Roles of IL-24 as Anti-Tumor Factor in Cancers

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Abstract

Cancer immunotherapy (CI) refers to methods that enable the use of the immune system and its components as cancer treatment. As a result of its capacity to inhibit cell proliferation, IL-24 has drawn a lot of interest. Additionally, by activating autophagy and death in cancer cells, IL-24 functions as a self-sufficient anti-cancer agent. A number of human cancers can become cytotoxic when exposed to IL-24. In the current investigation, ductal mammary gland carcinoma treated with various dosages of IL-24 (10, 50, and 100 pg/ml). The optimum cytotoxic concentration of IL-24 on ductal mammary gland carcinoma was determined using the MTT test. Using the AO/EB (Acridine-Orange and Ethedium Bromide) Technique It also enables the differentiation of healthy cells, necrotic cells, and cells that have undergone early and late apoptosis. LC-3, caspase 8, and caspase 9 levels of specific apoptosis and autophagy were measured utilizing a gene expression technique.

Keywords: health; patience; Anti-Tumor Factor

1. Introduction

The second greatest cause of death worldwide is cancer. Radiation, chemotherapy, and surgery are the three conventional cancer treatments that used routinely in hospitals (Sung et al., 2021). Breast cancer (mammary gland carcinoma) is the most frequently diagnosed cancer in women worldwide with more than 2 million new cases in 2020. It is the second most frequent cancer-related death among women worldwide. Breast cancer develops slowly, and the majority of cases are found through routine screenings.

2. Material

The laboratory equipment's and instruments:		
NO.	Equipment's/Instruments	Company/ Origin
	Autoclave	Stermite/ Germany
	Cell culture plates	Santa Cruz Biotechnology/ USA
	O2 incubator	Cypress Diagnostics/ Belgium
	Distilator system	Daihan LabTech/ Indonesia
	Hemocytometer	SantaCruz Biotechnology/ USA
	Fluorescent microscope	Leica / Germany
	Inverted microscope	
	Laminar flow hood class II	Dewar / China
	Magnetic stirrer	Heidolph/ Germany
	Micropipettes 5-50 µl ,100-1000 µl, 0.5-10 µl	Eppendorf / Germany
	Microtiter reader	Huma HR / USA
	Refrigerator	Hitachi/ Japan
	Screw cap bottles	Pyrex/ England
	Syringe Millipore 0.22um	Satorins membrane/ Germany
	T25 culture flask	SantaCruz Biotechnology/ USA
	Vortex	Thermolyne maxi mix plus/ USA
	Water Bath	CL010 CYAN /Belgium
18.	Absolute Ethanol	FLUKA / Switzerland
19.	Acridine Orange/Ethidium bromide staining kit	Himedia / Indian
20.	Deionized H2O	Pioneer/Korea
21.	DMSO (Dimethyl sulfoxide)	Santacruz Biotechnology / USA
22.	Dual Stain AO/EB	Gibco/USA
23.	Ethanol 96%	Teba/Iraq
24.	MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)	Bio-World / USA
25.	Penicillin / streptomycin 100x cat no (PS-B)	Capricorn / Germany
26.	RPMI 1640 (Roswell Park Memorial ,Institute) cat no (RPMI-HA),	Capricorn / Germany
27.	Phosphate buffered saline,Ph 7.2	Himedia / Indian
28.	Phenol red	Himedia / Indian
29.	Trypan blue	Himedia / Indian

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Gene expression materials and equipment's			
	Material	Cat. No.	Company
1	AddPrep Total RNA Extraction Kit	10119	Addbio/Korea
2	AddScript cDNA Synthesis Kit	22701	Addbio/Korea
3	Ethanol	3803686	Leica/ USA
4	Go Taq RT-qPCR	A6001	Promega/USA
5	Microfuge IB Centrifuge		BeckmanCoulter /Germany
6	Dry microtubes incubator		Ae /UK
7	Mx3005P Real-Time PCR System		Agilent /USA
8	Pipettes		DARWELL/ China
9	Vortex		Capp /China

3. Methods

Ductal mammary gland carcinoma treated with IL-24 and another Without IL-24
At concentration of 10, 50 and 100 pg./ml of media by using:

