Original Article

The role of PIM3 gene expression in thyroid and breast cancer

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Abstract

Cancer is one of the most significant health issues of our time, as well as a primary cause of death among people. A malignant tumor or malignant neoplasm is a simple definition. It's a disorder characterized by irregular cells that divide rapidly and uncontrollably

(Pim-3 Proto-Oncogene, Serine/Threonine Kinase) PIM3 expression promotes carcinogenesis and progression by blocking apoptosis and regulating cell cycle progression.

Our findings reveal that the PIM3 gene is overexpressed in thyroid and breast cancer compared to controls (presumably healthy individuals), which is consistenti with the PIM3 gene's action mechanism.

Keywords: Gene expression, PIM3, Thyroid cancer, Breast cancer

Introduction

Thyroid cancer, the most frequent endocrine system tumor, arises from follicular thyrocytes or parafollicular C- cells of the thyroid gland and represents a pattern of malignant change from benign adenomas and highly diversified squamous cell carcinoma to moderately differentiated carcinomas 1.

Breast cancer is the most frequent female cancer and the second most common cancer-related death in women. Each year, almost a million new instances of breast cancer are discovered. Both mortality and burden are exceptionally high. Over 50 years, it has become a serious global health concern. 2

Pim-3 Proto-Oncogene, Serine /Threonine Kinase) PIM3 this gene can prevent apoptosis and promote cell survival and protein translation. PIM3 gene is located in the nucleus of chromosome 22 on the long arm, region 13, band 3 sub band3 (22q13.33).3 The Pim group of kinases, which includes Pim-1, Pim-2, and Pim-3, stimulates the phosphorylation of the pro-apoptotic protein Bad. Pim is an oncogene with anti-apoptoticiproperties that work with the proto-oncogene Myc to promote tumor formation. Pim proteins can control tumor proliferation and the cell cyclei, as well as improve the anti-apoptotic capabilities of various tumorous and normal cells. Previous research has shown that Pim kinase stimulates the cell cycle'si progression from the G1 to the S phase and accelerates cellular proliferationi4,5.

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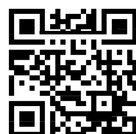
Quick Response Code: 	**Website:** www.pnrjournal.com
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2. Materials And Methods

This research was carried out between October 2021 and May 2022. All the research studies were carried out at the University of Technology and the Iraqi Hereditary Company. (IHC).

2.1. Study Groups

The overall number of people involved in the research was 150 people, with the respective study groups: - GROUP 1.100 patients' blood samples divided into 50 patient blood samples of Iraqi men and women diagnosed with thyroid cancer and 50 patient's blood samples of Iraqi women diagnosed with breast cancer aged between 40 and 50. (18-85 years). The samples were taken in Baghdad/Al-Ameli, Iraq's National Hospital for Cancer Treatmenti and clinical information was obtained from hospital archives and case-sheetirecords

Group 2: 50 samples were collected from seemingly healthy persons of both sexes ranging in age from 21 to 85 years.

2.3. Blood Sampling

2 ml of whole blood was obtained from each patient's venous blood and placed straight into EDTA-containing tubes under sterile circumstances.

Total RNA Extraction from Blood Samples

The RNA was extracted from whole blood samples of patients and healthy controls using the TransZolUp plus RNAi Kit 6.

2.4. cDNA Synthesis from mRNA

Using the Easy Scripti® One Step gDNA Removal and cDNA Synthesis Super Mix Kit (TransGen, Chinai), total RNA was reverse transcribed to complementary DNA (cDNA) 7,8. Based on the assembly specifications, the procedurei was carried out in a response volume of 20 µl. For reverse transcription, the total RNA volume was 20µl. Table 2 shows the thermal cycleri steps for cDNA reverse transcription settings. Table 1 lists the primers and probes utilized in this investigation, as well as their sequences.

Table 1. Thermal cycler steps

	Step 1	Step 2	Step 3
Temperature/ °c	25	42	85
Time	10min	15min	5seconds
	Random Primer (N9) binding	Anchored Oligo(dT)18 binding	Inactivate reverse transcriptase enzyme

Primers used in the study are shown in (Tablei2) for GAPDH and PIM3 genes .

