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DEVELOPMENT OF EFFECTIVE CHO-K1 HOST SYSTEM TARGETING AT NUTRIENT-REGULATED INSULIN-LIKE GROWTH FACTOR I (IGF-I) PATHWAY

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Biotechnology Engineering)

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MARCH 2014

ABSTRACT

An effective mammalian cell culture host system expressing therapeutic proteins is a combination of cell line's inherent characteristics with efficient use of nutrients that is able to provide desirable output of high cell viability. Insulin-like Growth Factor I (IGF-I) has been shown to have the ability to promote cell proliferation, while also having complex interactions with other constituents in the media. Therefore, it is hypothesized that if IGF-I pathway is effectively manipulated it could lead to achieving the desired high cell density culture. This present study is designed to develop a CHO-K1 based host system by understanding (IGF-I) gene and protein expression in this cell line and its expression relationship between constituents of media. It is confirmed that both IGF-I gene and protein are expressed in CHO-K1 cells, through reverse transcriptase real-time PCR analysis and enzyme-linked immunosorbent assay (ELISA) respectively. Using a three level Full Factorial Design, the optimal media composition of 10 % (v/v) serum and 0.500 mM glutamine was found to contribute to high cell density of 8.870 X 10^5 cells/ml in T-flask. The optimal media composition was validated and gave 12.500 x 10^5 cells/ml; an increase of 26.936 % from culturing in standard formulation of 10 % (v/v) serum and 2 mM glutamine. The culture with optimal media then reached 23.300 x 10^5 cells/ml (46.352 % increased) when scaled-up in 500 ml spinner vessel. The culture also reached higher cell density (16.600 x 10^5 cells/ml); increase of 24.699 % from 12.500 x 10^5 cells/ml) when adapted in zero glutamine. Results from Full Factorial Design showed that the quadratic term of glutamine plays an important role for high cell density. This also supports the observation that the cells reached high cell concentration when cultured in zero glutamine media. Based on multivariate data analysis (MVDA) using Principal Component Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA), the high cell density achieved in low glutamine was found to be correlated with high expression of IGF-I gene and protein. This may be governed through growth hormone signaling and IGF-I signaling pathway as shown in the pathway analysis performed in this work. In conclusion, the study showed that the IGF-I pathway which is known for its role in cell proliferation is responsive to the regulation by nutrients. The relationship between the reduced requirement of glutamine with the high expression of IGF-I gene and protein further improves the efficiency of the system. The host system (CHO-K1 cell line and the improved media formulation) obtained from this study can now serve as a platform for producing bio-products of interest.

خلاصة البحث

النظام الفعال المضيف لخلايا التدييات المعبر عن البروتينات العلاجية هو مزيج من الخصائص الوراثية للخلية مع الاستخدام الفعال للعناصر الغذائية التي هي قادرة على توفير الناتج المرغوب فيه من كمية الخلايا. شبيه الانسولين عامل (IGF-I) لديه القدرة على تعزيز انتشار الخلايا، مع وجود تفاعلات معقدة له أيضا مع المكونات الأخرى ضمن الوسط الموجود فيه .ولذلك، نخن نفترض أنه إذا تم التلاعب بمسار IGF-I بشكل فعال فإنه يمكن أن يؤدي إلى تحقيق المطلوب من كثافة عالية للخلايا .تم تصميم هذه الدراسة المعتمدة على النظام المضيف لتطوير CHO-K1 عن طريق فهم التعبير الجيني والبروتيني ل (IGF-I) وعلاقته بين الهيئات المكونة في الوسط الموجود فيه في هذا النوع من الخلايا. ولقد تم التأكد من كل من IGF-Iالتعبير الجيني والبروتينات المعبرة عنه في الخلاياCHO-K1 ، من خلال تحليل الناسخ العكسي في RT-PCR و فحص الانزيم المرتبط المناعي (ELISA)على التوالي .باستخدام ثلاث مستويات من التصمثم المصنع الكامل . تم العثور على الوسط الأمثل للتكوين الذي يمثل 10 % (v/v) مصل و0.500 ملم الجلوتامين للمساهمة في كثافة عالية من الخلايا X8.870 10^5 خلية / مل في القارورة .تم التحقق من صحة تكوين الوسط الأمثل وقدأعطى 12.500 × 10^5 خلية / مل؛ بزيادة قدرها26.936٪ من الخلايا المزروعة في صياغة مستوى 10٪ (V / V)مصل و 2 ملي من الجلوتامين . لقد تأكد ذلك في الخلايا المزروعة في الوسط الأمثل عندما وصلت الى 23.300 × 10^5 خلية / مل وكانت الزيادة أعلى كثافة بنسبة (46.352٪ زيادة) عندما تم تكييفها في الشريان الدوار .الخلايا المزروعة وصلت (16.600 × 10^5 خلية / مل)؛ بزيادة قدرها 24.699 ٪ من مستوى الخلايا 10^5 × 12.500/ مل)عندما كان قيمة الجلوتامين صفر .وأظهرت النتائج من النصميم الكامل المصنع أن مصطلح الدرجة الثانية من الجلوتامين يلعب دورا هاما لكثافة عالية في الخلايا .كما يدعم هذه الملاحظة أن الخلايا المزروعة بلغ ارتفاع تركيزها عاليا عندما كان قيمة الجلوتامين. صفؤا في الوسط الموجود فيه. اعتمادا على أساس تحليل البيانات متعدد المتغيرات (MVDA) باستخدام تحليل المركبات الرئيسية (PCA) و(PLS-DA)،ان الكثافة العالية التي تحققت في الخلية عند انخفاض الجلوتامين وجدت لتكون مرتبطة مع التعبير IGF-I للجينات والبروتينات .ويمكن من خلال هذه الإشارات التحكم في هرمون النمو IGF-I ومساره كما هو موضح في تحليل المسارات التي أجريت في هذا العمل .في الختام، أظهرت الدراسة أن مسار IGF-I والذي يعرف بدوره في انتشار الخلايا؛ يستجيب لتنظيم من قبل العناصر الغذائية .العلاقة بين انخفاض الاحتياجات من الجلوتامين مع التعبير IGF-Iللجينات والبروتينات يحسن زيادة كفاءة النظام.ويذيد كثافة الخلايا. للنظام المضيف CHO-K1) في الخلية مع صياغة الوسط المحسن الذي تم الحصول عليه من هذه الدراسة يمكن ان يستخدم الآن كمنصة لإنتاج المنتجات الحيوية المهمةز هذا النظام هو أيضا على استعداد لمزيد من الصقل في الوسط وبخاصة نحو المصل الخالي من الصياغة .

