Expression of Cdc25c in *Piper betle* treated drug resistant human colon cancer cells, HT-29

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RESEARCH BACKGROUND

Colon Cancer

- It is the third most common cancer among men and second most common in women (Torre et. al. 2015)
- About 1.4 million cases in total and 693,900 of them resulting in death only in year 2012 (Torre et. al. 2015).
- The existing treatment measures include 5-flurouracil based chemotherapy (Shakibaei et. al. 2014).

Piper betle

- *Piper betle* has shown its cytotoxic effect on HT-29 cells (Ng et. al. 2014).
- *Piper betle* locallly known as daun sirih, belongs to the family of Piperaceae (Rai et. al. 2011).
- It is one of the targeted cultivated plant under The Bioeconomy Malaysia 2015 (Mohd 2015)
- The phytochemicals found in *Piper betle* includes chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenol methyl ether, cadinene, γ-lactone, allyl catechol, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, p-cymene, terpinene, eucalyptol, carvacrol, sesquiterpenes, cadinene, caryophyllene, dotriacontanoic acid, hentriacontane, pentatriacontane, stearic acid, n-triacontanol, triotnacontane, piperlonguminine, allylpyrocatechol diacetate, isoeugenol, 1, 8-cineol, α-pinene, β-pinene, sitosterol, β-sitosteryl palmitate, γ-sitosterol, stigmasterol, ursolic acid, and ursolic acid 3β-acetate (Rai et. al. 2011)
- Its properties include anti-bacterial, anti-cariogenic, anti-fungal, anti-allergic, anti-diabetic, anti-inflammatory, hepatoprotective, cardioprotective, and immunomodulatory effects (Periyanayagam et. al. 2012).
- Past studies showed HT-29 cells appear as round shaped cells after treated with IC_{50} of *Piper betle* (Gnapragasan 2015; Hay 2015). Round shaped cells may indicate M-phase cells (Sakaue-Sawano et al 2007) that probably undergone mitotic arrest which subsequently leads to cell death (Elliott & Elliott 2009)

Cdc25c

- Cell cycle consists of four phases namely G_1, S, G_2, and M phase.
- To avoid genetic abnormalities, progression through the phases are subjected to controls and checkpoints. At the checkpoints, if the safety requirements are not met, the cycle will be halted.
- A complex between Cdk1 and cyclinB1 plays an important role in regulation of G2-M transition (Figure 1).
- Dephosphorylation of Cdk1 and hence activation of the Cdk1/cyclinB1 kinase is catalyzed by Cdc25c (Elliott & Elliott 2009). DNA damage may cause activation of Chk1 (Figure 1) which in turn phosphorylates and inactivates Cdc25C (DiPaola 2002). This will inhibit the Cdk 1 thus causing G2-M phase arrest (DiPaola 2002).
- Cdc25C as shown in previous study involved in Withaferin A (WA) induced G2-M phase cell cycle arrest. Result showed that the decreased level of Cdc25C protein which is likely to inhibit the activity of the Cdk1/cyclinB1 kinase complex caused the cell cycle arrest of WA-mediated G2-M (Stan et. al. 2008).

Paclitaxel

- Paclitaxel serves as a positive control in this study.
- Paclitaxel belongs to the taxane family of antitumor drugs.
- Paclitaxel is the first natural product that has been proven to stabilize microtubules (Abal et. al. 2003).
- By promoting the assembly of microtubules, paclitaxel obstructs their depolymerisation thus blocking the progression of cell cycle at the G2-M phase (Toiyama et. al. 2009).
- Study by Toiyama et. al. (2009) showed HT-29 cells treated with Paclitaxel at cytotoxic doses presented with a significant accumulation of cells in G2-M phase.

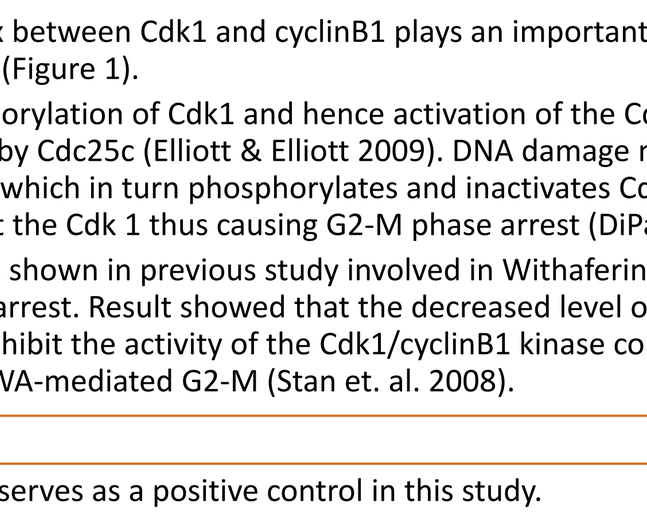

Figure 1: The late G2 checkpoint controlling cell-cycle progression from G2 to M phase. (DiPaola 2002).

