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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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BIBR1591 INDUCES APOPTOSIS IN BREAST CANCER CELL LINE AND INCREASES EXPRESSION OF DAPK1, AND NR4A3

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Abstract.

Background: Breast cancer, the world's most prevalent cancerous disease that threatens women, is mainly dependent upon ovarian endocrine secretion for its growth and development. Telomerase inhibitors have been widely studied for their use to treat various tumors. BIBR1591 is the first highly effective small molecule telomerase inhibitor that could inhibit telomerase of many types of cancer cells at sub micromolar concentration

Aim: Our research aimed to study the molecular mechanism and action of BIBR1591, trying to understand the telomerase inhibitor in breast cancer, focusing on its ability to induce apoptosis and alter the expression of specific genes.

Material and Methods: The MCF-7 breast cancer cell line was treated with BIBR1591 at different concentrations for 48 hours, and the effects on cell viability and apoptosis were evaluated using MTT and flow cytometry assays, respectively. The DAPK1, and NR4A3 gene expression levels were assessed by qPCR.

Results: Treatment with BIBR1591 resulted in a dose-dependent decrease in cell viability and a significant increase in apoptosis in the MCF-7 cells. Furthermore, the expression levels of CDH13, DAPK1, and NR4A3 genes were upregulated following treatment with BIBR1591.

Conclusion: Our research concludes that BIBR1591 has unique molecular anticancer effect as its expression of CDH13, DAPK1, and NR4A3 genes. These results support the potential use of BIBR1591 as a therapeutic option for breast cancer treatment.

Key words. BIBR1591, MCF-7 cells, breast neoplasms, telomerase, CDH13.

Introduction.

Breast cancer, the world's most prevalent cancerous disease that threatens women, is mainly dependent upon ovarian endocrine secretion for its growth and development. With the progress of modern medicine and the global aging trend, the incidence of malignant tumors is likely to increase over the next few decades [1,2]. Currently, surgery, radiotherapy, and chemotherapy remain the pillar strategies for anti-breast cancer therapy. However, most cancer cells display acquired resistance to current anti-cancer drugs. In recent years, the degradation of tumor resistance to endocrine therapy functions as the candidate strategy for the treatment of anti-breast cancer. It was reported that the use of telomerase inhibitors, either alone or in combination, reduced the proliferation of breast cancer by inhibiting telomerase activity in vitro and in vivo. Still, the molecular mechanism is unclear [3]. BIBR1591 is a small molecule telomerase inhibitor anticancer in several types of cancer, including breast cancer. In breast cancer, telomerase expression is significantly higher in tumor tissues than in adjacent normal tissues, and high telomerase activity is associated with poor prognosis and increased tumor aggression. Therefore, inhibiting telomerase activity using small molecules like BIBR1591 may offer a promising approach to breast cancer treatment [4,5]. Additionally, epigenetic silencing of tumor suppressor genes by promoter hypermethylation is an essential early mechanism in leukemogenesis and has been associated with poor breast cancer prognosis. Breast cancer is a heterogeneous disease with various subtypes that differ in their clinical behavior and response to treatment. Epigenetic changes, including DNA methylation and histone modifications, play a significant role in breast cancer development and progression. Many tumor suppressor genes and other genes that regulate apoptosis, growth pattern, and invasion are epigenetically silenced in breast cancer [6].

CDH13 (also known as H-cadherin and T-cadherin) is a member of the grief gene superfamily localized at 16q24. In vitro and in vivo studies have shown that CDH13 can function as a tumor suppressor gene in many types of cancer, including breast cancer. CDH13 hypermethylation has been detected in breast and lung cancers, pituitary adenomas, diffuse large B-cell lymphoma, and nasopharyngeal and cutaneous squamous cell carcinomas [7,8]. Loss of CDH13 expression has been associated with a more invasive phenotype and a worse prognosis in breast cancer [9-11].

Role of DAPK1, NR4A3, in Cancer Progression.

The DAPK family includes five members, but little research has focused on DAPK1, the initiator of the DAPK family. DAPK1, the most prominent member of the DAPK family, is a 160 kDa protein encoded by a gene located at 9p21.3. DAPK1 participates in the regulation of diverse cellular signal transduction and plays critical roles in cancer growth. Some research has shown that reduction or loss of DAPK1 is observed in liver cancer cells [12,13]. Hypermethylation of the DAPK1 promoter requires the involvement of specificity protein 1 (Sp1) in cell death. Loss of DAPK expression or CGI methylation in the promoter region of DAPK may be characteristic of highly invasive or metastatic breast tumors. NR4A3, also known as NR4A3 transcript variant 5, is a human gene that encodes a protein containing a nuclear hormone receptor domain, which is thought to function in the body's overall metabolism. Apoptosis is a kind of programmed cell death (PCD), a gene that controls the growth of normal cells by regulating programmed cell death or, occasionally, when induced by other cells that cause damage

through immunological reactions. PCD is the primary method to eliminate damaged cells [14]. Caspase is a cysteine-based opposing protease family member expressed in various host cells. Inhibition of its activation and proliferation or apoptosis plays an essential regulatory role in causing tumor formation. The mechanism of how BIBR1591 induces apoptosis in human tumor cells is not precise. Also, there is no report about the modulation of DAPK1, NR4A3, and Caspase by BIBR1591 in breast cancer cells [15].

