Exploring the effect of EX-527 as a selective SIRT1 inhibitor against MCF-7 breast cell line by modulating POLD1 activity

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Abstract:

Breast cancer is the most prevalent tumor diagnosed in women and the second cause of death around the world. Therefore, improving the prognosis and effectiveness of treatment for breast cancer requires more studies to understand the underlying molecular mechanisms of its pathogenesis. Sirtuin 1 is a NAD+-dependent class III histone deacetylase enzyme that is involved in metastasis, apoptosis, tumor growth, and other carcinogenesis pathways. The precise function of Sirtuin1 in relation to breast cancer is yet unknown, and its impact is still debatable. The purpose of this study was to examine the effect of sirtuin1 downregulation on the expression level of p53, the tumor suppressor protein, and on the DNA polymerase delta1 (POLD1), the gene encoding the catalytic and proofreading subunit p125 of DNA polymerase δ, in order to determine the significant function of sirtuin1 in breast cancer. The expression level of proteins was determined by the western blot method. The results of the current study revealed that downregulation of SIRT1by the EX-527 compound in MCF-7 breast cancer cell lines resulted in increased expression of p53 and decreased expression of POLD1. This study suggested that sirtuin1 co-operate with POLD1 in the molecular mechanism of breast cancer; therefore, SIRT1 could be a promising potential target for breast cancer treatment in the future.

Keywords: Breast Cancer; MCF-7; POLD1; p53; SIRT1 inhibitor.

استكشاف تأثير EX-527 كمثبط انتقائي لـ SIRT1 ضد خط خلايا الثدي MCF-7عن طريق تعديل نشاط ال POLD1 .

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خلاصة

الخلاصة: سرطان الثدي هو الورم الأكثر انتشارا بين النساء وثاني أكبر سبب للوفاة في جميع انحاء العالم. لذلك، فان تحسين تشخيص وفعالية علاج سرطان الثدي يتطلب المزيد من الدراسات لفهم الاليات الجزيئية الكامنة وراء التسبب في المرض. السيرتوين 1 هو عبارة عن انزيم هيستون ديسيتيلاز من الدرجة الثالثة المعتمد على النيكوتيناميد داي نيوكليوتايد والذي يشارك في انتشار الأورام الخبيثة وموت الخلايا المبرمج وتطور الورم ومسارات التسرطن الأخرى. الوظيفة الدقيقة للسيرتوين 1 فيما يتعلق بسرطان الثدي غير معروفة حتى الان ولا يزال تأثيرها محل جدل. كان الغرض من هذا البحث هو دراسة تأثير تقليل تنظيم السيرتوين 1 على مستوى تعبير بروتين P53 وعلى بوليميريز الحمض النووي دلتا 1 POLD1 وهو الجين الذي يشفر الوحدة الفرعية الحفزية p125 وتصحيح التجارب المطبعية من الحمض النووي δ من اجل تحديد الوظيفة الهامة للسيرتوين 1 في سرطان الثدي. تم تحديد مستوى التعبير عن البروتينات بواسطة اختبار الويسترن بلوت. كشفت نتائج الدراسة الحالية ان تقليل تنظيم السيرتوين 1 بواسطة مركب الEX-527 في خط خلايا سرطان الثدي ال MCF-7أدى الى زيادة التعبير عن

p53 وانخفاض التعبير عن. POLD1 اقترحت هذه الدراسة أن sirtuin1 يتعاون مع POLD1 في الآلية الجزيئية لسرطان الثدي. لذلك، يمكن أن يكون SIRT1 هدفا محتملا واعدا لعلاج سرطان الثدي في المستقبل.

الكلمات المفتاحية: سرطان الثدي;MCF-7 ; POLD1 ; p53;مثبط سيرتوين1.