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology Engineering).

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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ACKNOWLEDGEMENTS

First and foremost, praises to Allah and peace and blessings upon Prophet Muhammad s.a.w the last messenger. It has been two years since I started this research project under the supervision of Dr Yumi Zuhanis Has-Yun Hashim and Dr Azura Amid, and Alhamdulillah, I am grateful to Allah for blessing me with this great opportunity since the start and guiding me in every step of the way towards the completion of this research. My research has been a stepping stone in my career, and for that I would like to thank both my supervisor and my co- supervisor for giving me the opportunity to be a part of this project and for their guidance, help, and also feedback, not to be forgotten for being amazing teachers to me, providing me with support without me asking, giving me help in every way possible. Truly, without their teaching, guidance and support, this project would not have been completed successfully. I would to thank Ministry of Science, Technology and Innovation Malaysia (MOSTI) for giving me the opportunity to continue my studies by providing me a scholarship. Heartiest thanks I convey to my family, for the endless support that they have given to me during this period of my research.

For my colleagues, lab mates and my companions that I have gained during this particular time of my life, my research would not have been possible without you. I would like to thank Sister Salfarina Ezrina, for being a friend and also a colleague, whom has been so helpful. Not to be forgotten Sister Siti Nuramalis, Sister Yusilawati, Brother Azmir, Sister Najiah, Sister Alawiyah, Sister Siti Hajar, Sister Hidayah, Brother Jaafar and Brother Phirdaous. I would also like to thank the lab technicians for without them, Brother Hafizul, Brother Anuar and Brother Haslan, my work would not have gone as smoothly as possible. Special thanks I would like to give to Sister Nour for her help in translation of my English abstract to Arabic.

These two years have been a great experience, and I have gained so much, and without all these people, it would not have been possible. Many people were involved in the completion of this research, therefore my deepest gratitude to all whom has contributed and whom were involved directly and indirectly in the completion of this project. I apologize if I have not stated your name and thank you from the bottom of my heart. May Allah bless all of you and grant your good deeds with rewards.

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LIST OF ABBREVIATIONS

ACTB	Beta Actin
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BSA	Bovine Serum Albumine
BSC	Biosafety Cabinet
CHO-K1	Chinese Hamster Ovary K1
CS	Calf Serum
DOE	Design of Experiment
EGF	Epidermal Growth Factor
ELISA	Enzyme-linked Immunosorbent Assay
FBS	Fetal Bovine Serum
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GH	Growth Factor
GHR	Growth Factor Receptor
HEPES	N-2-Hydroxyethylpiperazine-N'-2-ethane Sulfonic Acid
IGF-I	Insulin-like Growth Factor I
IGF-IR	Insulin-like Growth Factor I Receptor
MVDA	Multivariate Data Analysis
NCBI	National Center for Biotechnology Information
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PLS	Partial Least Square
PLS-DA	Partial Least Square Discriminant Analysis
qPCR	Quantitative Polymerase Chain Reaction
RNA	Ribonucleic Acid
RPMI	Rosewell Park Memorial Institute
RSM	Response Surface Method
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide
UV	Ultraviolet
VIP	Variable of Importance
YSI	Yellow Spring Intruments

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

An effective host system to express therapeutic proteins is a system that is able to provide desirable outputs which may include high cell density, good growth rate, minimize consumption of media and high expression of the protein of interest. While many host systems can be used such as bacteria, plant, yeast, insect and mammalian cells, the latter is the optimal and preferred host system for the production of recombinant eukaryotic proteins for biopharmaceutical purposes. Its major benefit is the direct expression of the desired protein, including large and complex proteins like Factor VIII in the culture medium. Mammalian cell culture host also allows correct folding and posttranslational modifications for optimal biological activity of the protein produced (Martin & Harmsen, 2008; Hossler, Khattak & Li, 2009).

In order to develop an efficient mammalian cell culture host system, there are several strategies to be focused on. This includes improved design and the ability of the host cell line to offer potential product improvements, maximization of viable cell number, improvement of medium formulation, inclusion of exogenous growth factors, implementation of high performance reactor configurations and maximization of production rate per cell (Rader, 2008; Martin & Harmsen, 2008). Many earlier studies focused on a single strategy at a time. For example, Baserga (1993) and Sunstrom et al. (2000a) reported on the development of an efficient cell line which produced the constitutive expression of Insulin-like growth factor I (IGF-I) leading to self