Primer	Sequence (5′→3′ direction)
PIM3 Human qPCR expression Primer	
Forward	AGACCCTGACTTTCTCCTGC
Reverse	TTGAGAAAACCAAGTCCCGC
GAPDH –housekeeping gene	
Forward	TGAGAAGTATGACAACAGCC
Reverse	TCCTTCCACGATACCAAAG

2.5. Real-Time PCR (qRT–PCR)

The qRT-PCR test was performed using the Stratagene Real-time PCR System (Analytik Jena Technologies) with qPCR software [9]. The gene expression levels and fold changes were quantified through the measurement of the threshold cycle (Ct) using the 2xqPCR Master Mix Kits (Trans Gen, China) 10,11. The components are presented in table 3. Every reaction was performed twice and included a non-

Template control, non-amplification control, and non-primer control (NPC) as the negative controls.

2.6. The qPCR Reaction Run

The cycling protocol was set up following the thermali profile described in (Table 4).

2.7. Housekeeping Gene Amplification

The housekeeping gene glyceraldehydei3-phosphate dehydrogenase (GAPDH) was employed as an internal control in calculating the ΔCT value. Table 5 shows the temperature profile used in a qRTPCR process for GAPDH amplification.

2.8. qRT-PCR analysis of PIM3 gene expression

2.8.1. ΔCT

The expression ratio was calculated without a calibrator sample 2-ΔCt, according to the following equation:

ΔCT (test)= CT gene of interest (target, test) – CT internal control

Eventually, the expression ratio was calculated according to the following formula: 2- ΔCt = Normalized expression ratio

Table 3. Reaction Component and volume for RT PCR used in PIM3.genei Component

Component	Volume (μl)
Master mix Syper Green	10
Forward primer	1
Revers primer	1
CDNA	2
Nuclease-free water (N.F.W)	6
Total volume	20

Table 4. Thermal profile of PIM3, gene expression.

		Temperature	Time	cycle
Stage 1	Denaturation	95ºC	30 sec	1
Stage 2	Denaturation	95ºC	5 sec	
	Annealing	58	15 sec	35
	Extension	72ºC	20 sec	
Stage 3	Dissociation	72 ºC	1	1

Table 5. Thermal profile of GAPDH gene expression

Step	Temperature/ ºC	Duration/ Sec
Enzyme activation	95	30
Denature	95	5
Anneal	60	15
Extend	72	20

3. Results And Discussion

The Ct value of GAPDH, the house keeping gene used in the present study, is shown in Table (6). The Ct value for GAPDH in the healthy group was a mean ± SEM (21.53± 0.15). For the thyroid cancer g with a mean ± SEM (21.47 ± 0.06). In the breast cancer group, with a mean ± SEM (20.97± 0.07). A significant difference was found between these groups regarding the mean Ct value of GAPDH (P-value = 0.00).

Barber [12] used qRT-PCR to examine the expression of 1,718 genes in 72 different types of normal human tissue, using GAPDH as a reference gene. When used in clinical research, they discovered that GAPDH is a reliable technique for normalization in qRTPCR.

To improve on this, and even though there was a significant variance in the mean Ct value between groups in the current study, the variability of the total change in GAPDH expression was studied in separate study groups using the 2-Ct value and the ratio of 2-Ct of the different study groups to that of the control group, as shown in Table (7)

The 2-Ct value of the healthy group was 3.146E-07. for the thyroid group, it was 3.372E-07. And the breast cancer group was 3.372E-07. The computed ratio for gene fold expression was 1.00 for the healthy groups and 1.07 for the thyroid cancer group for the breast cancer group. 1.07. These minor variations in gene fold expression between the study groups render the GAPDH gene a functional control gene

Table 6. The housekeeping gene (GAPDH) Ct values in the three studied groups.

Groups	CT GAPDH house-keeping gene N	Mean ± SEM	Median
Thyroid Cancer	50	21.47 ± 0.06	21.36 a
Breast Cancer	50	20.97± 0.07	20.92 b
control	50	21.53± 0.15	21.10 a
Kruskal-Wallis P-valuei	0.00		

highly significant P ≤ 0.001 (***)

Table 7: Comparison of GAPDH Fold expression between study groups.