1. Introduction

In terms of female cancer incidence and mortality, breast cancer has taken the highest two ranks. The most current US cancer statistics analysis from 2018 shows that breast cancer accounts for 30% of all female cancer diagnoses, significantly more than any other cancer type [1]. Breast cancer has been diagnosed in several low-income countries, and the mortality rate from the disease is higher in these regions compared to more advanced nations [2]. In highly developed nations, such as the United Kingdom, the Netherlands, the USA, increasing usage of mammography screening has been one of the primary causes for the high incidence of breast cancer since the 1980s [3]. However, despite having relatively low incidence rates, certain Asian and African nations are experiencing rapidly increasing rates in breast cancer due to limited access to early detection and diagnosis, as well as changes in lifestyle and socio-economic growth [4,5]. Breast cancer occurrence can be influenced by genetics, as well as by environmental factors, radiation exposure, alcohol consumption, and dietary habits [6]. Human breast cancers are classified to several subtypes according to gene expression, such as progesterone receptor (PR), human epidermal growth 2 receptor (HER2), and estrogen receptor (ER). The most commonly utilized type of human breast cancer cell line is MCF-7, which is characterized by the expression of the progesterone receptor (PR) and the estrogen receptor (ER) [7-9]. Sirtuin 1 (SIRT1) is a class III histone deacetylase enzyme that is depending on nicotinamide adenine dinucleotide (NAD+) and it is the mammalian analogue of the yeast enzyme silent information regulator 2 (Sir2) [10]. Furthermore, SIRT1 is required for the development of cancer and its resistance to therapy because it deacetylates histones and non- histone proteins, which in turn controls the activity and function of several transcription factors and their transcriptional cofactors, such as c-Myc, p53, NF-kB, the FOXO family, and others, to control metabolic processes, DNA repair, cell growth, apoptosis, and the cell cycle. [11,12]. As a result, it is critical to understand the molecular roots of SIRT1 and its role in breast cancer pathogenesis. Numerous investigations have revealed that SIRT1 affects cancer cells bilaterally and may function as either a tumor suppressor or a tumor promoter [13,14]. Recent study revealed that SIRT1 is increased in breast cancer cells and is essential for the development and spread of cancer cells [15,16]. Furthermore, it has been documented that elevating NAD+ production in cancerous breast cells results in increased expression of SIRT1, which motivates the development and advancement of breast cancer by controlling the activities and functions of various oncogenic and transcription factors, particularly the tumor suppressor protein p53 tumors [17,18].

DNA polymerase delta 1 (POLD1) functions as a gene that codes for p125, a catalytic and processing subunit of DNA polymerase δ which is essential for DNA replication and genome integrity [19]. POLD1 and proliferating cell nuclear antigen (PCNA) cooperate in order to regulate the replication of DNA, cell division, and cell cycle [20]. As a direct regulatory target of SIRT1, p53, which controls the transcriptional activity of POLD1 [21]. According to a recent study, POLD1 participates in the development and propagation of breast cancer [22]. Further research is necessary to fully understand the

expression and function of POLD1 in breast cancer.

Thus, this study aimed to determine if SIRT1 may regulate POLD1 activity by influencing p53 function, which ultimately affects the onset and progression of breast cancer, so this provides a new insight to the molecular mechanism of SIRT1 in hormone-regulated breast tumor. This investigation employed the EX-527 compound as a potent and selective SIRT1 inhibitor.

2. Materials and methods

1.Chemicals and reagents

Two compounds were used to inhibit SIRT1, the EX-527 compound which was purchased from (Bidepharm, China), and Nicotinamide which was purchased from (Macklin, Shanghai, China) and used as reference SIRT1 inhibitor. Anti-SIRT1 (1:1000), anti-p53 (1:1000) and anti-GAPDH (1:5000) antibodies were purchased from (Elabscience, USA). Anti-POLD1 (1:1000) was purchased from (ELK Biotechnology, USA). Secondary antibody IgG-HRP goat anti-Rabbit was purchased from (Elabscience, USA).

2.Cell lines

MCF-7 cells (human breast cancer cell lines) were used as a model for breast cancer in this project. MCF-7 cell lines were supplied by ATCC. This study was performed in the tissue culture research laboratory of Mustansiriyah University/College of Pharmacy.

3. Storage and Thawing of Cell Lines

In a tank of liquid nitrogen, cells were maintained at -80 °C for a long term. Cells were thawed immediately within 1–2 minutes in water bath at 37 °C. Then, cells were resuspended with 10 ml of fresh media and collected by centrifugation at approximately 1000 rpm for 5 min. After centrifugation, the cell pellets were gently resuspended in complete media and transferred to a 75 cm^3 tissue culture flask that contained 15 ml of complete growth medium [23].

4. Cell Maintenance

Cells were grown and allowed to propagate in a complete Dulbecco's modified Eagle's medium (DMEM) with high glucose (Capricorn Scientific, GmbH), supplemented with 10% fetal bovine serum (FBS) (Capricorn Scientific, GmbH) and 1% antibiotic/antimycotic (Capricorn Scientific, GmbH). Cells were cultured at a density 2 × 10^3 cells/ml in 75 cm^3 culture flasks and kept at 37 °C in an incubator (Hermle, Germany) with 5% CO_2. When confluency was 80%, cells were subcultured by washing them with 3 ml of phosphate buffered saline (PBS, Capricorn Scientific, GmbH) to remove any residual medium. Cells were allowed to detach from the flask bottom by incubating them for 3 to 5 minutes at 37 °C with 3 ml of 0.05% trypsin (Capricorn Scientific, GmbH). After detachment, 3 ml of a new complete growth medium was added to inactivate trypsin, then the cell suspension was placed into a sterilized tube. The cells were centrifuged at 1000 rpm for 3-5 minutes. The supernatant in the tube was discarded, and the remaining cell pellets were resuspended in 1 ml of complete growth medium. In a T-75 flask containing 15 ml of complete growth medium, 10 µl of cell suspension was cultured [24,25].