MTT assay

Measured the dead and life cell to indicate the cytotoxicity IL-24 on Ductal Mammary Gland Carcinoma

Acridine-Orange and Ethedium Bromide assay

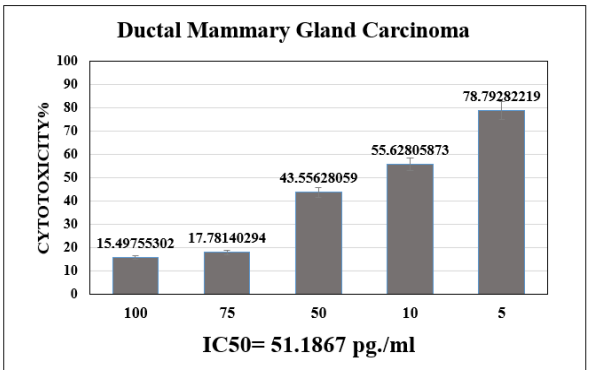
To indicate apoptosis in Ductal Mammary Gland Carcinoma

Gene expression

Measure the gene expression levels of L-C3 (Autophagy) and caspase 8 (Apoptosis) and caspase 9

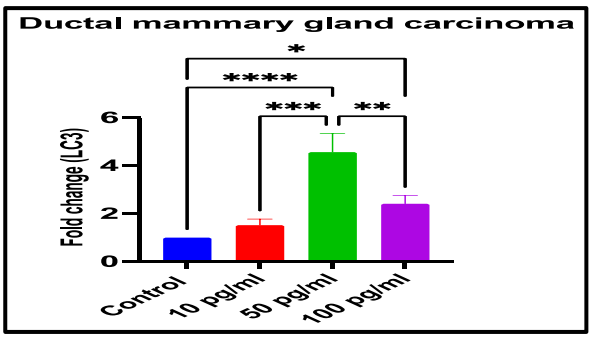
4. Results

MTT experiment for ductal mammary gland cancer indicated that 51.18 pg/ml of medium was the concentration that had the most inhibitory effect on the growth rate of ductal mammary gland carcinoma cells after 48 hrs. of exposure to IL-24. AO/EtBr assay of ductal mammary gland carcinoma, IL-24 at concentrations of 10, 50, and 100 pg./ml of media caused apoptosis in all ductal mammary gland carcinoma cells compared with non-treated ductal mammary gland carcinoma, and IL-24 at concentrations of 50 pg/ml of media caused apoptosis in the majority of ductal mammary gland carcinoma compared with other IL-24 concentrations. ductal mammary gland carcinoma cells treated with IL-24 at concentration of 10, 50 and 00 pg./ml of media showed significant expression in LC-3 gene compared with non-treated primary cell lines of ductal mammary gland carcinoma. However, treatment of IL-24 at concentration of 50 pg/ml of media showed a significant overexpression of LC-3 gene expression among all concentrations of IL-24 that used in the present study indicating that IL-24 induced autophagy in primary cell lines.

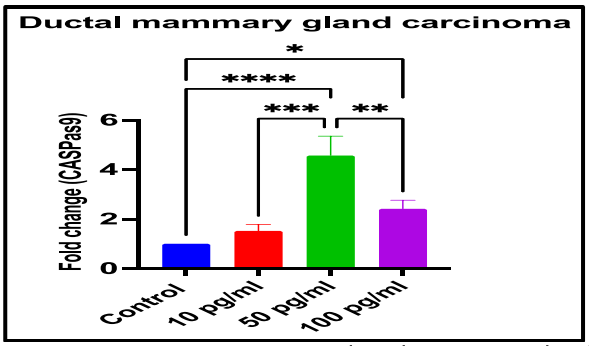


The cytotoxic effect of IL-24 against ductal mammary gland carcinoma after 48 hrs. of exposure

The fluorescence microscope image of apoptosis process activity of primary cell culture of ductal mammary gland carcinoma.



LC-3 gene expression in ductal mammary gland carcinoma cells.



Caspase 9 gene expression in ductal mammary gland carcinoma cells.

