UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF DEREGULATION IN PROTEIN EXPRESSION LEVEL OF ERLOTINIB-RESISTANT H1299 CELL LINES

SYUKRIYAH MAT DAUD

FBSB 2015 132

IDENTIFICATION OF DEREGULATION IN PROTEIN EXPRESSION LEVEL OF ERLOTINIB-RESISTANT H1299 CELL LINES

SYUKRIYAH BINTI MAT DAUD

162549

Dissertation submitted in partial fulfillment of the requirement for the course BMY 4999 Project in the Department of Microbiology
Universiti Putra Malaysia
JUNE 2015

IDENTIFICATION OF DEREGULATION IN PROTEIN EXPRESSION LEVEL OF ERLOTINIB-RESISTANT H1299 CELL LINES

SYUKRIYAH BINTI MAT DAUD

162549

DEPARTMENT OF MICROBIOLOGY
FACULTY BIOTECHNOLOGY AND BIOMOLECULAR SCIENCES
UNIVERSITI PUTRA MALAYSIA
2015

PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk "**Identification of deregulation in protein expression level of erlotinib-resistant H1299 cell lines** " telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh **Syukriyah Binti Mat Daud (162549)** sebagai syarat untuk kursus BMY 4999 projek.

Disahkan oleh:

…….. Tarikh: …….

Dr. Nurhidayah Binti Roslan

Penyelia

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

…… Tarikh: …….

Prof. Madya Dr. Muhajir Hamid

Ketua

Jabatan Mikrobiologi

Fakulti Bioteknologi dan Sains Biomolekul

Universiti Putra Malaysia

ABSTRACT

Erlotinib, a small-molecule epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, has been shown to be an effective solution in treating non-small cell lung cancer (NSCLC). However, most patients who initially show a good response to erlotinib treatment eventually develop resistant and experience progressive disease. There is no absolute answer on how the cells can develop resistance against the erlotinib. It was suggested that changes in the cell cycle could contribute to the acquired resistance of NSCLC. In this study, untreated H1299 and erlotinib-resistant H1299 (H1299-R) were used to compare proteins expression levels involved in cell cycle. The main cell cycle players that were investigated included Cyclin A and E, Cyclin-dependent kinases (CDK) 2, 4, and 6, p21, NFKB, and ß-actin. A calourimetric method, alkaline phosphatase, was used to detect the amount of proteins present in the sample. Although the concentration of the protein loaded into the gel was increased from 75 µg to 85 µg, the results obtained were similar. Both cell lines possessed ß-actin and Cyclin A proteins only.

ABSTRAK

Erlotinib, molekul kecil sebagai perencat epidermal growth factor receptor (EGFR) tyrosine kinase, telah terbukti menunjukkan penyelesaian yang berkesan untuk merawat non-small cell lung cancer (NSCLC). Walaubagaimanapun, kebanyakan pesakit yang pada asalnya bertindak balas dengan erlotinib akhirnya menjadi tahan dan mengalami penyakit yang berterusan. Tiada jawapan yang pasti bagaimana sel-sel tersebut boleh menimbulkan tahan terhadap erlotinib. Ada yang mencadangkan bahawa perubahan dalam kitaran sel mungkin yang menyumbang kepada mendapat tahan pada NSCLC. Untreated H1299 dan erlotinib-resistant H1299 (H1299-R) telah digunakan untuk membandingkan paras ekspresi protin yang terlibat dalam kitaran sel. Pemain utama dalam kitaran sel yang dikaji termasuk cyclin A dan E, cyclin-dependent kinases (CDK) 2, 4, dan 6, p21, NFKB dan Beta actin. Kaedah kalorimetrik, alkaline phosphatase telah digunakan untuk menentukan jumlah protin dalam sel. Walaupun kepekatan protin yang dimasukkan ke dalam gel telah dinaikkan dari 75 µg kepada 85 µg, keputusan yang diperoleh adalah sama. Kedua-dua sel mempunyai ß-actin dan Cyclin A.

ACKNOWLEDGEMENT

Firstly, I would like to thank my project supervisor, Dr. Nurhidayah binti Roslan for her guidance and supervision as well as support throughout the completion of this project. I would like to give my deepest appreciation for her helpfulness and co-operation in sharing ideas and answering many uncertainties that arise during the course of the study.

Next, I would like to extend my gratitude to master students, Joanne, Kak Fairuz and Kak Masytah who willing to help me with the techniques and skills during the implementation of this project. Also for the support, courage and advised for me to complete this project successfully.

