UNIVERSITI PUTRA MALAYSIA

COMPARISON BETWEEN DEATH RECEPTORS EXPRESSION LEVEL ON (H460) HUMAN LUNG CANCER CELL LINES AND (MRC-5) NORMAL LUNG CELL LINE

MUHRA ROMODAN OMER

lp
FPSK2 2020 50

COMPARISON BETWEEN DEATH RECEPTORS EXPRESSION LEVEL ON (H460) HUMAN LUNG CANCER CELL LINES AND (MRC-5) NORMAL LUNG CELL LINE .

MUHRA ROMODAN OMER

A THESIS SUBMITTED AS PARTIAL REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE (BIOMEDICAL SCIENCES)

DEPARTMENT OF BIOMEDICAL SCIENCES FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA

2020

© COPYRIGHT UPM

ABSTRACT

COMPARISON BETWEEN DEATH RECEPTORS EXPRESSION LEVEL ON (H460) HUMAN LUNG CANCER CELL LINES AND (MRC-5) NORMAL LUNG CELL LINE .

Muhra Romoda Omer [1] , Kamal Shaik Fakiruddin [2,3] , Khadijat Abubakar Bobbo [2], Shaiful Qushairi Suarni [2], Syahril Abdullah [1,2]

[1] Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Selangor.

[2] UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Selangor.

[3] Cancer Research Centre, Institute for Medical Research (IMR), National Institute of Health (NIH), Ministry of Health Malaysia, 40170 Shah Alam, Selangor

[4] Faculty Of Biotechnology And Biomolecular Science, Universiti Putra Malaysia, 43400 UPM, Selangor.

Introduction: Lung cancer is one of the most common cancer types in both developed and developing countries. There are two types of lung cancer; the Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). The NSCLC is more frequent and represents about 80% - 85% of lung cancer cases, while the SCLC represents about 10% - 15% of lung cancer cases. Most of the lung cancer related deaths are due to cancer metastasis and late stage diagnoses. Current lung cancer treatments exhibit a considerable number of side effects which necessitate the urgent need for an alternative treatment. One of the propective treatments is the use of Tumour Necrosis Factor-Related Apoptosis Ligand (TRAIL) protein, which effectiveness is highly dependent on the TRAIL cognate surface death receptors 4 and 5 (DR4 and DR5) on NSCLC, but not in normal cells. The level of the receptors on NSCLC has been determined by Fakiruddin et al, (2019), which correlates the effectiveness of TRAIL to the cancer cell. However, the results would need to be further verified using normal lung cell as a control. .

Objective: To re-evaluate the findings by Fakiruddin et al., (2019) by comparing the levels of the receptors from NSCLC H460 cells to normal MRC-5 lung cell.

Hypothesis: Lung cancer cells express higher level of the death receptors than normal lung cells.

Methodology: Two types of lung cells (H460 and MRC-5) were used in this study to compare the expression level of the receptors. NSCLC (H460) and normal lung cell (MRC-5) were cultured and maintained in T75 flask with RPMI medium with supplements necessary for each cell line. When both cell lines reached the targeted confluency ~80%, the cells were harvested into single cell suspension and stained with allophycocyanin (APC)-conjugated TRAIL-R1 (DR4) and TRAIL-R2 (DR5) for flow cytometry analysis of TRAIL agonist (DR4 and DR5).

© COPYRIGHT UPM

Results: The NSCLC H460 cell line showed higher expression level of DR5 than DR4. However, the normal lung cell (MRC-5) exhibited lower expression of both receptors (DR4 and DR5) when compared to H460.
Conclusion: Since the result have shown that the presence of death receptors (DR4 and DR5) in varying proportions in NSCLC is higher than MRC-5 death receptors expression that will facilitate and enable targeting death receptors in cancer stem cell and cancer cells which will cause Apoptosis to the cells but not normal cells.

Keywords: *lung cancer, NSCLC, death receptors, TRAIL agonist*

© COPYRIGHT UPM

ACKNOWLEDGEMENT

In the name of Allah The Most Merciful .