5. Discussion

In current study was conducted to investigate the activity of IL-24 at different concentrations (10, 50 and 100 pg./ml of media) was as anticancer agent against ductal mammary gland carcinoma. MTT assay results of IL-24 treatment to ductal mammary

gland carcinoma cells showed that IL-24 at concentration of 50 pg./ml of media was the most cytotoxic concentration ductal mammary gland carcinoma cells on compared with other concentrations. IL-24 induces apoptosis in tumor cell by modulating various signaling pathways that is cell-type dependent. Although signaling pathways triggered by IL-24 have been the focus of intensive studies for over 20 years (Pelicano et al., 2004; Menezes et al., 2014). Treatment of interleukin -24 lead to apoptosis, where several studies showed (Bhutia et al.,2013). Over expression of interleukin -24 lead to apoptosis, where several studies showed (Bhutia et al.,2013) in breast cancer stem cells, it was observed that IL-24 induced apoptosis selectively in cancer stem cells without affecting normal stem cell growth one of the important mechanisms of IL-24-mediated cell death is induction of ER stress. Although ER stress can regulate both pro- and anti-apoptotic pathways, prolonged periods of intense ER stress shift the balance towards apoptosis (Pelicano et al.,2004; Menezes et al.,2014). In current study, all concentrations of IL-24 succussed to induce apoptosis in ductal mammary gland carcinoma, however AO/EB assay result of IL-24 treatment to ductal mammary gland carcinoma showed that 50 pg./ml of media concentration of IL-24 was most effective concentration compared with other concentrations (10 and 100 pg./ml of media). In present study, the gene expression result of LC-3 gene showed the IL-24 induced autophagy in ductal mammary gland carcinoma. in ductal mammary gland carcinoma, LC-3 gene expression was significantly increased in ductal mammary gland carcinoma cells that treated with IL-24 at concentration of 50 pg./ml of media compare with others concentrations. In the current study, caspase 9 gene expression did not show a significant expression in non-treated primary cell lines of ductal mammary gland carcinoma compared with same cell lines that treated with IL-24, which showed significant caspase 9 gene expression. While, in ductal mammary gland carcinoma, treatment of IL-24 at concentration of 50 pg./ml of media showed a significant caspase 9 gene expression compared with other concentrations. caspase 8 gene expression was under detection range of gene expression indicating IL-24 did not induce extrinsic apoptosis due to low expression in caspase 8 gene in ductal mammary gland carcinoma.

References

Dairkee, S. H., Deng, G., Stampfer, M. R., Waldman, F. M., & Smith, H. S. (1995). Selective cell culture of primary breast carcinoma. Cancer Res. 55:2516–2519

Leake, R. E., Freshney, R. I., & Munir, I. (1987). Steroid responses in vivo and in vitro. In Green, B., & Leake, R. E., (eds.), Steroid hormones: A practical approach. Oxford: IRL Press at Oxford University Press, pp. 205–218.

Speirs, V., Green, A. R., & White, M. C. (1996). Collagenase III: A superior enzyme for complete disaggregation and improved

Pelicano, H., Carney, D., & Huang, P. (2004). ROS stress in cancer cells and therapeutic implications. *Drug resistance updates*, *7*(2), 97-110.

Menezes, M. E., Shen, X. N., Das, S. K., Emdad, L., Guo, C., Yuan, F., ... & Fisher, P. B. (2015). MDA-7/IL-24 functions as a tumor suppressor gene in vivo in transgenic mouse models of breast cancer. *Oncotarget*, *6*(35), 36928.

Bhutia, S. K., Das, S. K., Azab, B., Menezes, M. E., Dent, P., Wang, X. Y., ... & Fisher, P. B. (2013). Targeting breast cancer-initiating/stem cells with melanoma differentiation-associated gene-7/interleukin-24. *International journal of cancer*, *133*(11), 2726-2736.

Birgisdottir, Å. B., Lamark, T., & Johansen, T. (2013). The LIR motif–crucial for selective autophagy. *Journal of cell science*, *126*(15), 3237-3247.

Briukhovetska, D., Dörr, J., Endres, S., Libby, P., Dinarello, C. A., & Kobold, S. (2021). Interleukins in cancer: from biology to therapy. *Nature Reviews Cancer*, *21*(8), 481-499. Qiu, Y., Su, M., Liu, L., Tang, Y., Pan, Y., & Sun, J. (2021). Clinical application of cytokines in cancer immunotherapy. *Drug Design, Development and Therapy*, 2269-2287.