5. Cell counting

After trypsinization, MCF-7 cells were counted by mixing 10 µl of 0.4% trypan blue (Elabscience®, USA) with 10 µl of cell suspension in a 1.5 ml Eppendorf tube (1:1 dilution), then maintained at room temperature for approximately 3 minutes. Next, 10 µl of the mixture was inserted into one of the dual Bio-Rad counting slide chambers. The slide was placed into the TC20 ™ automated cell counter (Bio-Rad), which

automatically counted viable cells. The information obtained from the automated cell counter was used to re-seed a flask and culture plates at the necessary cell density [26].

6. SIRT1 Inhibition

MCF-7 cells were plated in a 6-well plate at a density of 1×10^5 cells/well and incubated at 37 °C with 5% CO_2. After 24 h, cells were treated with 25.3 µM and 98.32 µM of EX-527 and Nicotinamide compounds, respectively, to induce inhibition of SIRT1, then incubated for 72 hours for protein extraction.

7. Protein extraction

The RIPA lysis buffer was applied to extract total proteins from cells based on the manufacturing guidelines of the western blot detection kit (Elabscience®, USA). Cells were sonicated for 1 min at 2 s' sonication for full lysis and to reduce sample viscosity. Cells were placed in a cold centrifuge for about 10 minutes at 12,000 rpm, and the supernatant of every sample was obtained in order to measure the protein concentration. An assay kit of bicinchoninic acid protein (Elabscience®, USA) was used to measure the protein content in each sample, in compliance with the manufacturer's guidelines.

8. Western blot assay

Cell lysate was mixed with 5× SDS loading buffer (Elabscience®, USA) and allowed to boil for about 5-10 minutes on a dry heating block at 95 °C, followed by centrifugation for 2 minutes at 12000 rpm. The same amounts of protein were then separated electrophoretically after being transferred onto SDS-PAGE gels containing 10% SDS. Then, the isolated proteins were transported to a polyvinylidene difluoride membrane (PVDF, Millipore, Elabscience®, USA). Membranes were blocked with 5% (w/v) powder of non-fat skim milk (Elabscience®, USA) that was dissolved in TBS-T (Elabscience®, USA) and incubated at room temperature for 90 min. After that, primary antibodies (anti-SIRT1 and anti-p53, Elabscience®, USA) and anti-POLD1 (ELK Biotechnology, USA) were added and incubated with the membranes. Primary antibodies were diluted according to manufacturing instructions at a ratio of 1:1000 in 5% non-fat skim milk powder that was dissolved in TBS-T. The membranes then incubated overnight at 4°C on a rocker. In the next day, the membranes were washed 3 times (15 minutes each time) with 1X TBS-T buffer. Then, the secondary antibody (anti-Rabbit IgG-HRP, Elabscience®, USA) was added and incubated with the membranes for 1 hour at room temperature on a rocker. The secondary antibody was diluted at a ratio of 1:5000 in 2% nonfat dry skim milk dissolved in 1X TBS-T buffer. The membranes were washed again with 1X TBS-T buffer for 15 minutes with gentle shaking and repeated three times to remove any residual antibody. To observe the bands, chemiluminescence (Elabscience®, USA) was used. The Western blot data were quantified using band densitometry analysis with the ChemiDoc TM XRS Plus imaging system (Bio-Rad Laboratories, France) [27, 28]. As an internal loading control, GAPDH was used.

3.Statistical analysis

GraphPad Prism 8.1 software was used for statistical analysis of all data. The significant differences between groups were measured by a t-test. Asterisks represent the significant difference compared with untreated cells, $p<0.05$.

4. Results

1. SIRT1 protein expression in MCF-7 cell lines

Western blot analysis of SIRT1 expression in MCF-7 cell lines. Following cell treatment with 25.3 µM EX-527 and 98.32 µM

Nicotinamide compounds, the amount of SIRT1 expression in these groups relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) significantly decreased ($p<0.05$), as compared with the control group **(Figure 1)**.