hTERT and its Significance in Cancer.

Human telomerase is a type of reverse transferase located at the end of eukaryotic chromosomes, synchronized with telomeric DNA to extend telomeres during cell division. hTERT is the most important component of human telomerase and is widely present in 85%-90% of tumor tissues, showing a high expression level. When telomerase is active in normal human cells, it is almost completely inactive. The reasons for its reactivation are the inactivation of hTERT, the expression of the regulatory protein hTERT, hTERT itself, and the synthesis of the RNA component hTR. The first three steps and the smallest subunits of hTERT have great implications for tumor formation and hTERT changes. As an extremely important biomarker of telomerase activity, hTERT has been found in a large number of clinical studies. The high expression level of hTERT is associated. High expression of hTERT can be used as a target in the treatment and early diagnosis of breast cancer. Our research aimed to study the molecular mechanism and action of BIBR1591, trying to understand the telomerase inhibitor in breast cancer.

Materials and Methods.

Cell culture:

Breast cancer cell lines (MCF7) were obtained from the Iraqi Center for Genetics and Cancer Research. They got these types of cells from Germany in 2018. The cells were kept in a humidified incubator at 37°C and 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. It was incubated in a 37°C oven containing control of cell viability. The Trypan Blue (Bio. Ind) staining test performed proliferation [16].

Cytotoxicity Analysis:

The crystal violet assay was used to evaluate the effect of BIBR1591 on the viability of breast cancer cells. To put it briefly, 5,000 cells were placed into each well of 96-well plates, and the plates were then incubated for 24 hours. Following that, the cells were exposed to BIBR1591 at different doses (0, 5, 10, 20, and 40 μM) for 48 hours. After treatment, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet solution for 15 minutes. Excess crystal violet stain was washed off with water, and the plates were air-dried. A microplate reader was used to detect the absorbance at 570 nm after the crystal violet stain that was attached to the cells was dissolved in 10% acetic acid. The mean absorbance value was computed for analysis after each concentration was tested three times.

The percentage of surviving cells was calculated by normalizing the absorbance of treated cells to that of untreated control cells [17].

As a result, BIBR1591 was applied at concentrations ranging from 1 μM to 100 μM. Untreated cells were used as a control group. At the end of 24, 48, and 72 hours, the formazan dye change caused by WST-1 due to glycolytic NAD(P)H production by the mitochondrial respiratory system in living cells was quantitatively measured with the "Multiskan FC microplate" device. The IC50 dose of BIBR1591 on MCF7 cells was calculated in a time- and dose-dependent manner with CalcuSyn Version 2.0 software [18].

Detection of Apoptosis by Flow Cytometry:

The detection of DNA fragmentation, a marker of apoptotic degradation, and phosphatidylserine translocation determined the apoptotic effect of BIBR1591 on the cell line. They were determined by the Annexin V-FITC (BD Pharmingen) method. Cells were placed in 6-well plate cells/mL concentration and treated with an IC50 BIBR1591 for 48 hours. The BD Accuri C6 Flow Cytometer (BD Biosciences) evaluated results relative to drug-free control groups. The data were acquired, and the percentage of cells in different stages of apoptosis (early apoptosis, late apoptosis, and necrosis) was evaluated relative to drug-free control groups using BD Accuri C6 software.

Analysis of Total RNA Isolation, cDNA (Complementary DNA) Synthesis, and Gene Expression Changes in MCF7 cells treated with IC50 dose of BIBR1591 were determined by real-time polymerase chain reaction (RT-PCR) method after 48 hours. At the end of the incubation, the RNeasy Plus Mini Kit from Qiagen was utilized for total RNA extraction, and the RT2 First Strand Kit was employed for cDNA synthesis (Qiagen). RT-PCR was carried out using a Light Cycler 480 II (Roche) device. The results were evaluated by the 2-ΔΔCT method. Genes with a fold change of more than ±2 fold.