Group	Means Ct of GAPDH	2-Ct	experimental group/ Control group	The fold of gene expression
Group 2 Thyroid Ca.	21.5	3.372E-07	3.372E-07/3.146E-07	1.07
Group 2 Breast Ca.	21.5	3.372E-07	3.372E-07/3.146E-07	1.07
Group 1 control healthy	21.6	3.146E-07	3.146E-07/3.146E-07	1.00

Each quantification PCR reaction was run in triplicate for each sample. Samples from the healthy and cancer groups were used in each run. Plots of each run (i.e., amplification plots and dissociation curves) were also recorded. Figures 1, 2, 3, and 4 in the appendix present the amplification plots and dissociation curves for GAPDH and PIM3, respectively.

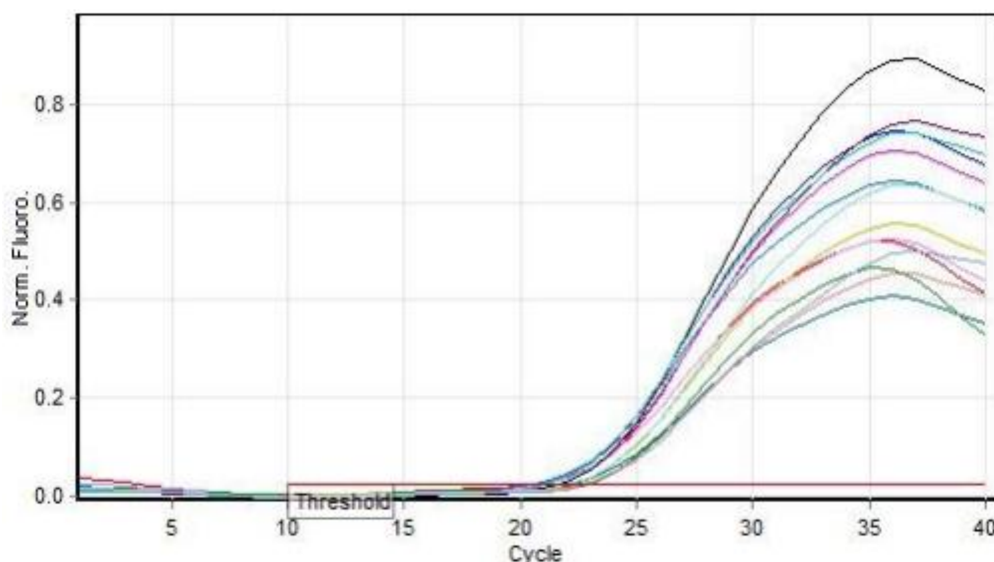

Figure 1. GAPDH amplification plots by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen rotor Gene qPCR system.

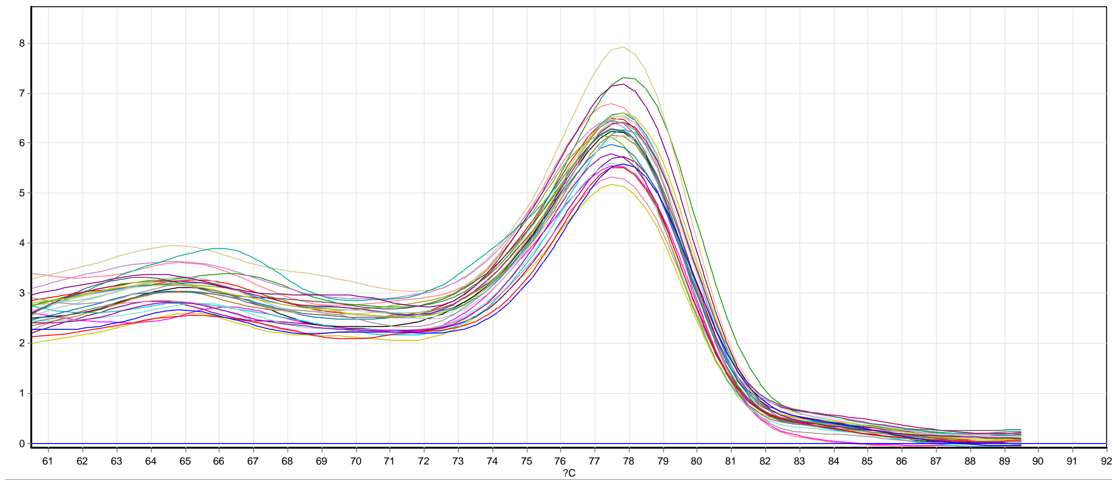

Figure 2. GAPDH dissociation curves by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene qPCR machine.