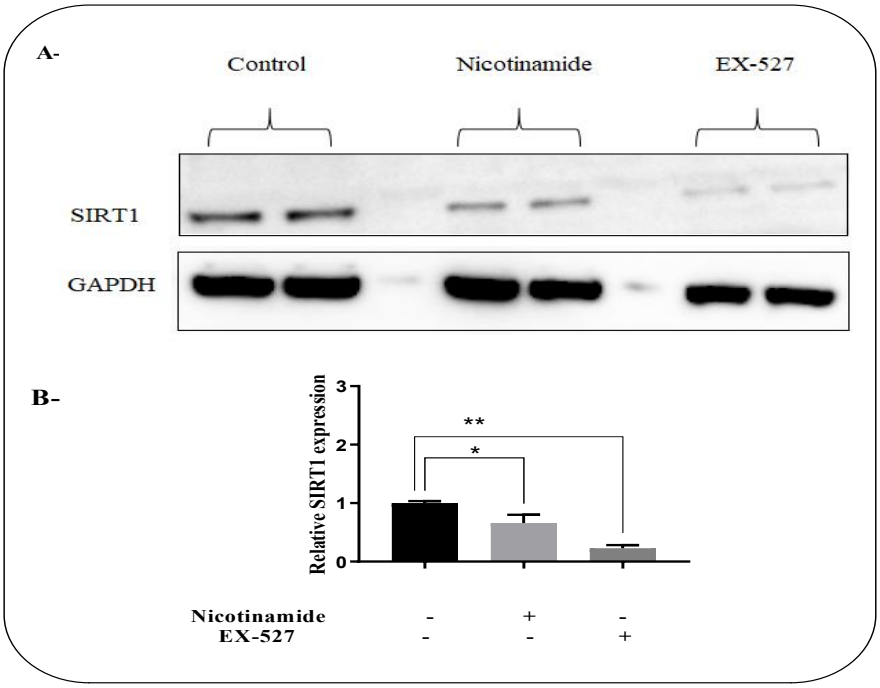

Figure 1: The effect of EX-527 on the expression of SIRT1 in MCF-7 cell lines. (A) Cells were incubated with EX-527 and Nicotinamide compounds for 48 h, and cell lysate was analyzed by western blot. (B) EX-527 induced a decrease in the expression level of SIRT1 in MCF-7 cells, and protein bands were quantified by ImageJ software; GAPDH was used as a loading protein control. The significant differences between groups were measured by a t-test.

2. P53 protein expression in MCF-7 cell lines

Figure 2 illustrates the elevated p53 expression in MCF-7 cell lines after being treated with a 25.3 µM EX-527 compound in comparison to the control group ($p< 0.05$). Additionally, the expression of p53 was raised in MCF-7 cell lines after being treated with a 98.32 µM Nicotinamide compound, as shown in **Figure 2 A, B,** $p< 0.05$, suggesting that p53 levels reacted effectively to the downregulation of the expression and function of SIRT1.

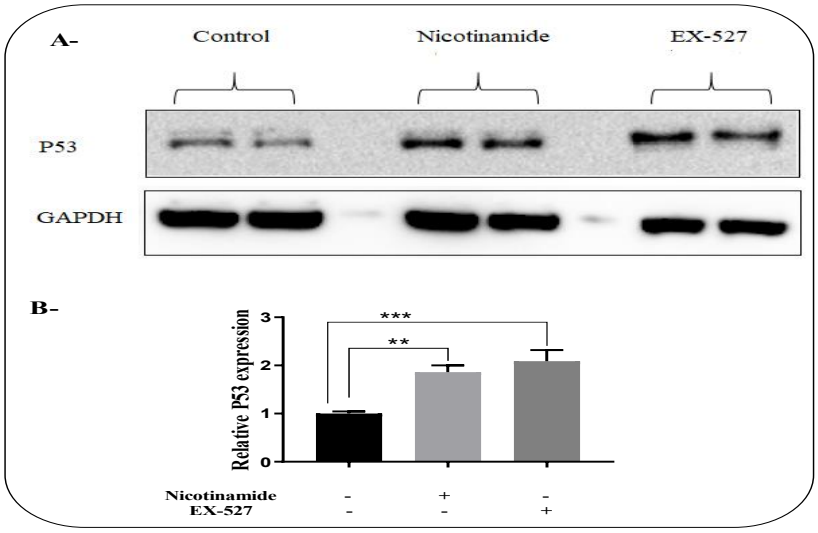

Figure 2: The effect of EX-527 compound on p53 expression in MCF-7 cell lines. (A) Cells were incubated with EX-527 and Nicotinamide for 48 h, and cell lysate was analyzed by western blot. (B) EX-527 increased p53 expression in MCF-7 cells, and protein bands were measured by ImageJ software; GAPDH was used as a loading protein control. The significant differences between groups were measured by a t-test.

