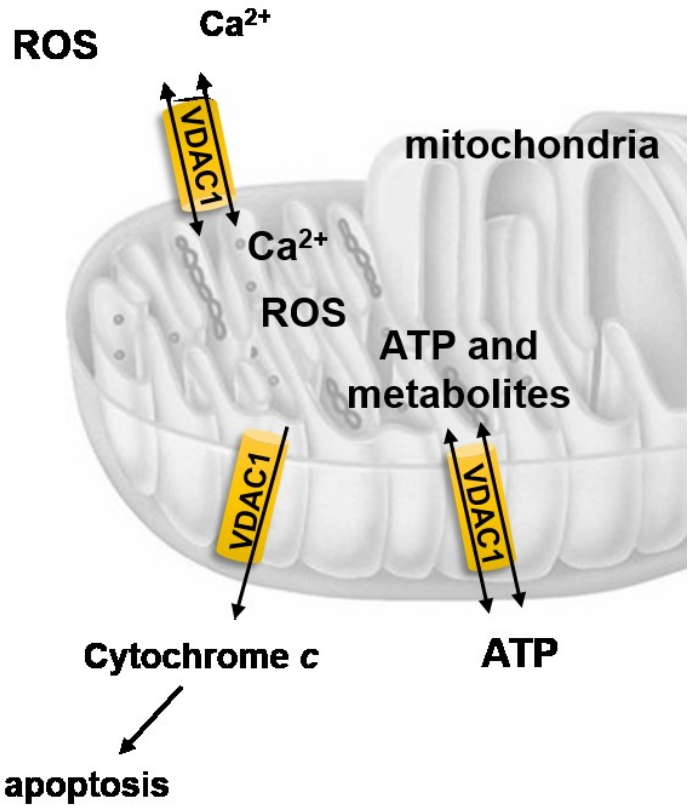

Figure 1.11. VDAC1 major functions in the mitochondria.
The VDAC1 channel is located on the outer mitochondrial membrane and regulates the transfer of metabolites between the mitochondria and the cytosol. These include ROS, calcium and ATP which are key regulators in mitochondrial function. VDAC1 has also been shown to be a major regulator in mitochondria-mediated apoptosis through trafficking pro-apoptotic proteins to the cytosol such as cytochrome *c*.

1.4.5. Summary of “priming”

Interestingly, it is through mitochondrial sensitization that the novel chemotherapy potential of “priming” is observed (Chonghaile *et al.* 2011, Reed 2011). Could these natural compounds that are sensitizing the mitochondria to damage also be used as a pre-treatment to chemotherapy in a “priming” mechanism? The VDAC1 regulator has been shown to not only be regulated by CBD and cisplatin which could prove vital in altering the mitochondrial membrane potential (Yang *et al.* 2006, Rimmerman *et al.* 2013). Sensitising the mitochondria by “priming” the cells with CBD prior to the chemotherapy agent cisplatin could prove to enhance cancer cell death.

1.5. Hypothesis

“Priming” with cannabidiol (CBD) enhances chemotherapeutic treatment of cancer

1.6. Aims

- Optimise CBD’s potential as a “priming” agent in cancer cells.
- To explore the novel mechanism of “priming” in cancer cells.
- To derive the mode of action for “priming” with CBD in cancer cells.