
**UNIVERSITI PUTRA MALAYSIA**

***ESTABLISHMENT OF A CHO CELL LINE BY OVER-EXPRESSION OF THE X-LINKED INHIBITOR OF APOPTOSIS***

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**By**

**JANE LIEW CHIAR JENN**



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**JANE LIEW CHIAR JENN**

**July 2012**

**Chairman: Associate Professor Tey Beng Ti, PhD**

**Faculty: Institute of Bioscience**

In mammalian cell culture, serum deprivation inhibits cell growth and induces apoptosis. Adapting host cells to serum-free medium is difficult and time-consuming, therefore anti-apoptosis engineering of mammalian cells were used to account the problems caused by serum deprivation. The X-linked inhibitor of apoptosis protein (XIAP) is the most effective member of the inhibitor of apoptosis protein (IAP) family, where it inhibits apoptosis by retarding the downstream caspase activity in the apoptosis chain cascade. In an attempt to investigate the ability of XIAP to delay serum-deprived-induced-apoptosis, stable CHO-K1 cell lines expressing the XIAP proteins were established by introducing the XIAP gene into the host genome. Transfected cells with high expression of XIAP were selected by conducting the MTT assay and flow cytometric analysis. Selected cell line was cultured and closely examined under several serum-deprived conditions. During exposure to serum-

deprived medium, cells expressing XIAP showed decreased level of apoptosis and a higher number of viable cells compared to the control cell lines. The viability of control cells dropped to 40% after 2 days of serum deprivation, while the XIAP expressing cells still maintained at a viability over 90%, where caspase-3 activity was found to be inhibited. Meanwhile, the study also showed that CHO-K1-XIAP cells exhibited a slower growth profile, where most of the cells were found to be located in the G0/G1 phase. The results suggest that the over-expression of XIAP induces G0/G1 growth arrest, where proliferation was retracted, leading to a 50% reduction in maximum cell density.

In the following attempt to investigate the productivity of the established cell line, the CHO-K1-XIAP cell line was then co-transfected with cycle-3 green fluorescent protein (GFP), as a model protein to access the productivity of the cells. Cells were cultured under serum-deprived condition with addition of sodium butyrate (NaBu) treatment in various concentrations. NaBu is widely used to boost up the commercial production of recombinant proteins, but was found to be cytotoxic and causes rapid apoptotic cell death in mammalian cell cultures. Upon subjecting the cells to serum-deprived and NaBu-induced condition, the control significantly declines in cell viability and GFP production. The CHO-K1-GFP expressing XIAP remained robust and continued to express GFP for the initial 2 days, having a 52% increment of high GFP producers. Moreover, the results indicated that the intracellular levels of GFP were further increased while cells became progressively growth-arrested, shifting 33% of the low GFP producers to high GFP producers. Although XIAP over expression

could not effectively inhibit NaBu-induced apoptosis, however, a 35% reduction of caspase-3 activation was observed in CHO-K1-XIAP-GFP cells at its termination point.

Taken together, host cells solely expressing a single type of anti-apoptotic protein are found to be inadequate in regard to extension of culture longevity and enhancement of protein production. Therefore, for a better and more effective apoptosis inhibition, a combination of caspases inhibitor and mitochondrial dysfunction should be considered in future studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENUBUHAN BARISAN SEL CHO OLEH PENGEKSPRESAN BERLEBIHAN X-LINKED INHIBITOR OF APOPTOSIS**

Oleh

**JANE LIEW CHIAR JENN**

**Julai 2012**

**Pengerusi: Profesor Madya Tey Beng Ti, PhD**

**Fakulti: Institut Biosains**

Dalam kultur sel mamalia, deprivasi serum akan menghalang pertumbuhan sel dan mendorong apoptosis. Penyesuaian sel-sel perumah kepada medium tanpa serum adalah sukar dan memerlukan jangka masa yang panjang. Oleh yang demikian, anti-apoptosis kejuruteraan sel mamalia telah digunakan untuk mengambil kira masalah-masalah yang disebabkan oleh deprivasi serum. *X-linked inhibitor of apoptosis protein* (XIAP) adalah ahli yang paling berkesan dalam kumpulan *inhibitor of apoptosis protein* (IAP), di mana ia menghalang apoptosis dengan memperlahankan aktiviti hiliran *caspase* di dalam rantaian apoptosis. Dalam usaha untuk menyiasat keupayaan XIAP untuk melambatkan induksi apoptosis yang disebabkan oleh deprivasi serum, barisan sel CHO-K1 stabil telah ditubuhkan dengan memperkenalkan gen XIAP ke dalam genom sel perumah. Sel-sel transfeksi dengan ekspresi XIAP yang tinggi dipilih dengan menggunakan *MTT assay* dan analisis *flow*