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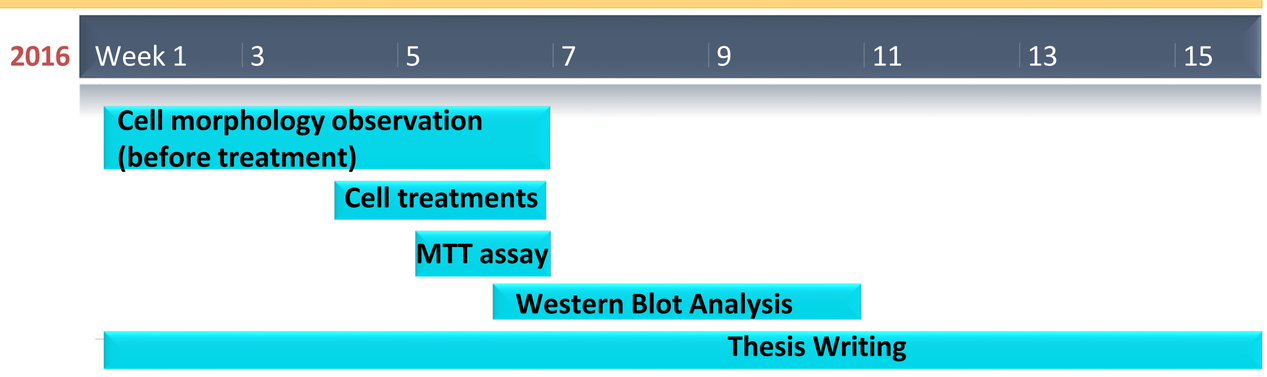

RESEARCH QUESTIONS

What is the modulation effect of *Piper betle* on cell cycle that subsequently leads to cancer cell death?

OBJECTIVES

To determine the expression of Cdc25c in *Piper betle* treated drug resistant colon cancer cells.

HYPOTHESIS/ EXPECTED OUTCOME

Piper betle treated HT-29 cells express high abundance of M-phase regulatory protein compared to untreated cells.

METHODOLOGY

HT-29 colon cancer cells (American Type Cell Culture) will be cultured in McCoy's 5A medium + 10% (v/v) Fetal Bovine Serum and incubated in the condition of 37ºC and 5% CO_2.

Morphology of the cells before treatment will be observed under inverted microscope.

Control HT-29 (without treatment)	HT-29 cells treated with Paclitaxel	HT-29 cells treated with *Piper betle* extract

Morphology of the cells after treatment will be observed under inverted microscope.

MTT assay: HT-29 cells treated with Paclitaxel will be plated at 10,000 cells per well and incubated for 6 to 24 hours. Ten (10) µL yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) will be added and incubated for 2 to 4 hours until purple precipitate is visible. 100 µL Detergent Reagent will be added and left in the dark for 2 hours. The absorbance is read at 570nm to determine the cell viability.

Western blot (Figure 2): Cells will be lysed using a lysis buffer and centrifuged. The supernatant will be collected and protein concentrations will be determined by the Lowry Protein Assay. Samples will then be separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% gel, and transferred to a polyvinylidene fluoride (PVDF) membrane using the Trans-Blot Turbo Transfer System (Bio-Rad). Blocking agent will be used to block unspecific sites. Primary and secondary antibodies will be added. Visualization by chemiluminescence will be done (Figure 2).

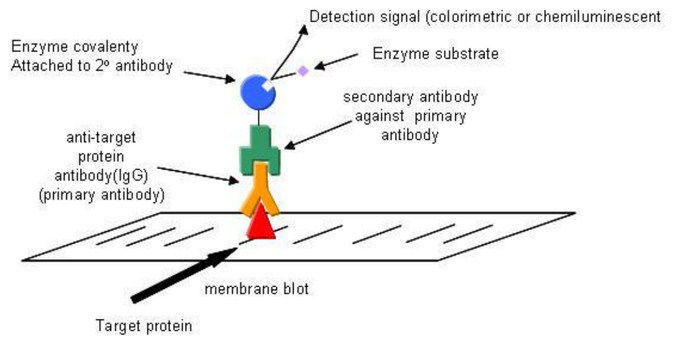

Figure 2: The schematic diagram of the principles of western blotting (College of Saint Benedict & Saint John's University 2006).

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