Not forgetting my laboratory partner, Noor Khafizah who always together with me during completion this project. I would like to sincerely thank you very much for your help, support, co-operation, tolerance during at first until at the end of this project. Also to my group members, Maisarah and Ain for always share the ideas and co-operations all the time.

I also wish to extend my special thanks to my supportive and understanding family members, especially my beloved parents Mat Daud bin Ab. Rahman and Sabariah binti Ibrahim, who always support me, give encouragement, loves and prayers for me to complete this project successfully.

Last but not least, I would like to thank all those who were involved directly or indirectly for the completion of this meaningful and wonderful project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The lungs are a pair of spongy, air-filled organs located on either side of the chest. Their principal function is to perform gaseous exchange between the body and the surroundings. They are used to supply oxygen, and to release carbon dioxide from the bloodstream.

Cancer is now believed to result from perturbations in the cell cycle that result in unlimited proliferation and an inability of a cell to undergo differentiation and/or apoptosis (Malumbres and Barbacid, 2009). However, the exact mechanism of the cancer is not yet known, and different cancers seem to have different triggers.

Lung cancers have been classified into two major types, which are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). However, NSCLC is the most frequently diagnosed type of lung cancer and is the leading cause of cancer-related mortality worldwide (Travis et al., 1995; Gridelli et al., 2007).

According to the American Cancer Society, most lung cancer statistics include both small cell and non-small cell lung cancers (NSCLC). Lung cancer is the second most common cancer in both men and women (not counting skin cancer). Lung cancer accounts for about 27% of all cancer deaths and is by far the leading cause of cancer death among both men and women. The mortality rate of lung cancer is much higher than that of colon, breast, and prostate cancers combined.

Studies show that mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR) gene of NSCLC are the one of the causes of the tuour occurence. The over expression of EGFR has been reported and implicated in the progression of many human maglinant tumours, including NSCLC (Inamura et al., 2010).

Surgical resection, one of the treatments for NSCLC case, is not the best solution as it is only viable for certain types of early stage cancer. As the majority of lung cancer cases are diagnosed in the late stages, it is not always an option. Standard treatment such as chemotherapy, radiotherapy, and surgery has reached a plateau in this disease (Gridelli et al., 2007). Chemotherapy with active agents recognized as a better treatment. Because of that, more research has been conducted on targeting the EGFR as a treatment.

Increased EGFR expression is known to correlate with poor clinical outcome in patients with NSCLC. The EGFR has been considered a potential therapeutic target because of this. In recent years, several compounds that have been develop to target the EGFR signaling pathway have demonstrated significant anticancer activity (Noonberg and Benz, 2000; Herbst and Bunn, 2003).

The two reversible EGFR tyrosine kinase inhibitors approved for the treatment of advanced NSCLC are elotinib and gefitinib. Of these, treatment using erlotinib has shown a significant improvement in median survival, quality of life, and related symptoms in an unselected population of advanced NSCLC patients (Gridelli et al., 2007).

Erlotinib (Tarceva; Genentech, South San Fransisco, CA), is a synthetic anilinoquinazoline compound that acts by competitive inhibition. It competes with adenosine triphosphate (ATP) at the TK domain of the receptor, resulting in an inhibition of the EFGR signaling pathway (Machetti et al., 2005). Other than that, erlotinib also has significant anti tumour activity as a first-line treatment (Gridelli et al., 2007).

Although erlotinib has been approved as a therapy for advanced and chemoresistant NSCLC, however the development of resistance against this drug can reduce the drug's efficacy in treatment. Most of the patients who initially respond to erlotinib eventually become resistant and experience progressive disease within a year or two (Gazdar, 2009).

Development of resistance against erlotinib limits the chances of surviving the cancer, which already has a poor overall survival rate. Hence, other treatments that involve the manipulation of the cellular mechanisms may serve as an alternative to help to solve this problem. One of the alternatives is to study the deregulation of protein expression in the erlotinib-resistant NSCLC cell-lines. The objectives of the study are:

1. To optimize the amount of total lysate required to detect selected proteins in H1299 cell lines.
2. To evaluate the level of proteins expression of main cell cycle players in H1299 cell lines.
3. To compare between protein expression level of the selected cell cycle players in H1299 and H1299R cell lines.