First and Foremost praise is to ALLAH, the Almighty, the greatest of all, on whom ultimately we depend for sustenance and guidance. I would like to thank Almighty Allah for giving me the opportunity, determination, and strength to do my research. His continuous grace and mercy was with me throughout my life and ever more during the tenure of my research. I would like to express my deepest thanks to my supervisor and advisor Associate professor Dr. Syahril Abdullah for all the help, patience, support, and motivation. I can't carry on with my acknowledgment without thanking my great parents who might not be physically here but they have always been with me spiritually, they have always supported me with prayers and motivations, who have always told me that I can do anything even in the time where I didn't believe in myself. For sure I wouldn't be here without my super-parents support. I would also like to thank my mentors Khadijat Abubakar Bobbo and Mr.Kamal who was always there when I feel lost to guide me and show me the right way I would also want to thank two of my friends Wafa Ghashoot and Nafis Ahmed for all the time support, they say "friend in need is a friend indeed" . lastly, I would like to extend my deepest appreciation to my lecturers and my university University Putra Malaysia for giving me the chance to explore more and learn the way I have always dreamed. Alhamdullah.

© COPYRIGHT UPM

TABLE OF CONTENT

Table of Contents

ABSTRACT 4

ACKNOWLEDGEMENT 6

APPROVAL 7

DECLARATION 8

TABLE OF CONTENT 9

CHAPTER 1 11

INTRODUCTION 11

BACKGROUND OF THE STUDY 1.1: 11

PROBLEM STATEMENT 1.2: 13

RESEARCH OBJECTIVES 1.3: 14

HYPOTHESIS 1.4 14

CHAPTER TWO ERROR! BOOKMARK NOT DEFINED.

LITERATURE REVIEW Error! Bookmark not defined.

CHAPTER THREE 15

METHODOLOGY 15

CELL CULTURE MAINTENANCE 3.1.2: 15

CHAPTER FOUR 18

RESULTS 18

CHAPTER FIVE 19

DISCUSSION 19

© COPYRIGHT UPM

CHAPTER SIX 21

CONCLUSION **21**

REFERENCES 22

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

BACKGROUND OF THE STUDY 1.1:

About a hundred years ago Lung cancer was firstly reported while nowadays it is one of the most common deaths leading disease globally for both genders. in 2018 it claimed about 1. 6 million worldwide death and a global prevailing rate of almost 1.8 million ((Cortes-Dericks &Galetta & Elmallah and Micheau). Although great advances in different treatment options that have been discovered over the past decades, the prognosis remains demoralized. The high-level motility and aggressiveness of lung cancer cells cause therapies either to fail or to show serious side effects that cannot be neglected. As the incidence of lung cancer patients number soars, the efforts on finding new treatment options to treat cancer and lower the side effect are also rising. Early diagnosing of cancer and screening with low dose computed tomography decrease the mortality chance. Lung cancer is a disease that has no particular symptoms or signs at an early stage. Therefore, most patients are diagnosed with an advanced stage which makes the recovery more complicated Spiro, ((S. G.)
Lung cancer had been classified into two different types. It’s very crucial to determine the type because the pathogenesis of lung cancer of the two types must be treated differently. The first type is Small cell lung cancer (SCLC) which makes up less than 20% of lung cancers in total cases this type is mostly caused by tobacco smoking. Small cell lung cancer (SCLC) often starts in the bronchi, then rapidly grows and spreads to other organs, including the lymph nodes. Small cell lung cancer has two stages: Limited stage: is the first stage where cancer is found in one side of the lung and some cases it might include the nearby lymph nodes. The second stage is the Extensive stage: where Cancer has already spread to the other lung, and mostly to other organs in the body. The second type of lung cancer is non-small cell lung cancer (NSCLC) which makes up 80-85% of the total cases. The staging system which is mostly used for NSCLC is the American Joint Committee on Cancer (AJCC) TNM system, which is based on 3 information keys : (T): The size of the main tumour and its extent, (N): The spread range to nearby lymph nodes (M): the spread (metastasis) to distant sites. Stages and grades must be determined before starting any treatment method because classifications play a significant role in estimating different treatment methods. (Amin, M. B., & Edge, S. B. (Eds.).