*cytometric*. Barisan sel transfeksi yang dipilih dikultur dan dikaji dengan teliti di bawah beberapa keadaan deprivasi serum yang berlainan. Semasa pendedahan kepada medium deprivasi serum, sel-sel yang mengekspresi XIAP menunjukkan penurunan dalam apoptosis dan mempunyai nombor sel-sel hidup yang lebih tinggi berbanding dengan barisan sel kawalan. Viabiliti sel kawalan menurun kepada 40% selepas 2 hari deprivasi serum, manakala sel yang mengekspresi XIAP masih mengekalkan viabiliti yang lebih daripada 90%, di mana aktiviti *caspase-3* didapati dihalangkan. Sementara itu, kajian ini juga menunjukkan bahawa sel-sel CHO-K1-XIAP mempamerkan profil pertumbuhan yang perlahan, di mana kebanyakan sel-sel terletak dalam fasa G0/G1. Keputusan ini mencadangkan bahawa pengekspresan berlebihan XIAP mendorong perhentian penumbuhan sel, di mana proliferasi sel ditarik balik dan membawa kepada pengurangan sebanyak 50% dalam kepadatan sel maksimum.

Dalam usaha seberikut untuk menyiasat produktiviti barisan sel yang ditubuhkan, barisan sel CHO-K1-XIAP kemudiannya ditransfeksi dengan *cycle-3 green fluorescent protein* (GFP), sebagai protein model untuk mengakses produktiviti sel. Barisan sel tersebut dikultur di bawah keadaan deprivasi serum dengan penambahan butirat natrium (NaBu) dalam pelbagai kepekatan yang berlainan. NaBu digunakan secara meluas untuk meningkatkan pengeluaran komersial protein rekombinan, tetapi didapati bersifat sitotoksik dan menyebabkan kematian apoptosis yang pesat dalam kultur sel mamalia. Apabila sel ditundukkan kepada keadaan deprivasi serum dan penambahan NaBu, viabiliti dan pengeluaran GFP sel kawalan merosot dengan

ketara. Manakala, sel-sel CHO-K1-GFP yang mengekspresi XIAP kekal teguh dan terus mengkespresi GFP untuk 2 hari yang pertama, di mana 52% sel populasi merupakan pengeluar GFP yang tinggi. Selain daripada itu, keputusan menunjukkan bahawa tahap intraselular GFP terus meningkat apabila penumbuhan sel berhenti secara progresif, di mana 33% pengeluar GFP yang rendah beralih menjadi pengeluar GFP yang tinggi. Walaupan pengekspresan berlebihan XIAP tidak berupaya untuk menyekat apoptosis yang disebabkan oleh NaBu secara berkesan, pengurangan pengkatifan *caspase-3* sebanyak 35% telah diperhatikan dalam sel-sel CHO-K1-XIAP-GFP pada titik penamatan.

Sebagai kesimpulannya, sel-sel perumah yang mengkespresi hanya sejenis protein anti-apoptotis adalah tidak mencukupi untuk lanjutan usia sel yang panjang dan pengeluaran protein yang tinggi. Oleh yang demikian, bagi perencatan apoptosis yang lebih baik dan lebih berkesan, gabungan perencat-perencat *caspase* dan disfungsi mitokondria perlu dipertimbangkan dalam kajian masa depan.

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I certify that a Thesis Examination Committee has met on 9 July 2012 to conduct thte final examination of Jane Liew Chiar Jenn on her thesis entitled "Establishment of a CHO Cell Line by Over-expression of the X-linked Inhibitor of Apoptosis" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Seow Heng Fong, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Muhajir bin Hamid, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Ling Tau Chuan, PhD**
Professor
Faculty of Sciences
Universiti Malaysia
(Internal Examiner)

**Hideki Yamaji, PhD**
Associate Professor
Graduate School of Engineering
Kobe University
(External Examiner)

_____
**SEOW HENG FONG, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 September 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Tey Beng Ti, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

**Tan Wen Siang, PhD**
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Noorjahan Banu Mohamed Alitheen, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)



_____

**BUJANG BIN KIM HUAT, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any others degree at University Putra Malaysia or at any other institution.

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**JANE LIEW CHIAR JENN**

Date: 9 July 2012

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