

Evaluation of therapeutic effects of KC7F2, temozolomide, and its combination on genes involved in the Wnt signal pathway in the U87MG glioma cell line

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Research Article

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Abstract

Glioma is the most common malignant primary brain tumor that survives less than 12 months after diagnosis. The Wnt signaling pathway creates a complex network of proteins that have different effects on cells, so they can be involved in many cancers, including gliomas. Drug resistance is seen in many cancer treatments. Therefore, we investigated the combined effect of Temozolomide (TMZ) and KC7F2 on cell survival and changes in gene expression in the Wnt signal pathway.

The effects of TMZ and KC7F2 on cancer cell viability and proliferation were investigated by WST-1 method. Isobologram analysis was performed to evaluate the combined effects of TMZ and KC7F2 on U87MG glioma cell line. Also, Quantitative RT-PCR was used to evaluate the mRNA expression level of genes related to the Wnt signal pathway in treated cancer cells and control groups.

IC₅₀ TMZ and KC7F2 values in U87MG cell lines were determined to be 461 μ M and 19 μ M in 48 hours, respectively. Combined effects of TMZ and KC7F2 on the U87MG cell line show the CI values for TMZ and KC7F2 were found as 0.746. It was found that KC7F2+TMZ increased APC, CCND1, CXXC4, DAAM1, FBXW11, FRZB, FZD3, FZD8, LEF1, LRP5, LRP6, PYGO1, RHOA, RHOU, RUVBL1, VANGL2, WIF1, WNT10A, WNT16, WNT3A, and WNT6 while decreasing NKD1, PRICKLE1, DKK1, WNT1 expressions in U87MG cell lines.

The results show that the combination of TMZ and KC7F2 can be a promising anti-cancer agent for the treatment of glioblastoma through the Wnt / β -catenin signaling pathway. However, further studies are needed to understand all angles.

Introduction

Glioma is the most common malignant primary brain tumor that develops inside the skull. This malignant mass can include astrocytes, oligodendrocytes, or a combination of these two types of cells. The incidence of glioma varies considerably based on age, sex and race. Glioblastoma multiforme (GBM; WHO Astrocytoma Grade IV) is the most common and deadly subtype of glioma in adults. Extensive treatments include surgery, radiotherapy, chemotherapy and immunotherapy. The extent of tumor resection and the age of diagnosis are prognostic factors for glioblastoma [1]. Survival rates vary in glioma subtypes, but a relative five-year survival ratio of gliomas has been suggested based on population-based studies. However, Pilocytic astrocytoma (grade I) has the highest relative survival [2].

The family of Wnt signaling pathways are evolutionarily conserved in different species and play an important role in the growth, differentiation, proliferation, and survival of embryonic stem cells [3, 4]. The association of the Wnt signaling pathway with cancer was established when Int-1, as one of the proteins in this pathway, showed proto-oncogenic properties, and it was found that mutations in this protein lead to breast cancer in mice [5]. The Wnt signaling pathway creates a complex network of proteins that have different effects on cells. One of the most important of these proteins is β -catenin, which plays a pivotal role in the Wnt signaling pathway [6, 7]. If this pathway is inactive, β -catenin levels will be kept lower by a

set of proteins. In fact, β -catenin phosphorylation by GSK-3 β , along with APC and Axin, destroys it, resulting in a low level of β -catenin in the nucleus [8–10]. And the LEF / TCF family transcription factor, along with other proteins, binds to DNA and inhibits gene expression. TCF / LEF is a subset of transcription factors that can act as inhibitors or activators of transcription depending on the complex that makes them up [11, 12].

In contrast, the activation of disheveled (Dvl) protein, which breaks down the degradation complex, results in β -catenin dephosphorylation and can be transferred to the nucleus. In the nucleus, β -catenin bind to family members of the TCF / LEF transcription factor and stimulate transcription factors [12, 13]. And the expression of important growth regulatory genes, including cyclin D1 and c-Myc increases [14]. The role of this pathway in some malignancies, including breast cancer, pancreatic, and colorectal cancer, has been identified, so that activation of the Wnt signaling pathway and increased protein expression can be important factors in these diseases [15].

DNA alkylating agents are the oldest class of anticancer drugs. They are actively used and maintain their importance for a variety of cancers, including brain tumors. Alkylating agents damage DNA by making large and small additions with nucleic acid bases [16]. The most promising drug for brain tumors is temozolomide (TMZ). TMZ is a prodrug of imidazole derivatives. It is a second-generation alkylating chemotherapy agent. Because TMZ is lipophilic, it effectively crosses the blood-brain barrier. Inhibits the growth of cancer cells, slows their growth [17, 18]. O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, plays a role in the resistance of tumor cells to alkylating agents. MGMT is expressed in glioma and its contribution to TMZ resistance is known [17].