ELISA:

Using the mTOR ELISA kit (Abcam, cat. no. ab176657), the mTOR (pSer2448) level was measured. As previously mentioned, cells were lysed in RIPA buffer, and mTOR was identified in accordance with the manufacturer's guidelines [19].

Statistical analysis:

SPSS 21.0 was used for all statistical analyses (IBM Corp.). The mean ± standard deviation (SD) is used to display the data. Dunnett's post hoc test was used to detect significance differences after a one-way ANOVA. A statistically significant difference was defined as $P<0.05$.

Results.

Cytotoxicity Results The cytotoxic effect of BIBR1591 in the MCF7 cell line is shown in Figure 1 and Table 1. BIBR1591 IC50 concentration was determined as 60 μM at the 48th hour.

Apoptosis analysis results: The Annexin V-FITC method was used to evaluate the apoptotic and necrotic effects of MCF7 cells treated with BIBR1591 IC50 concentration (60 μM) for 48 hours compared to the control group. BIBR1591 increased apoptosis in MCF7 cells compared to the control (Figure 2) and (Table 2).

Table 1. *Illustrates the relationship between the concentration in molary of BIBR1591 and the cell proliferation inhibition percentage.*

Concentration	Cytotoxic effect
10µM	17%
20 µM	21%
50 µM	44%
100 µM	59%

Table 2. *Shows the apoptosis percentage on the flow cytometer Q3- LL side.*

Group	apoptosis in Q3- LL
Control group (only ethanol and DMSO)	**12.6 %**
BIBR1591	**51.3%**

Table 3. *Illustrates the percentage of expression.*

	Control	BIBR1591	P value
CDH13	0.24%	7.14%	0.023*
DAPK1	-0.02%	6.83%	0.064
NR4A3	0.34%	7.16%	0.034*
hTERT	-0.04%	-2.7%	0.049*

P value less than 0.05 is considered significant.

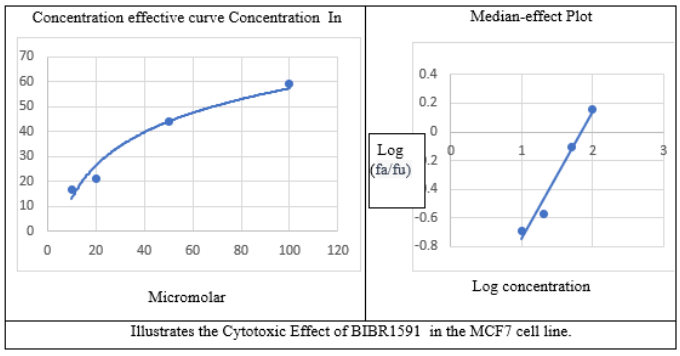

Figure 1. *Illustrates the Cytotoxic Effect of BIBR1591 in the MCF7 cell line.*

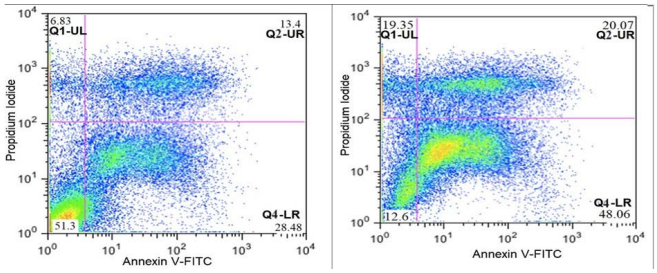

Figure 2. *Flow cytometer results in the percentage of apoptosis and necrosis.*

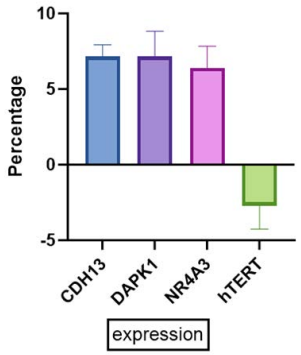

Figure 3. *Illustrates the percentage of expression in the treated sample.*

Calculation:

In the context of cell proliferation inhibition, Fa refers to the fraction affected, which represents the percentage of cells affected by a particular treatment or drug. In other words, it is the fraction of cells that have stopped dividing or have been killed due to the treatment.