REFERENCES

Balak M. N., Gong Y., Riely G. J., Somwar R., Li A. R. and Zakowski M. F. (2006). Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clinical Cancer Research*. 12: 6494-6501.

Blume-Jensen P. and Hunter T. (2001). Oncogenic kinase signalling. *Nature*. 411: 355-365.

Chellappan S. S., Giordano A. and Fisher P. B. (1998). Role of cyclin dependent kinase and their inhibitors in cellular differentiation and development. *Current Topics in Microbiology and Immunology*. 227: 57-103.

Ciardiello F. and Tortora G. (2008). EGFR antagonists in cancer treatment. *The New England Journal of Medicine*. 358(11): 1160-1174.

Cohen M. H., Williams G. A. and Sridhara R. (2003). FDA drug approval summary: Gefitinib (ZD1839)(Iressa) tablets. *Oncologist*. 8: 303-306.

Demetri G.D., Von M.M., Blanke C.D., Van den A.A.D., Eisenberg B., Roberts P.J., Heinrich M.C., Tuveson D.A., Singer S., Janicek M., Fletcher J.A., Silverman S.G., Silberman S.L., Capdeville R., Kiese B., Peng B., Dimitrijevic S., Drunker B.J., Corless C., Fletcher C.D., and Joensuu H. (2002). Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *The New England Journal of Medicine*. 347(7): 472-480.

Esposito V., Baldi A., Tonini G., Vincenzi B., Santini M., Ambrogi V., Mineo T. C., Persichetti P., Liuzzi G., Montesarchio V., Wolner E., Baldi F.,and Groeger A. M. (2004). Analysis of cell cycle regulator proteins in non-small cell lung cancer. *Journal of Clinical Pathology*. 57(1): 58-63.

Fong K. M., Sekido Y., and Minna J. D. (1999). Molecular pathogenesis of lung cancer. *The Journal of Thoracic and Cardiovascular Surgery*. 118: 1136-1152.

Fry W. A., Philips J. L. and Menck H. R. (1999). Ten-year survey of lung cancer treatment and survival in hospitals in the United States: a national cancer data base report. *Cancer*. 86: 1867-1876.

Fukuoka M., Yano S., Giaccone G., Tamura T. and Nakagawa K. (2003). Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small cell lung cancer. *Journal of Clinical Oncology*. 21: 2237–46.

Furihata M., Ohtsuki Y., Sonobe H., Shuin T., Yamamoto A., Terao N. and Kuwahara. (1998). Prognostic significance of cyclin E and p53 protein overexpression in carcinoma of the renal pelvis and ureter. *British Journal of Cancer*. 77: 783-788.

Gazdar A. F. (1994). The molecular and cellular basis of human lung cancer. *Anticancer Research.* 14: 261-267.

Gazdar A. F. (2009). Activating and resistance mutations of EGFR in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. *Oncogene*. 28: 24-31.

Gridelli C., Bareschino M.A., Schettino C., Rossi A., Maione P., and Ciardiello F. (2007). Erlotinib in non-small cell lung cancer treatment: Current status and future development. *The Oncologist*. 12: 840-849.

Han J.Y., Park K., Kim S., Lee D. H., Kim H. Y. and Kim H. T. (2012). First SIGNAL: first-line single-agent iressa versus gemcitabine and ciplastin trial in never-smokers with adenocarcinoma. *Journal of Clinical Oncology*. 10(1): 1122-1128.

Hatakeyama M. and Weinberg R. A. (1995). The role of RB in cell cycle control. *Progress in Cell Cycle Research*. 1: 9-19.

Herbst R. S and Bunn P. A. (2003). Targeting the epidermal growth factor receptor in non-small cell lung cancer. *Progress in Cell Cycle Researh*. 9(16): 5813-5824.

Hoffman P. C., Maeur A. M. and Vokes E. E. (2000). Lung cancer. *Lancet*. 355: 479-485.

Inamura K., Ninomiya H., Ishikawa Y., and Matsubara O. (2010). Is the epidermal growth factor receptor status in lung cancer reflected in clinicopathologic features? *Archives of Pathology and Laboratory Medicine*. 134: 66-72.

Jackman D. M., Yeap B. Y., Sequist L. V., Lindeman N., Holmes A. J. and Joshi V. A. (2006). Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. *Clinical Cancer Research*. 12: 3908-3914.

Jemal A., Siegel R., Ward E., Hao Y., Xu J., and Thun M. J. (2009). Cancer statistics. *A Cancer Journals for Clinicians*. 59(4): 225-249.