© COPYRIGHT UPM

(2017).) The current treatment options for lung cancer can either be chemotherapy, radiotherapy, or surgery alone or in a combination of two of them (& & Galetta) However, with the existence of all the treatment options the mortality rate is still increasing dramatically. The poor prognosis is causing relapsing for patients and lowering their chance of living this is resulting in a less than 15% chance 5-year survival rate (. (Myers and Wallen) The poor prognosis has been linked with the presence of a minimal percent (0.1 to 0.8%) of cells known as the cancer stem cells (CSCs), and they can develop drug resistance to these treatments ((Jiang et al.) Although the existence of different options to cure cancer, discovering a new treatment strategy is urgent due to the considerable number of side effects that had been reported by the current treatment. One of the prospective treatments is the use of Tumour Necrosis factor-related Apoptosis Ligand (TRAIL) protein. also known as Apo-2 ligand (1995 & Pitti et al. 1996)) it is a member of the structurally related TNF family of cytokines and exists as either a type II membrane protein or as a soluble protein.

© COPYRIGHT UPM

PROBLEM STATEMENT 1.2:

The number of side effects that are being reported due to current cancer treatment continues to escalate. Therefore, discovering new treatment is urgent at this point.
TRAIL is a promising therapy, it can induce apoptosis in cancer cells that are often hard to treat, these features make TRAIL a promising agent for anti-cancer therapy. (Mohr, Chu, Brooke, & Zwacka).
TRAIL proved that it could get attached to the death receptors in the cancer cell (CSC) inducing apoptosis pathway either extrinsic pathway or intrinsic pathway (Fakiruddin et al.).
Therefore, more investigation needed to be done due to a limitation of information about TRAIL effect on the normal cell line. Therefore, this present retrospective study aims to determine and re-evaluate the findings by (Fakiruddin et al.) that H460 cell line expresses a higher level of death receptor 5 (DR5) than death receptor 4 (DR4) and compares the findings with normal lung cells (MRC-5).

© COPYRIGHT UPM

RESEARCH OBJECTIVES 1.3:

- To determine the level of expression of the death receptors on normal lung cells (MRC-5).
- Comparing the expression levels of the death receptors from NSCLC (H460) cells (H460) to normal lung cell (MRC-5).

HYPOTHESIS 1.4

- Lung cancer cells (H460) express higher level of the death receptors than normal lung cells (MRC-5).

© COPYRIGHT UPM

CHAPTER THREE
METHODOLOGY

The human lung fibroblast cell line (MRC-5) and the cancer human lung large cell line (H460) were utilized in this study in vitro as models of cancer and normal lung cell lines respectively.

CELL CULTURE 3.1.1:

One type of human non-small cell lung cancer cell lines (H460) was used in this study. large cell lung cancer (H460) (cat no: JCRB1407), were purchased from Cell Bank Australia (Westmead, NSW, Australia).). Another cell line that was used in this study is the normal lung cell line (MRC-5) obtained from (UPM-MAKNA*)*.

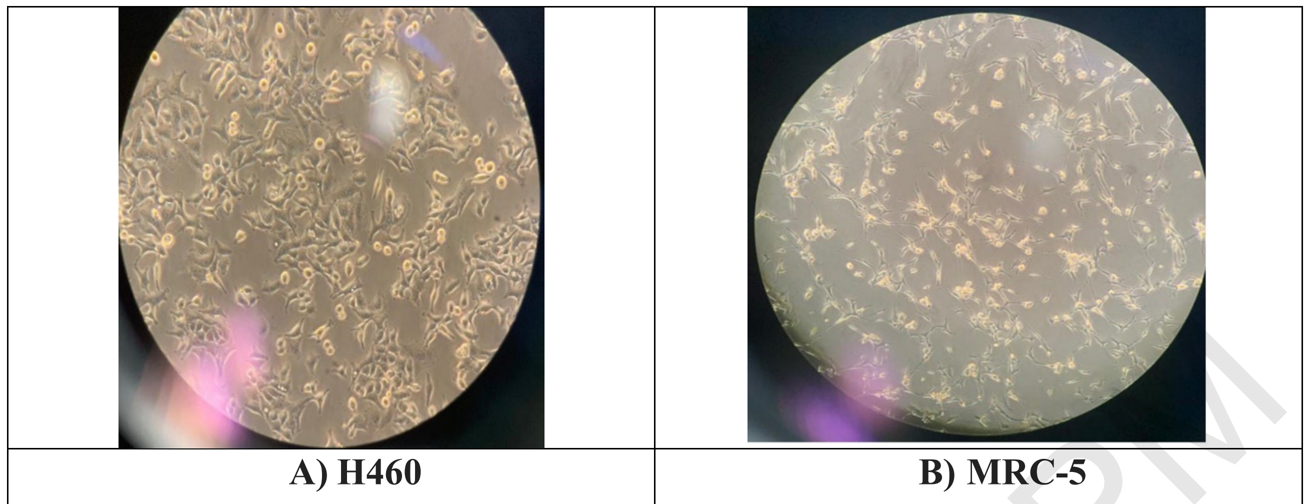
CELL CULTURE MAINTENANCE 3.1.2:

(H460) cells were maintained in RPMI-1640 medium supplemented with 15% heat-inactivated fatal bovine serum (FBS), 1% penicillin/streptomycin and 0.8 µg/mL insulin (4 mg/mL stock.
(MRC-5) cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

The culture was maintained in the atmosphere of 5% CO_2 at 37°C. The cells were cultured in 25-cm^2 cell culture flask (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and harvested using 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) when cells reached 80% confluence. All culture reagents were obtained from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Cells were observed daily under the microscope to examine the general health (the morphology and behavior). The media was changed every three days until the cells reached 70% to 80% confluence. Cells were sub-cultured when reached 70% confluence to maintain log phase growth.

© COPYRIGHT UPM



A) H460	B) MRC-5

FIGURE 1. A) (H460) cell line under 10X10 magnification, cultured in 25-cm2 flasks (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA).
. B) (MRC-5) cell line under 100x10 magnification cultured in 25-cm2 flasks (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

SUBCULTURE 3.1.4:

During the subculture, the previous media was discarded from the flask. The cells were washed with 2ml of PBS for 25-cm2 flasks (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA) two times. 1ml of – EDTA was added to 25-cm2 flasks (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and then cells were incubated for 5 minutes to detach the cells from the flask. Upon cell detachment, 2ml of media was added into the culture flasks to inactivate the activity of trypsin. The 3ml of cell suspension was aspirated into a 15- ml centrifuge tube, and centrifuges at 1000 rpm for 5 minutes. The supernatant was discarded and the cell pellet was resuspended in a fresh pre-warm media, cell count was performed and cells are plated in a new 75-cm2 flask (Nunc, Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing pre-warmed media. The flask was shacked gently to assure cell spreading in the flask, and observed under a microscope before placing it in a 5% CO_2 incubator at 37°C.

© COPYRIGHT UPM

FLOW CYTOMETRY ANALYSIS 3.2:

CELL PREPARATION FOR FLOW CYTOMETRY 3.2.1:

Cells were maintained for three days in 75-cm2 flasks (Nunc, Thermo Fisher Scientifific, Inc., Waltham, MA, USA). when cells reached ~80% they were harvested using 2ml of 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA) for 5 minutes in a 5% CO_2 incubator at 37°C. After the cells detach, they transferred to a 15ml centrifuge tube, 1 mL DPBS with 2% FBS was added to the cells in the tube. The tube was then centrifuged at 1000 rpm for 5 minutes. This procedure was repeated two times to get rid of the remaining trypsin. After that cells were re-suspended in 100uL DPBS with 2% FBS. Then the tube was tapped to break the cell pellet.

CELL STAINING PROCEDURE 3.2.2:

After breaking the cells through tapping, cells were distributed into 4 cryopreserve tubes, the first tube was unstained used as a control, the second tube was stained with 10uL allophycocyanin (APC)-conjugated TRAIL-R1 (DR4) (cat no: FAB347A; Mouse IgG1), the third tube was stained with 10uL TRAIL-R2 (DR5) (cat no: FAB6311A; Mouse IgG2B), the fourth tube was stained with 10uL of allophycocyanin (APC)-conjugated TRAIL-R1 (DR4) (cat no: FAB347A; Mouse IgG1), TRAIL-R2 (DR5) (cat no: FAB6311A; Mouse IgG2B). The tubes then were carefully tapped and inside a 4-degree fridge/dark for 20min. After the incubation period, 1mL of DPBS with 2% FBS was added into the tube to wash away unbound antibody, then the cells were centrifuged at 1000 rpm for 5 minutes. After counternutation the cells, the supernatant was discarded and 500uL DPBS with 2% FBS was added and cells were subjected f flow cytometry analysis. Flow cytometry analysis was performed in (Faculty of Biotechnology and Biomolecular Science, UPM).