On the other hand, fu refers to the fraction unaffected, which represents the percentage of cells that are still capable of dividing or growing despite the treatment. It is calculated as 1 - Fa. Using the table 1 provided, we can calculate fa and fu for each concentration:

At 10 µM, fa = 17% and fu = 83%
At 20 µM, fa = 21% and fu = 79%
At 50 µM, fa = 44% and fu = 56%
At 100 µM, fa = 59% and fu = 41%
Next, we can calculate fa/fu for each concentration:
At 10 µM, fa/fu = 0.17/0.83 = 0.2048
At 20 µM, fa/fu = 0.21/0.79 = 0.2658
At 50 µM, fa/fu = 0.44/0.56 = 0.7857
At 100 µM, fa/fu = 0.59/0.41 = 1.4390

Gene expression changes:

At the end of 48 hours, MCF7 cells treated with an IC50 dose of BIBR1591 showed a significant decrease in hTERT expression compared to the control, while DAPK1 and NR4A3, which are apoptotic and tumor suppressor genes, were observed. Table 3 and Figure 3 show an increase in the expression of CDH13.

Discussion.

The research of research demonstrates that BIBR1591 is an effective Chemotherapeutic drug against breast cancer. The results highlight the ability of BIBR1591 to induce apoptosis and modulate the expression of critical genes involved in tumor suppression and cell death, such as DAPK1, NR4A3, and CDH13.

Apoptosis induction:

The significant increase in apoptosis observed in the MCF-7 cells treated with BIBR1591, as evidenced by the flow cytometry results, suggests that BIBR1591 effectively induces cell death in breast cancer cells. The increase in apoptosis from 12.6% in the control group to 51.3% in the treated group indicates that BIBR1591 is a potent inducer of apoptosis in breast cancer cells. This aligns with previous studies that have shown the efficacy of telomerase inhibitors in triggering programmed cell death in various cancer types [19,20].

Gene expression modulation:

One of the most important results obtained from this research is the decrease in levels of DAPK1, NR4A3, and CDH13 after BIBR1591 treatment, knowing that these genes play an important role in cell death and tumor suppression.

DAPK1: DAPK1 acts as a pro-apoptotic protein and an important mediator for the action of the pro-apoptotic protein p53. In unstressed cells, p53 is an inactive protein due to its binding to the E3 ubiquitin-protein ligase. Our result showed that there is an increase in DAPK1 expression for about (6.83-fold) this result suggests that BIBR1591 may exert its anti-tumor effects in part by enhancing the expression of this crucial apoptosis regulator. This result is in line with other previously

published research in this regard that Telomerase inhibitors increase its expression, but it's conducted for the first time in the MCf7 cell line treated with BIBR1591 [21-24].

NR4A3: R4A members have also demonstrated the ability to cause apoptosis in different cell lines with accumulation in the G1 phase of the cell cycle, with a concomitant decrease of cells in the S and G2/M phases. The role of all NR4A members in the apoptotic process has been described in many studies utilizing overexpression or silencing of mRNA using commercial siRNA or shRNA the research showed a significant increase its expression about the 7.16-fold increase in NR4A3 expression observed in this study indicating that BIBR1591 may activate apoptotic pathways through NR4A3, further contributing to its anti-cancer effects. This is consistent with findings in other studies where NR4A3 activation has been linked to increased apoptosis in cancer cells [25-29].

CDH13: also known as T-cadherin, is involved in cell adhesion and has been shown to function as a tumor suppressor. The dramatic upregulation of CDH13 (7.14-fold) in response to BIBR1591 treatment suggests that this gene may play a critical role in mediating the anti-tumor effects of BIBR1591 in breast cancer. Restoring CDH13 expression could reverse the invasive phenotype often seen in aggressive breast cancers [30-36].

Conclusion.

The development of modern technology has helped us understand the molecular and pathological conditions of diseases, including breast cancer, which has made it easier for us to choose novel drugs and experiment with existing drugs. The current study aimed to study the molecular mechanism and action of BIBR1591, trying to understand the telomerase inhibitor in breast cancer, focusing on its ability to induce apoptosis and alter the expression of specific genes. The research showed that the inhibitor could exhibit an inhibitory effect on breast cancer cell proliferation. Additionally, the study's identification of specific genes upregulated by BIBR1591 provides further insight into the drug's mechanism of action in regulating apoptosis and invasion. Further preclinical and clinical studies are necessary to evaluate the effectiveness and safety of BIBR1591 as a therapeutic agent for breast cancer. Although additional experimental and clinical testing will be needed to further evaluate the therapeutic efficacy of the inhibitor against breast cancer, these data support a potential use of such a drug for the treatment of hTERT-active breast cancers and could contribute to the development of novel anti-BRCA agents. The study provides a rationale for the use of this compound for the treatment of many heterogeneous types of breast cancer cells. Further studies should shift towards determining its maximally tolerated dose, its toxicity to normal cells, the frequency of treatment, and other investigations aimed at repositioning hTERT inhibitors as anticancer therapy for breast cancer.

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Author contribution.

ZA, A Y N: draft writing; S T: data analysis; HAA; AAR: editing, proofreading, response to reviewer.

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Conflict of interest.

None

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