Brunelle, J. K., & Letai, A. (2009). Control of mitochondrial apoptosis by the Bcl-2 family. *Journal of cell science*, *122*(4), 437-441.

Buzas, K., Oppenheim, J. J., & Howard, O. Z. (2011). Myeloid cells migrate in response to IL-24. *Cytokine*, *55*(3), 429-434.

Campbell, K. J., & Tait, S. W. (2018). Targeting BCL-2 regulated apoptosis in cancer. *Open biology*, *8*(5), 180002.

Carneiro, B. A., & El-Deiry, W. S. (2020). Targeting apoptosis in cancer therapy. *Nature reviews Clinical oncology*, *17*(7), 395-417.

Chada, S., Bocangel, D., Ramesh, R., Grimm, E. A., Mumm, J. B., Mhashilkar, A. M., & Zheng, M. (2005). mda-7/IL24 kills pancreatic cancer cells by inhibition of the Wnt/PI3K signaling pathways: identification of IL-20 receptor-mediated bystander activity against pancreatic cancer. *Molecular therapy*, *11*(5), 724-733.

Chada, S., Sutton, R. B., Ekmekcioglu, S., Ellerhorst, J., Mumm, J. B., Leitner, W. W., ... & Mhashilkar, A. M. (2004). MDA-7/IL-24 is a unique cytokine–tumor suppressor in the IL-10 family. *International immunopharmacology*, *4*(5), 649-667.

Cho, H., Wu, M., Zhang, L., Thompson, R., Nath, A., & Chan, C. (2013). Signaling dynamics of

palmitate-induced ER stress responses mediated by ATF4 in HepG2 cells. *BMC systems biology*, *7*(1), 1-13.

Chowdhury, D., & Lieberman, J. (2008). Death by a thousand cuts: granzyme pathways of programmed cell death. *Annual review of immunology*, *26*, 389.

Chowdhury, D., & Lieberman, J. (2008). Death by a thousand cuts: granzyme pathways of programmed cell death. *Annual review of immunology*, *26*, 389. Pfeffer, C. M., & Singh, A. T. (2018).

Chowdhury, D., & Lieberman, J. (2008). Death by a thousand cuts: granzyme pathways of programmed cell death. *Annual review of immunology*, *26*, 389.

Csapo, M., & Lazar, L. (2014). Chemotherapy-induced cardiotoxicity: pathophysiology and prevention. *Clujul medical*, *87*(3), 135.

D'Errico, G., Machado, H. L., & Sainz, B. (2017). A current perspective on cancer immune therapy: step-by-step approach to constructing the magic bullet. *Clinical and translational medicine*, *6*(1), 1-10.

Dairkee, S. H., Deng, G., Stampfer, M. R., Waldman, F. M., & Smith, H. S. (1995). Selective cell culture of primary breast carcinoma. Cancer Res. 55:2516–2519

Dasari, S. K., Bialik, S., Levin-Zaidman, S., Levin-Salomon, V., Merrill, A. H., Futerman, A. H., & Kimchi, A. (2017). Signalome-wide RNAi screen identifies GBA1 as a positive mediator of autophagic cell death. *Cell Death & Differentiation*, *24*(7), 1288-1302.

Dasari, S. K., Schejter, E., Bialik, S., Shkedy, A., Levin-Salomon, V., Levin-Zaidman, S., & Kimchi, A. (2017). Death by over-eating: The Gaucher disease associated gene GBA1, identified in a screen for mediators of autophagic cell death, is necessary for developmental cell death in Drosophila midgut. *Cell Cycle*, *16*(21), 2003-2010.

Dash, R., Azab, B., Quinn, B. A., Shen, X., Wang, X. Y., Das, S. K., ... & Fisher, P. B. (2011). Apogossypol derivative BI-97C1 (Sabutoclax) targeting Mcl-1 sensitizes prostate cancer cells to mda-7/IL-24–mediated toxicity. *Proceedings of the National Academy of Sciences*, *108*(21), 8785-8790.