© COPYRIGHT UPM

CHAPTER FOUR
RESULTS

FLOW CYTOMETRY ANALYSIS 4.1:

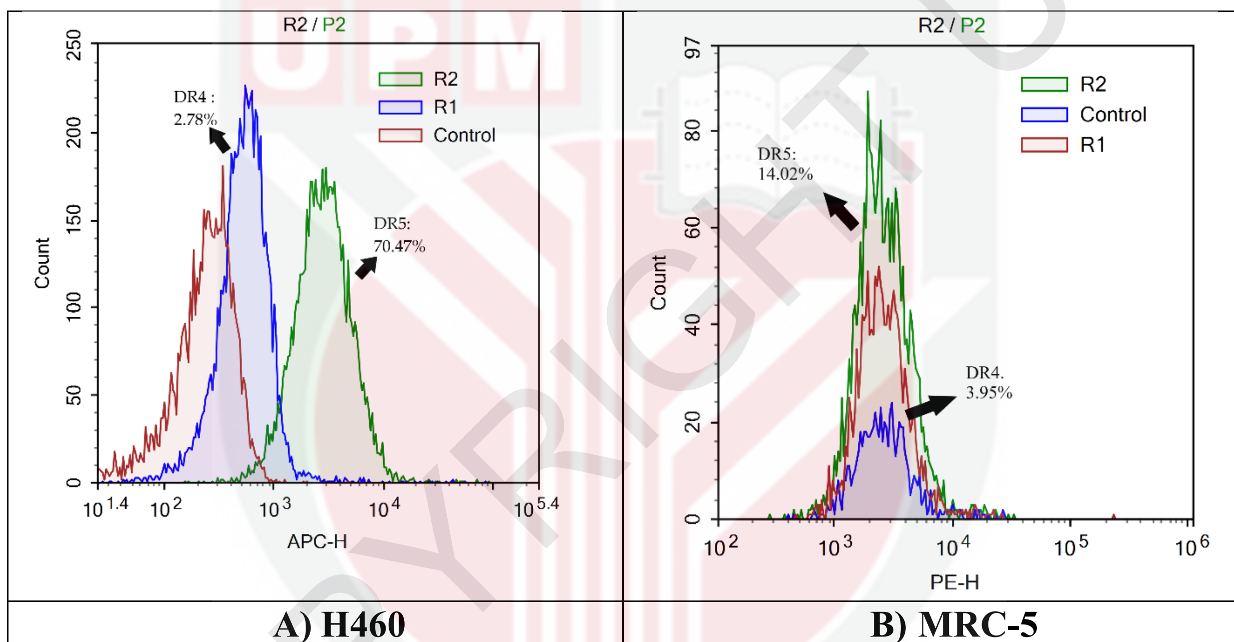


Figure 2. Analysis of TRAIL receptors expression [agonist receptors (DR4 and DR5]). by flow cytometry. **A)** (H460) Non-small cell lung cancer (NSCLC) in cryopreserve tube at density (2.0 x10^6 cells) stained with 10uL of allophycocyanin (APC)-conjugated TRAIL-R1 (DR4) TRAIL-R2 (DR5). **B)** (MRC-5) normal lung cell lines in cryopreserve tube at (2.0 x10^6 cells) density stained with10uL of allophycocyanin (APC)-conjugated TRAIL-R1 (DR4, TRAIL-R2 (DR5).