Kobayashi S., Ji H., Yuza Y., Meyerson M., Wong K. K. and Tenen D. G. (2005). An alteration inhibitor overcomes resistance caused by a mutation of the epidermal growth factoe receptor. *Cancer Research*. 65: 7096-7101.

Kris M. G., Natale R. B. and Herbst R. S. (2003). Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *The Journal of the American Medical Association*. 290: 2149–2158.

Ling Y. H., Li T., Yuan Z., Haigentz M., Weber T. K., and Perez-Soler R. (2007). Erlotinib, an effective epidermal growth factor receptor tyrosine kinase inhibitor, induces p27KIP1 up-regulation and nuclear translocation in association with cell growth inhibition and G1/S phase arrest in human non-small-cell lung cancer cell lines. *Molecular Pharmacology*. 72(2): 248-258.

Maemodo M., Inoeu A., Kobayashi K., Sugawara S., Oizumi S. and Isobe H. (2010). Gefitinib or chemotherapy for non-small cell lung cancer with mutated EGFR. *The New England Journal of Medicine.* 362: 2380-2388.

Malumbres M. and Barbacid M. (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nature Reviews Cancer*. 9: 153-166.

Marchetti A., Martella C., Felicioni L., Barassi F., Salvatore S., Chella A., Camplese P.P., Iarussi T., Mucilli F., Mezetti A., Cuccurullo F., Sacco R., and Buttitta F. (2005). EFGR mutations in non-small lung cancer : Analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *Journal of Clinical Oncology*. 4(23): 857-865.

Martin S. J. and Pereira J.R. (1999). Clinical factors and prognosis in non-small cell lung cancer. *Journal of Clinical Oncology*. 22: 453-457.

Matsushime H., Roussel M. F., Ashmun R. A., and Sherr S. S. (1991). Colony-stimulating factor 1 regulated novel cycling during the G1 phase of the cell cycle. *Cell*. 65: 701-713.

Mitsudomi T., Morita S., Yatabe Y., Negoro S., Okamoto I. and Tsurutani J. (2010). Gefinitib versus ciplastin plus docetaxel in patients with non-small cell lung cancer harbouring mutations of the epidermal growth factor receptor. *Lancet*. 11: 121-128.

Moody S.E., Sarkisian C.J., Hahn K.T., Gunther E.J., Pickup S., Dugan K.D., Innocent N., Cardiff R.D., Schnall M.D., and Chodosh L.A. (2002). Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell*. 2(6): 451-461.

Müller-Tidow C., Metzger R., Kügler K., Diederichs S., Idos G., Thomas M., Dockhorn-Dworniczak B., Schneider P. M., Koeffler H. P., Berdel W. E. and Serve H. (2001). Cyclin E is the only cyclin-dependent kinase 2-associated cyclin that predicts metastasis and survival in early stage non-small cell lung cancer. *Cancer Research*. 61(2): 647-653.

Nielsen N. H., Arnerlov C., Cajander S., and Landberg G. (1998). Cyclin E expression and proliferation in breast cancer. *Analytical Cellular Pathology*. 17: 177-188.

Noonberg S. B and Benz C. C. (2000). Tyrosine kinase inhibitors targeted to the epidermal growth factor receptor subfamily: role as anticancer agents. *Drugs*. 59(4): 753-767.

Ohashi K., Maruvka Y. E., Michor F. and Pao W. (2013). Epidermal growth factor receptor tyrosine kinase inhibitor-resistant disease. *Journal of Clinical Oncology*. 31: 1070-1080.

Paez J.G., Janne P.A., Lee L.C., Tracy S., Greulich H., Gabriel S., Herman P., Kaye F.J., Lindeman N., Boggon T.J., Naoki K., Sasaki H., Fujii Y., Eck M.J., Sellers W.R., Johnson B.E., and Meyerson M. (2004). EFGR mutation in lung cancer : Correlation with clinical response to gefitinib therapy. *Science*. 304: 1497-1500.

Pao W., Miller V., Zakowski M., Doherty J., Politi K., Sarkaria I., Singh B., Heelan R., Rusch V., Fulton L., Mardis E., Kupfer D., Wilson R., Kris M., and Varmus H. (2004). EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proceedings of the National Academy of Sciences*. 36(101): 13306-13311.

Patel J. D., Bach P. B. and Kris M. G. (2004). Lung cancer in US women: a contemporary epidemic. *The Journal of the American Medical Association*. 291(14): 1763-1768.