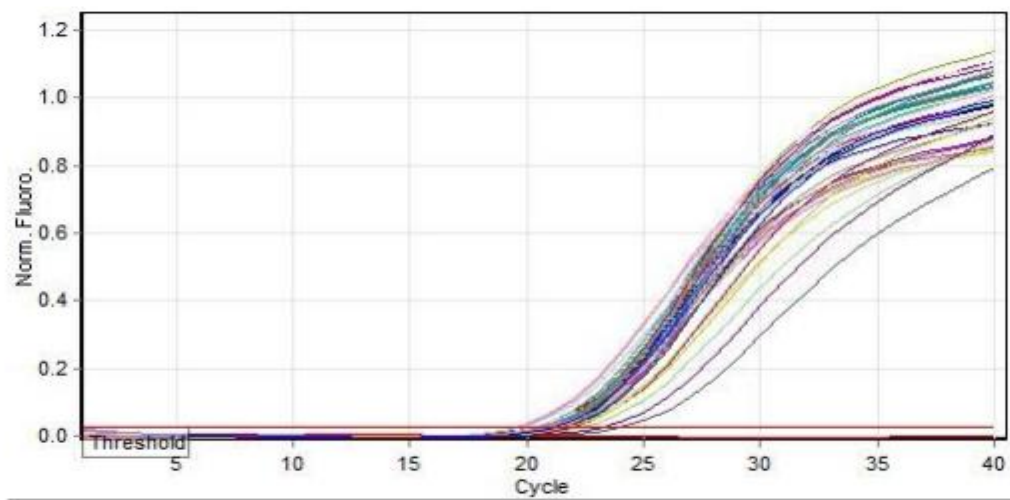

Figure 3. PIM3 amplification plots by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene qPCR machine.

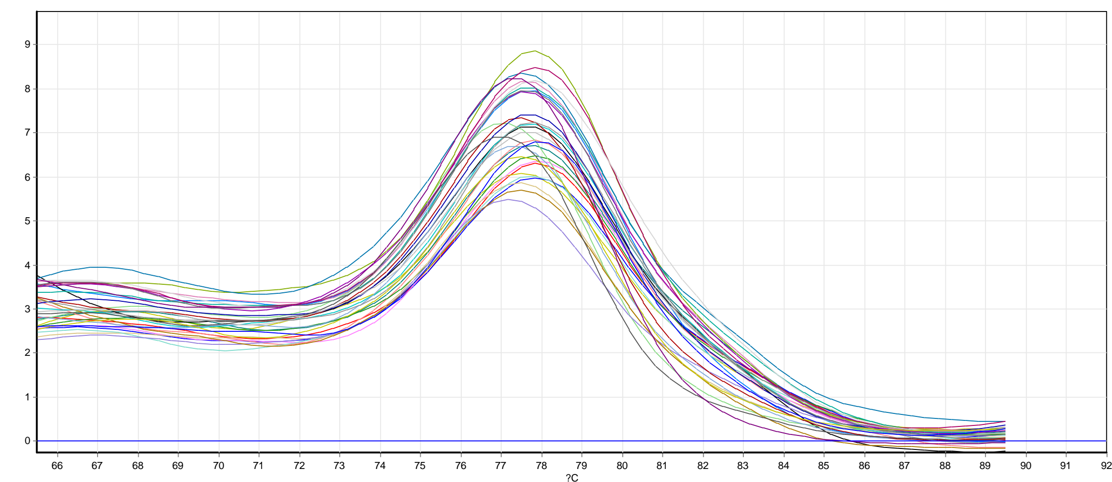

Figure 4. PIM3 dissociation curves by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene qPCR machine.