© COPYRIGHT UPM

CHAPTER FIVE

DISCUSSION

Lung cancer is one of the highly prevalent and deadly cancers in the United States and globally for males and females (GLOBOCAN 2019).TRAIL is a cell signalling protein that can be produced or secreted by the majority of normal tissue cells in the human body. Number of cells such as the residual stem cell and immune system cells including the cytotoxic T lymphocyte and monocytes express this ligand (Almasan & Ashkenazi, 2003; Naval et al., 2019), which has the ability to bind to 5 different groups of receptors, three of them are anti-apoptotic known as decoy receptors and two are pro-apoptotic or conative death receptors (Almasan & Ashkenazi, 2003; Dubuisson & Micheau, 2017; Kretz et al., 2019; Naval et al., 2019)

TRAIL can cause apoptosis in a variety of cancer cells and transformed cells by binding to several death receptors and induce apoptosis via recruitment of the death-inducing signalling complex (DISC) which will activate the caspase cascade causing cell apoptosis (Guohua Pan et al., 1997). TRAIL and its receptors have been the target of several anti-cancer therapeutics since the 1990s (. (Mariani and Krammer 1998)). In (NSCLC) cell line drug resistance varies from one type to another, and it can determine the cell apoptosis rate. Studies have reported that the Molecular mechanism(s) is responsible for the drug resistance in non-small cell lung cancer (NSCLC). In a previous study of XIAP-mediated protection of H460 lung cancer cells against cisplatin in 2010,

© COPYRIGHT UPM

results from the study exhibit that cisplatin (50 μM)-induced cell death (apoptosis) level was higher in A549 and CH27 cell lines than in H460. (Cheng, et al., 2010). The high protein levels of X-linked inhibitor-of-apoptosis protein (XIAP) seen in H460 cells inspected to play a key role in the cisplatin resistance of H460 cells regulation. (Cheng, et al., 2010). (XIAP) has the ability to bind and suppress the activities of caspase 3 in H460 cells and lead to apoptosis inhibition of these cells. Cheng, et al., Since TRAIL can be considered as one of the safest and non-toxic treatments to normal cells, in this study we used (MRC-5) as a model to examine the TRAIL effect of normal lung cell line targeting the expression level of death receptors. Our result showed that death receptor expression on the normal cell is lower than (H460)lung cancer cell line. Death receptor 4 (DR4) expression as low as 3.95% however it's still higher than the death receptor 4 (DR)expression in (H460) cell line which shows 2.78%. While Death receptor 5 (DR5) expression showed higher expression 14.02% in (MRC-5) and 70.47% in (H460) cell line. Nevertheless, some non-small cell lung cancer (NSCLC) cells are particularly resistant to the effects of TRAIL.

© COPYRIGHT UPM

CHAPTER SIX

CONCLUSION

Over all , This experiment shows that normal lung cells can also express varying proportions, of the death receptors, higher (DR5) expression than (DR4) in both cell lines (MRC-5) and (H460). However, the function of DR4 and DR5 during TRAIL apoptosis pathway activation is quite distinct. Even though TRAIL is able to target cancer cells (H460) better since death receptor expression is higher as it was seen in this study result and number of other studies, but it might also have a reduced effect on the normal cell line (MRC-5). Therefore, TRAIL can be one of the potential therapeutic applications in NSCLC in the future , but more studies have to be done to estimate its effect on normal cells.