Perez-Soler R., Chachoua A., Hammond L.A., Rowinsky E.K., Huberman M., Karp D., Rigas J., Clark G.M., Santabarbara P., and Bonomi P. (2004). Determinants of tumor response and survival with erlotinib in patient with non-small cell lung cancer. *Journal of Clinical Oncology*. 22(16): 3238-3247.

Planas-Silva M. D. and Weinberg R. A. (1997). The restriction point and control of cell proliferation. *Current Opinion in Cell Biology*. 9(6): 768-72.

Rusch V., Klimstra D., Venkatraman E., Pisters P. W., Langenfeld J. and Dmitrovsky E. (1997). Overexpression of the epidermal growth factor receptor and its ligand transforming growth factor alpha is frequent in resectable non-small cell lung cancer but does not predict tumor progression. *Clinical Cancer Research*. 3(4): 515-22.

Sekido Y., Fong K. M. and Minna J. D. (1998). Progress in understanding the molecular pathogenesis of human lung cancer. *Biochimica et Biophysica Acta*. 1378: 21-59.

Sharma S. V., Bell D. W., Settleman J. and Haber D. A. (2007). Epidermal growth factor receptor mutations in lung cancer. *Nature Reviews Cancer*. 7(3): 169-181.

Sherr C. J. (1994). G1 phase progression: cycling on cue. *Cell.* 79: 551-555.

Sherr C. J. (1996). Cancer cell cycles. *Science.* 274: 1672-1677.

Sherr C. J and Roberts J. M. (1999). CDK inhibitors: Positive and negative regulators of G1-phase progression. *Genes & Development*. 13: 1501-1512.

Shigematsu H., Lin L., Takahashi T., Nomura M., Suzuki M., Witsuba I.I, Fong K.M., Lee H., Toyooka S., Shimizu N., Fujisawa T., Feng Z., Roth J.A., Herz J., Minna J.D., and Gazdar A.F. (2005). Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *Journal of National Cancer Institutes*. 97(5): 339-346.

Shivapurkar N., Reddy J., Chaudhary P. M. and Gazdar A. F. (2003). Apoptosis and lung cancer: A review. *Journal of Cellular Biochemistry*. 88: 885-898;

Siegel R., Naishadham D., and Jemal A. (2013). Cancer statistics. *A Cancer Journal for Clinicians*. 63(1): 11-30.

Sordella R., Bell D.W., Haber D.A., and Settleman J. (2004). Gefitinib-sensitizing EFGR mutations in lung cancer activate anti-apoptotic pathways. *Science.* 305: 1163-1167.

Tartarone A., Lazzari C., Lerose R., Conteduca V., Improta G., Zupa A., Bulotta A., Aieta M. and Gregor V. (2013). Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. *Lung cancer*. 81: 328-336.

Thibault R. M., Galluzzi1 L., Olaussen K. A., Zermati Y., Tasdemir E., Robert T., Ripoche H., Lazar V., Dessen P., Harper F., Pierron G., Pinna G., Araujo N., Harel-Belan A., Armand J. A., Wong T. W., Soria J. C., and Kroemer G. (2007). A Novel Epidermal Growth Factor Receptor Inhibitor Promotes Apoptosis in Non–Small Cell Lung Cancer Cells Resistant to Erlotinib. *The Journal of Cancer Research*. 67(13): 6253–6262.

Travis W. D, Travis L. B, and Devesa S. S. (1995). Lung cancer. *Cancer*. 75(1): 191-202.

Wei Z., An T., Wang Z., Chen K., Bai H., Zhu G., Duan J., Wu M., Yang L., Zhuo M., Wang Y., Liu X. and Wang J. (2014). Patients harboring epidermal growth factor receptor (EGFR) double mutations had a lower objective response rate than those

with a single mutation in non-small cell lung cancer when treated with EGFR-tyrosine kinase inhibitors. *Thoracic Cancer*. 5(2): 126–132.

Yam C. H., Fung T. K. and Poon R. Y. C. (2002). Cyclin A in cell cycle control and cancer. *Cellular and Molecular Life Sciences*. 59(8): 1317-1326.

Zhao C., Christine M., Fillmore P. S., Hammerman C. F., and Kim K. W. (2014). Non-small-cell lung cancers: a heterogeneous set of diseases. *Nature Reviews Cancer*. 14: 535–546.