The current work used a quantitative RT-PCR technique to examine PIM3 mRNA expression and compare it to that of the healthy group, thyroid cancer group, and breast cancer group. The fold change in gene expression was calculated using relative quantification.

This is evaluated by normalizing Ct values and computing the Ct, which is the difference in the mean Ct values of the replica of PIM3 cDNA amplification in each case and that of GAPDH.

To determine the geneiexpression folds concerning the housekeeping genesi, the 2-Ct of each group was compared to the controligroup. The Results are showniin Tablei (8). The genei expression fold in the breast cancer group was once and a half times higher than in the healthy group. The thyroid cancer group was once and a quarter times higher than the healthyi group. These results indicateia significantly increased expression of the PIM3 genei in theseigroups.

Our results show high expression in the PIM3 genei in all types of cancers under study, which agrees with the mechanical work of the PIM3 gene.

The gene expression of PIM3 in thyroid cancer is higher than in apparently healthy individuals. This result agrees with 13,14,15. Overexpression of PIM kinases has been observed in a wide range of human cancers, including hematologic cancers, prostatei cancer, pancreatic cancer, gastric cancer, head and neck cancer, colon cancer, and liver cancer. PIM kinase deregulation has been strongly linked to tumor genesis via collaboration with MYC, mediating survival signaling and regulating cell-cycle progression.

PIM3, a proto-oncogene PIM3 expression, contributes to tumor genesis and progression by inhibiting apoptosis, sustaining cell survival, and regulating cell cycle progression.16,17 Recently, multiple publications indicated that Pim-3 might also be involved in cell invasion. PIM3, for example, is involved in endothelial cell spreading and migration, as well as tumor necrosis factor (TNF)-induced angiogenesisi.PIM3 overexpression accelerates ovarian cancer cell proliferation and migration 18,19 .

Table 8: Fold of PIM3 expression Depending on 2-ΔCtMethod

Groups	Means Ct of PIM3	Means Ct of GAPDH	ΔCt (Means Ct of PIM3 - Means Ct of GAPDH)	2-ΔCt	experimental group/ Control group	The fold of gene expression
Group 2 Thyroid Ca.	21.560	21.500	0.060	0.959	0.959/0.779	1.231
Group 2 Breast Ca.	21.140	21.500	-0.360	1.284	1.284/0.779	1.647
Group 1 control healthy	21.960	21.600	0.360	0.779	0.779/0.779	1.000

Ethics

All operations that involved human participants in this study were carried out in compliance with the ethical norms of the University of Technology in Baghdad, Iraq.

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Compliance with Ethical Standards statements

Ethical approval :Science Department, Biotechnology section, University of Technology, Baghdad, Iraq, and Iraqi Hereditary Company (IHC), Baghdad, Iraq certifies the ethical approval, Funding details (In case of Funding) :I am responsible for paying the financing, Conflict of interest : There is no conflict of interest, Informed Consent: Science Department, Biotechnology section, University of Technology, Baghdad, Iraq, and Iraq Hereditary Company (IHC), Baghdad, Iraq Agreed

References

Samar Abdul Raheem Shaghati .[2015]. Detection of Parvovirus B19 by Immunohistochemistry in Patients with Thyroid Tumors A Thesis Submitted to the Council of College of Science AL-Mustansiriyah University in Partial Fulfillment of The Requirements For The Degree of Doctorate of Philosophy in Biology/ Zoology.

Supreetha. A*1, Ashwini. M2, Yashaswini.[2021] IJSDR | Volume 6 Issue 1.A Review: Breast Cancer, a Global Threat to Women.

Naofumi Mukaida,1,3 Ying-Ying Wang1 and Ying-Yi Li .(2011). Roles of Pim-3, a novel survival kinase, in tumorigenesis. Japanese Cancer Association. DOI: 10.1111/j.1349-7006.2011.01966.x

Rong-Xin Zhang1,2,3, Zhong-guo Zhou1,3,5, Shi-xun Lu1,2,4, Zhen-hai Lu1,2,3, De-sen Wan1,2,3, Zhi-Zhong Pan1,2,3, Xiao-jun Wu1,2,3 & Gong Chen1,2,3 |.(2017) Pim-3 as a potential predictor of

chemoradiotherapy resistance in locally advanced rectal cancer patients. Scientific Reports. 7: 16043 |

Milella, M., Falcone, I., Conciatori, F., Cesta Incani, U., Del Curatolo, A., Inzerilli, N., Nuzzo, C. M., Vaccaro, V., Vari, S., Cognetti, F., & Ciuffreda, L. (2015). PTEN: Multiple Functions in Human Malignant Tumors. Frontiers in oncology, 5, 24.