KC7F2 is the second generation of HIF-1 α inhibitor described by the VanMaier group. Cells are less sensitive to this agent [19]. KC7F2 is cytotoxic against cancer cells under normoxic conditions and increases toxicity by increasing HIF-1 α levels under hypoxic conditions [20]. In this study, we investigated the effects of temozolomide, KC7F2 and their combination on cell viability in U87MG glioma cell line. Also, changes in the expression of genes involved in the Wnt signal pathway were examined.

Material And Method

2.1 Cell line, chemicals, and culture conditions

The Glioblastoma U87 MG cell line used in our study was obtained commercially from the ATCC. For the culture of the desired cell line, DMEM medium with a volume of 500 ml was prepared. The total volume of DMEM medium contained 5 ml of penicillin-streptomycin (Biological Industries), 2 mM glutamine (Biological Industries), and 50 ml of Fetal Bovine Serum (FBS) (Biological Industries). To grow the cells, they were inoculated in the prepared medium and incubated at 37 ° C, with 95% humidity, and 5% CO₂ conditions. The proliferation dynamics of the cells were regularly monitored by inverted microscopy at 24 and 48 h intervals. KC7F2 (Sigma) in the logarithmic dose range of 1-30 (μ M) and Temozolomid (TMZ) (Sigma) active ingredients in the logarithmic dose range of 100-500 (μ M) were applied to cells. The

materials we used were first dissolved in DMSO (Sigma) while they were lyophilized. Therefore the main stock was prepared for both active ingredients. Control cell lines were evaluated by applying other conditions in the same way without the use of the substance.

2.2 Cytotoxicity analysis

Effects of the active agents (TMZ and KC7F) on cells for determining the IC₅₀ values were specified using Colorimetric WST-1 Assay Kits (Roche). For cytotoxicity analysis, glioma U87MG cells were implanted at 3.2×10^3 cell/ml in 96 well-plate and incubated for 24 h. After a 24-hour incubation period, KC7F2 (10-30 μ M) and TMZ (100-500 μ M) were administered to the cells. At the end of each incubation period of 24, 48 and 72 h, 10 μ l of WST-1 solution was added to the wells and incubated for 1 hour. Quantitative measurements of formazan dye intensity produced by living cells after incubation were performed every 15 minutes during the course within a microplate reader (Multiskan FC, Thermo) in the reference range of 620 nm and 450 nm absorption. Cells that were not treated with substance (TMZ and KC7F) were defined as controls. Three replicates were evaluated for each drug and their doses and control group.

2.3 Isobologram analysis

Isobologram analysis was performed to evaluate the combined effects of TMZ and KC7F on U87MG glioma cell line. To perform this analysis, the U87MG cells (3.2×10^3 cell/ml) were seeded into the 96-well plate. After the 24 h incubation period, different dose combinations of TMZ and KC7F2 which were based on the IC₅₀ doses of the agents, were administered to the cells for 24, 48 and 72 hours. The analysis was performed spectrophotometrically by WST-1 test in the same way as cytotoxicity analysis. ED₅₀, ED₇₅, ED₉₀ and Combination Index (CI) values were calculated.

2.4 Gene expression analysis

Changes in the expression level of genes involved in the Wnt signal pathway in U87MG cells treated with TMZ, KC7F2, and their combination were determined by real-time PCR at 48 h. To analyze changes in gene expression, cells (3.2×10^3 cell/ml) were implanted in 6-well plates and the specified dose of drugs was applied with appropriate dilutions for the cells. TMZ, KC7F2, and their combination were not applied to cells that were accepted as a control group. Total RNA was isolated from the cells using the RNeasy® Mini Kit (Qiagen Cat. No: 74104)) and cDNA synthesis was realized using RT2 First Strand Kit (Qiagen Cat. No: 330401). Expression changing of the 84 genes associated with the Wnt signal pathway was examined using RealQ Plus 2x Master Mix Green (Qiagen Cat. No: A323402) and Light Cycler 480 Instrument II (Roche).

2.5 Data analyses

IC₅₀ values of TMZ, KC7F2, and their combination in U87MG glioma cell line was performed using Graph pad Prism v8.0.2. Change of gene expression in U87MG glioma cell line was performed by xCELLigence® RTCA (Roche) software. Fold changes of the genes were calculated by $2^{-\Delta\Delta Ct}$ method, the fold

change values $p < 0.05$ were considered as significant. ED50, ED75, ED90, and Combination Index (CI) values were calculated by using CalcuSyn software (Biosoft).