3. POLD1 expression in MCF-7 cell lines

As shown in **Figure 3,** POLD1 expression was decreased in MCF-7 cell lines treated with 25.30 µM EX-527 compound compared to control group ($p < 0.05$). While MCF-7 cell lines treated with 98.32 µM Nicotinamide did not affect the expression of POLD1, suggesting that Nicotinamide has another pathway to supress the MCF-7 cell line when inhibited SIRT1 protein.

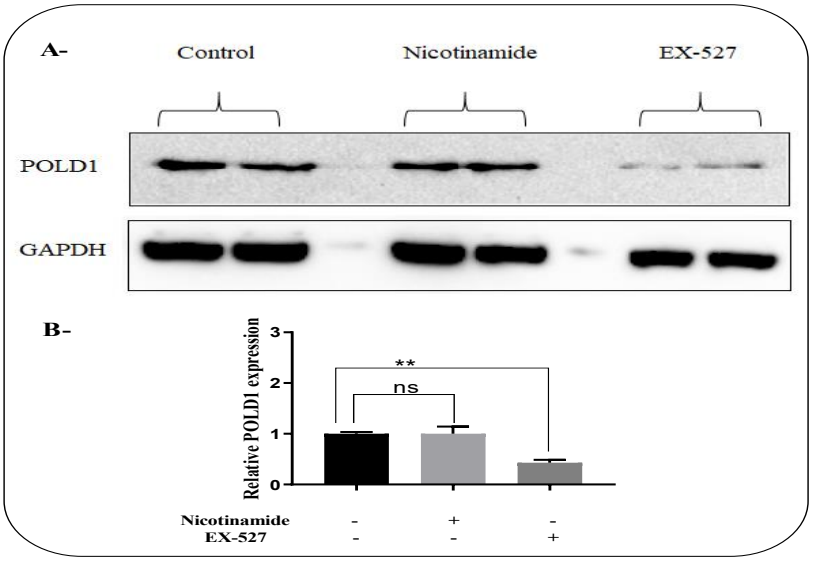

Figure 3: The effect of EX-527 compound on POLD1 expression in MCF-7 cell lines. (A) Cells were incubated with EX-527 for 48 h, and cell lysate was analyzed by western blot. The significant differences between groups were measured by a t-test. (B) EX-527 induced decreasing in the expression level of POLD1 in MCF-7 cells, and protein bands were quantified by ImageJ software; GAPDH was used as a loading protein control. Ns: Non-significant.

5. Discussion

Breast cancer is one of the most frequently diagnosed cancers in females around the world. In addition, a hematogenous spread can occur quickly in the early stages, predisposing the women's health to a serious risk. Many therapies are available currently for breast cancer, including surgery, chemotherapy, radiation, and targeted biotherapy, which can eradicate cancer cells but can also result in undesirable effects [29]. Sirtuin1 has a dual function in the pathophysiology of breast cancer by activating many proteins, including transcription factors, functional proteins, and multiple tumor suppressor genes. Therefore, SIRT1 can act as a tumor inhibitor or activator in breast cancer, depending on whether its target is downstream or upstream [30-32]. The SIRT1 mainly deacetylates the enzyme activity of tumor-suppressing proteins, such as p53, p21, and forkhead box (FOXO3). Recent study revealed that sirtinol, the traditional inhibitor of sirtuins, inhibited SIRT1 in breast cancer cells and resulted in activating p53 and enhancing p21 expression. This suggests that SIRT1 plays an oncogenic function in breast tumors [33].

The western blot results in this investigation demonstrated that SIRT1 protein was overexpressed in MCF-7 cells **(Figure 1)**. On the other hand, SIRT1 expression was significantly downregulated in MCF-7 cells treated with 25.3 µM of EX-527 compound compared to the downregulation caused by Nicotinamide. SIRT1 primarily controls the function of the tumor suppressor protein p53. SIRT1 could serve as a cancer promoter because it deacetylates p53, which prevents p53 from acting as a cancer suppressor [34]. Alterations in the process of transcription and post-translation of p53, especially acetylation, cause the transactivation of a number of genes, encourage cell death, and regulate the cell cycle [35]. Many cancer cells are influenced by the SIRT1/p53 signaling pathway. Studies have shown that downregulating SIRT1 by sirtinol and siRNA causes an elevation in p53 acetylation level and cellular death [36].