© COPYRIGHT UPM

REFERENCES

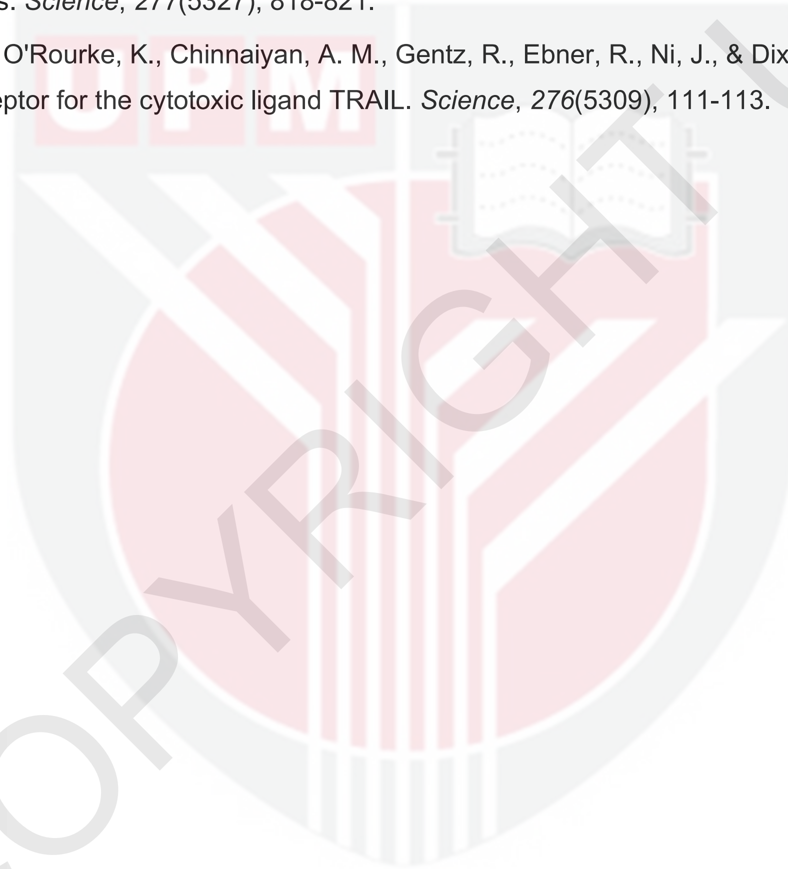
- Fakiruddin, K. S., Lim, M. N., Nordin, N., Rosli, R., Zakaria, Z., & Abdullah, S. (2019). Targeting of CD133+ Cancer Stem Cells by Mesenchymal Stem Cell Expressing TRAIL Reveals a Prospective Role of Apoptotic Gene Regulation in Non-Small Cell Lung Cancer. *Cancers*, *11*(9), 1261.

- Cheng, Y. J., Jiang, H. S., Hsu, S. L., Lin, L. C., Wu, C. L., Ghanta, V. K., & Hsueh, C. M. (2010). XIAP-mediated protection of H460 lung cancer cells against cisplatin. *European journal of pharmacology*, *627*(1-3), 75-84.
- Ishii, H., Iwatsuki, M., Ieta, K., Ohta, D., Haraguchi, N., Mimori, K., & Mori, M. (2008). Cancer stem cells and chemoradiation resistance. *Cancer science*, *99*(10), 1871-1877.
- Romaszko, A. M., & Doboszyńska, A. (2018). Multiple primary lung cancer: A literature review. *Adv Clin Exp Med*, *27*(5), 725-730.
- Virmani, A. K., Fong, K. M., Kodagoda, D., McIntire, D., Hung, J., Tonk, V., ... & Gazdar, A. F. (1998). Allelotyping demonstrates common and distinct patterns of chromosomal loss in human lung cancer types. *Genes, Chromosomes and Cancer*, *21*(4), 308-319.
- LiF, T., & MassagueJ, K. (2007). Beyondtumorigenesis: cancerstemcellsin metastasis. *Cell Res*, *17*, 3-14.
- Wiley, S. R., Schooley, K., Smolak, P. J., Din, W. S., Huang, C. P., Nicholl, J. K., ... & Goodwin, R. G. (1995). Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity*, *3*(6), 673-682.
- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.
- Rami-Porta, R., Ball, D., Crowley, J., Giroux, D. J., Jett, J., Travis, W. D., ... & International Staging Committee. (2007). The IASLC Lung Cancer Staging Project: proposals for the

© COPYRIGHT UPM

revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. *Journal of Thoracic Oncology*, *2*(7), 593-602.

- Hallissey, M. T., Dunn, J. A., Ward, L. C., Allum, W. H., & Group, B. S. C. (1994). The second British Stomach Cancer Group trial of adjuvant radiotherapy or chemotherapy in resectable gastric cancer: five-year follow-up. *The Lancet*, *343*(8909), 1309-1312.
- Sheridan, J. P., Marsters, S. A., Pitti, R. M., Gurney, A., Skubatch, M., Baldwin, D., ... & Goddard, A. D. (1997). Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science*, *277*(5327), 818-821.
- Sheridan, J. P., Marsters, S. A., Pitti, R. M., Gurney, A., Skubatch, M., Baldwin, D., ... & Goddard, A. D. (1997). Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. *Science*, *277*(5327), 818-821.
- Pan, G., O'Rourke, K., Chinnaiyan, A. M., Gentz, R., Ebner, R., Ni, J., & Dixit, V. M. (1997). The receptor for the cytotoxic ligand TRAIL. *Science*, *276*(5309), 111-113.



© COPYRIGHT UPM