Chi, S., Xue, J., Li, F., Zhu, C., Yu, Y., Li, H., Wang, X., Zhang, Y., Yang, J., Zhou, S., Yang, L., Ji, C., & Liu, X. (2016). Correlation of Serum Soluble Interleukin-7 Receptor and Anti-C1q Antibody in Patients with Systemic Lupus Erythematosus. Autoimmune diseases, 2016, 8252605.

Li, P., Du, J., Goodier, J. L., Hou, J., Kang, J., Kazazian, H. H., Jr, Zhao, K., & Yu, X. F. (2017). Aicardi-Goutières syndrome protein TREX1 suppresses L1 and maintains genome integrity through exonuclease-independent ORF1p depletion. Nucleic acids research, 45(8), 4619–4631

Li, S., Tian, Y., Wu, K., Ye, Y., Yu, J., Zhang, J., Liu, Q., Hu, M., Li, H., Tong, Y., Harberd, N. P., & Fu, X. (2018). I am modulating plant growth-metabolism coordination for sustainable agriculture. Nature, 560(7720), 595–600.

Abed SA, Al-Khafaji HM. 2021Design Of Primer And Probe To Detect SNP Rs 1892901 In Fosl-1Gene In Different Types Of Cancer In Iraqi Population. Syst Rev Pharm.;12(1):296-300.

Wei Y, Hu W, Wang Q, Zeng H, Li X, Yan Y, et al. Identification, transcriptional and functional analysis of heat-shock protein 90s in banana (M USA acuminata L.)

Yang T-b, Liu J, Yuan L-y, Zhang Y, Li D-q, Agnarsson I, et al. 2017

Molecular identification of spiders preying on Empoasca vitis in a tea plantation. Sci Rep.;7(1):1-10

Barber RD, Harmer DW, Coleman RA, Clark BJ. 2005 GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics.;21(3):389-95.

Arrouchi, H., Lakhlili, W., & Ibrahimi, A. (2019). A review on PIM kinases in tumors. Bioinformation, 15(1), 40–45

Nawijn, M. C., Alendar, A., & Berns, A. (2011). For better or for worse: the role of Pim oncogenes in tumorigenesis. Nature reviews. Cancer, 11(1), 23–34.

Block, K. M., Hanke, N. T., Maine, E. A., & Baker, A. F. (2012). IL-6 stimulates STAT3 and Pim-1 kinase in pancreatic cancer cell lines. Pancreas, 41(5), 773–781.

Zheng, H. C., Tsuneyama, K., Takahashi, H., Miwa, S., Sugiyama, T., Popivanova, B. K., Fujii, C., Nomoto, K., Mukaida, N., & Takano, Y. (2008). Aberrant Pim-3 expression is involved in gastric adenoma-adenocarcinoma sequence and cancer progression. Journal of cancer research and clinical oncology, 134(4)

Mukaida, N., Wang, Y. Y., & Li, Y. Y. (2011). Roles of Pim-3, a novel survival kinase, in tumorigenesis. Cancer science, 102(8), 1437–1442.

Li, Y. Y., Popivanova, B. K., Nagai, Y., Ishikura, H., Fujii, C., & Mukaida, N. (2006). Pim-3, a proto-oncogene with serine/threonine kinase activity, is aberrantly expressed in human pancreatic cancer and phosphorylates to block bad-mediated apoptosis in human pancreatic cancer cell lines. Cancer Research, 66(13), 6741–6747.

Liu, J., Qu, X., Shao, L., Hu, Y., Yu, X., Lan, P., ... & Zhang, C. (2018). Pim-3 enhances melanoma cell migration and invasion by promoting STAT3 phosphorylation. Cancer biology & therapy, 19(3), 160-168.