This study showed that downregulation of SIRT1 by the EX-527 compound in MCF-7 cells could significantly upregulate p53, much higher than Nicotinamide **(Figure 2)**. Additionally, by targeting multiple genes, the transcription factor p53 assists in inhibiting the replication of DNA until the process of DNA repair is performed [37]. It is crucial to determine the genes that p53 targets and that are involved in regulating the DNA replication process since DNA synthesis is one of the fundamental mechanisms that regulates cellular functions. The POLD1, which codes for the p125 protein, a catalytic and processing subunit of DNA polymerase delta 1, is one of the genes that is specifically targeted by p53. It has been established that p125 is crucial for enabling cancer cells to acquire resistance to DNA damage, which promotes the cell cycle and promote cell proliferation [38]. Numerous studies have suggested a relationship between poor prognosis and enhanced tumor invasion and proliferation in hepatic cell carcinoma and breast cancer events when POLD1 is overexpressed [39,40].

The findings of the current study showed that there is an appositive relationship between SIRT1 and POLD1 expression, that is, increased expression of SIRT1 in MCF-7 cells is associated with increased expression of POLD1. Moreover, downregulation of SIRT1 by the EX-527 compound in MCF-7 cells resulted in decreased expression of POLD1 **(Figure 3)**.

6. Conclusion

In conclusion, the results of this study demonstrated that decreased expression of SIRT1 by the EX-527 compound in MCF-7 cells led to an increased expression level of p53 and a decreased expression level of

POLD1, which could be linked to the inhibitory effect of p53 on POLD1.Therefore, targeting SIRT1 could have a potential value in treating breast cancer.

7. Conflict of interests

No conflict of interest was declared by the authors

8. Funding source

The authors did not receive any source of fund.

9. Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

10. Acknowledgments

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6. References

1- Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International journal of cancer. 2019 Apr 15;144(8):1941-53. doi: 10.1002/ijc.31937.

2- DeSantis CE, Ma J, Goding Sauer A, Newman LA, Jemal A. Breast cancer statistics, 2017, racial disparity in mortality by state. CA Cancer J Clin. 2017 Nov;67(6):439-448. doi: 10.3322/caac.21412.

3- Srivastava S, Koay EJ, Borowsky AD, De Marzo AM, Ghosh S, Wagner PD, Kramer BS. Cancer overdiagnosis: a biological challenge and clinical dilemma. Nat Rev Cancer. 2019 Jun;19(6):349-358. doi: 10.1038/s41568-019-0142-8.

4- Joko-Fru WY, Jedy-Agba E, Korir A, Ogunbiyi O, Dzamalala CP, Chokunonga E, Wabinga H, Manraj S, Finesse A, Somdyala N, Liu B, McGale P, Jemal A, Bray F, Parkin DM. The evolving epidemic of breast cancer in sub-Saharan Africa: Results from the African Cancer Registry Network. Int J Cancer. 2020 Oct 15;147(8):2131-2141. doi: 10.1002/ijc.33014.

5- Lei S, Zheng R, Zhang S, Chen R, Wang S, Sun K, Zeng H, Wei W, He J. Breast cancer incidence and mortality in women in China: temporal trends and projections to 2030. Cancer Biol Med. 2021 May 18;18(3):900–9. doi: 10.20892/j.issn.2095-3941.2020.0523.

6- Tan GZ, Li M, Tan X, Shi ML, Mou K. MiR-491 suppresses migration and invasion via directly targeting TPX2 in breast cancer. European Review for Medical & Pharmacological Sciences. 2019 Nov 15;23(22). doi: 10.26355/eurrev_201911_19566.

7- Theodossiou TA, Ali M, Grigalavicius M, Grallert B, Dillard P, Schink KO, et al. Simultaneous defeat of MCF7 and MDA-MB-231 resistances by a hypericin PDT-tamoxifen hybrid therapy. NPJ Breast Cancer. 2019 Apr 10;5:13. doi: 10.1038/s41523-019-0108-8.

8- Grubczak K, Kretowska-Grunwald A, Groth D, Poplawska I, Eljaszewicz A, Bolkun L, et al. Differential Response of MDA-MB-231 and MCF-7 Breast Cancer Cells to In Vitro Inhibition with CTLA-4 and PD-1 through Cancer-Immune Cells Modified Interactions. Cells. 2021 Aug 10;10(8):2044. doi: 10.3390/cells10082044.

9- Huang KS, Wang YT, Byadgi O, Huang TY, Tai MH, Shaw JF, et al. Screening of Specific and Common Pathways in Breast Cancer Cell Lines MCF-7 and MDA-MB-

231 Treated with Chlorophyllides Composites. Molecules. 2022 Jun 20;27(12):3950. doi: 10.3390/molecules27123950.

10- Sauve AA, Wolberger C, Schramm VL, Boeke JD. The biochemistry of sirtuins. Annu Rev Biochem. 2006;75:435-65. doi: 10.1146/annurev.biochem.74.082803.133500.

11- Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. Biochem J. 2007 May 15;404(1):1-13. doi: 10.1042/BJ20070140.

12- Jin X, Wei Y, Liu Y, Chen Y, Zhao B, Huang J, Yu H, Li C. High expression of SIRT1 associates with the doxorubicin resistance of breast cancer through the activation of Akt. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2020 Jan 1;20(1):94-102. doi: 10.2174/1871520619666191028100405.

13- Liu T, Liu PY, Marshall GM. The critical role of the class III histone deacetylase SIRT1 in cancer. Cancer Res. 2009 Mar 1;69(5):1702-5. doi: 10.1158/0008-5472.CAN-08-3365.

14- Joudah MS, Al-Sudani BT, Arif IS. SIRT1 activators as novel therapy for cancer. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2019 Aug 1;19(3):28-41. https://doi.org/10.32947/ajps.v19i3.572

15- Jin X, Wei Y, Xu F, Zhao M, Dai K, Shen R, Yang S, Zhang N. SIRT1 promotes formation of breast cancer through modulating Akt activity. Journal of Cancer. 2018;9(11):2012. doi: 10.7150/jca.24275

16- Y.R. Chung, H. Kim, S.Y. Park, I.A. Park, J.J. Jang, J.Y. Choe, Y.Y. Jung, S.A. Im, H.G. Moon, K.H. Lee, K.J. Suh, T.Y. Kim, D.Y. Noh, W. Han, H.S. Ryu, Distinctive role of SIRT1 expression on tumor invasion and metastasis in breast cancer by molecular subtype, Hum. Pathol. 46 (2015) 1027–1035. https://doi:10.1016/j.humpath.2015.03.015.185.

17- Behrouzfar K, Alaee M, Nourbakhsh M, Gholinejad Z, Golestani A. Extracellular NAMPT/visfatin causes p53 deacetylation via NAD production and SIRT1 activation in breast cancer cells. Cell biochemistry and function. 2017 Aug;35(6):327-33. doi: 10.1002/cbf.3279.

18- Solomon JM, Pasupuleti R, Xu L, McDonagh T, Curtis R, DiStefano PS, et al. Inhibition of SIRT1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. Mol Cell Biol. 2006 Jan;26(1):28-38. doi: 10.1128/MCB.26.1.28-38.2006.

19- Antoniali G, Marcuzzi F, Casarano E, Tell G. Cadmium treatment suppresses DNA polymerase δ catalytic subunit gene expression by acting on the p53 and Sp1 regulatory axis. DNA Repair.2015 Nov;35:90-105. doi: 10.1016/j.dnarep.2015.08.007.

20- H. Xu, P. Zhang, L. Liu, M.Y. Lee, A novel PCNA-binding motif identified by the panning of a random peptide display library, Biochemistry 40 (2001) 12e20. doi: 10.1021/bi010103+.

21- Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, et al. Negative control of p53 by Sir2alpha promotes cell survival under stress. Cell. 2001 Oct 19;107(2):137-48. doi: 10.1016/s0092-8674(01)00524-4.

22- L. Zhang, W.P. Yang, X. Zhu, C.Y. Wei, p53 inhibits the expression of p125 and the methylation of POLD1 gene promoter by downregulating the Sp1-induced DNMT1 activities in breast cancer, OncoTargets Ther. 9 (2016) 1351e1360. doi: 10.2147/OTT.S98713.

23- Agarwal V, Singh M. Resuscitation of Frozen Cell Lines. InAnimal Cell Culture: Principles and Practice 2023 Feb

1 (pp. 163-171). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-031-19485-6_11

24- Zainab A. Jabbar, Monther F. Mahdi and Basma M.Abd Razik (2023) "Synthesis, Characterization, ADME Study and Anti-proliferative evaluation against MCF-7 breast cancer cell line of new analog of a 4-aminophenyl quinazolinone derivative", Al Mustansiriyah Journal of Pharmaceutical Sciences, 23(4), pp. 411–428. doi: 10.32947/ajps.v23i4.1096.

25- Thompson ML, Kunkel EJ, Ehrhardt RO. Cryopreservation and thawing of mammalian cells. eLS. 2014. doi: 10.1002/0471142735.ima03gs99.

26- Kerschbaum HH, Tasa BA, Schürz M, Oberascher K, Bresgen N. Trypan blue—Adapting a dye used for labelling dead cells to visualize pinocytosis in viable cells. Cell. Physiol. Biochem. 2021 Jun 1;55:171-84. doi: 10.33594/000000380.

27- Mishra M, Tiwari S, Gomes A V. Protein purification and analysis: next generation Western blotting techniques. Expert Rev Proteomics. 2017;14(11):1037–53. doi: 10.1080/14789450.2017.1388167.

28- Talib LJ, Al-Sudani BT, Al-Abbassi MG. Aptamer Validation by Western Blot–an overview. Al Mustansiriyah Journal of Pharmaceutical Sciences. 2020;20(4):122-31. https://doi.org/10.32947/ajps.v20i4.782

29- Brackstone M, Townson JL, Chambers AF. Tumour dormancy in breast cancer: an update. Breast Cancer Res. 2007;9(3):208. doi: 10.1186/bcr1677.

30- Asaka R, Miyamoto T, Yamada Y, Ando H, Mvunta DH, Kobara H, et al. Sirtuin 1 promotes the growth and cisplatin resistance of endometrial carcinoma cells: a novel therapeutic target. Lab Invest. 2015 Dec;95(12):1363-73. doi: 10.1038/labinvest.2015.119.

31- Latifkar, A.; Ling, L.; Hingorani, A.; Johansen, E.; Clement, A.; Zhang, X.; Hartman, J.; Fischbach, C.; Lin, H.; Cerione, R.A.; et al. Loss of Sirtuin 1 Alters the Secretome of Breast Cancer Cells by Impairing Lysosomal Integrity. Dev. Cell **2019**, 49, 393–408.e7. doi: 10.1016/j.devcel.2019.03.011.

32- Wang, R.-H.; Zheng, Y.; Kim, H.-S.; Xu, X.; Cao, L.; Luhasen, T.; Lee, M.-H.; Xiao, C.; Vassilopoulos, A.; Chen, W.; et al. Interplay among BRCA1, SIRT1, and Survivin during BRCA1-associated tumorigenesis. Mol. Cell **2008**, 32, 11–20. doi: 10.1016/j.molcel.2008.09.011.

33- Santolla MF, Avino S, Pellegrino M, De Francesco EM, De Marco P, Lappano R, Vivacqua A, Cirillo F, Rigiracciolo DC, Scarpelli A, Abonante S. SIRT1 is involved in oncogenic signaling mediated by GPER in breast cancer. Cell death & disease. 2015 Jul;6(7):e1834-. doi: 10.1038/cddis.2015.201.

34- Abdolvahabi Z, Nourbakhsh M, Hosseinkhani S, Hesari Z, Alipour M, Jafarzadeh M, Ghorbanhosseini SS, Seiri P, Yousefi Z, Yarahmadi S, Golpour P. MicroRNA-590-3P suppresses cell survival and triggers breast cancer cell apoptosis via targeting sirtuin-1 and deacetylation of p53. Journal of Cellular Biochemistry. 2019 Jun;120(6):9356-68. doi: 10.1002/jcb.28211.

35- H. Vaziri, S.K. Dessain, E. Ng Eaton, S.I. Imai, R.A. Frye, T.K. Pandita, L. Guarente, R.A. Weinberg, hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase, Cell 107 (2001) 149e159. doi: 10.1016/s0092-8674(01)00527-x.

36- Peck B, Chen C-Y, Ho K-K, et al. SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. Mol Cancer Ther. 2010a;9:844-855. doi: 10.1158/1535-7163.MCT-09-0971.

37- Speidel D. The role of DNA damage responses in p53 biology. Archives of

toxicology. 2015 Apr;89(4):501-17. doi: 10.1007/s00204-015-1459-z.

38- Song J, Hong P, Liu C, Zhang Y, Wang J, Wang P. Human POLD1 modulates cell cycle progression and DNA damage repair. BMC Biochem. 2015;16(1):1–8. doi: 10.1186/s12858-015-0044-7.

39- Sanefuji K, Taketomi A, Iguchi T, Sugimachi K, Ikegami T, Yamashita Y, Gion T, Soejima Y, Shirabe K and Maehara Y: Significance of DNA polymerase delta catalytic subunit p125 induced by mutant p53 in the invasive potential of human hepatocellular carcinoma. Oncology. 79:229–237. 2010. doi: 10.1159/000322374.

40- Qin Q, Tan Q, Li J, Yang W, Lian B, Mo Q, Wei C. Elevated expression of POLD1 is associated with poor prognosis in breast cancer. Oncology Letters. 2018 Nov 1;16(5):5591-8. doi: 10.3892/ol.2018.9392.