Results

3.1 Evaluation of the Effects of KC7F2, Temozolomide, and its combination on U87MG cell viability

The effects of TMZ and KC7F2 on cell viability on U87MG cell lines were evaluated via WST-1. In order to determine the IC50 content of TMZ and KC7F2, KC7F2 dilutions (10-30 μM) and TMZ dilutions (100-500 μM) were evaluated by WST-1 test at 24, 48, and 72 h for U87MG cell lines. IC50 TMZ and KC7F2 values in U87MG cell lines were determined to be 461 μM and 19 μM in 48 hours, respectively (Figure 1) [21].

3.2 Evaluation of the combined effects of TMZ and KC7F2 on the U87MG cell line

According to the results, the CI values of TMZ and KC7F2 were found as 0.746 and the doses were 208.71 μM for TMZ and 8.60 μM for KC7F2 and the effects were accepted as additive [21].

3.3 Evaluation of the Effects of KC7F2, Temozolomide, and its combination on gene expression levels

When U87MG cell lines were exposed to IC50 KC7F2 (19 μM) for 48 hours, KC7F2 was found to alter the expression of Wnt signal pathway genes compared with controls. It was found that KC7F2 increased APC expression 3.9-fold, FZD8 expression 3.8-fold, RHOU expression 3.2-fold, VIF1 expression 3.3-fold, RHOA expression 2.1-fold while decreasing AES, AXIN2, CCND2, CXXC4, DIXDC1, DVL1, FZD7, NKD1, PRICKLE1, WISP1, WNT1, WNT2 expressions 6.6, 3.6, 5, 4.6, 3.3, 3.8, 3, 6.6, 6.6, 3.6, 4.6, and 4.3 folds, respectively, in U87MG cell lines (figure 2).

When U87MG cell lines were exposed to IC50 TMZ (461 μM) for 48 hours, TMZ was found to alter the expression of Wnt signal pathway genes compared with controls. It was found that TMZ increased APC expression 4.2-fold, DAAM1 expression 4.2-fold, FRZB expression 3.6-fold, FZD8 expression 6.8-fold, PYGO1 expression 3.3-fold, WNT1 expression 19-fold, and WNT3A expression 3.5-fold while decreasing AES, RHOU, WNT2, WNT6, WISP1 expressions 6.6, 6.6, 5, 5.6, and 2 folds, respectively, in U87MG cell lines (figure 2).

When U87MG cell lines were exposed to KC7F2+TMZ for 48 hours, It was found that KC7F2+TMZ increased APC expression 5.9-fold, CCND1 expression 3.3-fold, CXXC4 expression 4.6-fold, DAAM1 expression 5.7-fold, FBXW11 expression 3.3-fold, FRZB expression 3.4-fold, FZD3 expression 4.6-fold, FZD8 expression 7.1-fold, LEF1 expression 3.1-fold, LRP5 expression 8.1-fold, LRP6 expression 3.8-fold, PYGO1 expression 3.8-fold, RHOA expression 3.1-fold, RHOU expression 4.5-fold, RUVBL1 expression 4-

fold, VANGL2 expression 7.3-fold, WIF1 expression 13.2-fold, WNT10A expression 6-fold, WNT16 expression 10.8-fold, WNT3A expression 6-fold, WNT6 expression 4.9-fold while decreasing NKD1, PRICKLE1, DKK1, WNT1 expressions 4, 4.6, 2.3, and 2 folds, respectively, in U87MG cell lines (figure 2).

Discussion

Glioblastoma (GBM) is one of the most invasive brain tumors in adults. GBM treatment is a combination of surgery, chemotherapy (usually temozolomide), and radiotherapy [22]. Treatment is usually designed to kill cancer cells and often, massive cell death occurs shortly after the treatment. Even after these treatments, GBM recurs and has a poor prognosis [23]. The average life expectancy after diagnosis is less than 2 years, and less than 10% of people survive more than five years [24]. GBM is highly resistant to most treatments due to its heterogeneous nature and the presence of TMZ and RT-resistant cancer stem cells [17]. Numerous studies have shown the importance of using drugs in combination (simultaneous use of a combination of two drugs) to reduce the growth of cancer cells and lead cancer cells to apoptosis [25–27]. Therefore, we used KC7F2 to ameliorate the effects of TMZ. The combined effects of TMZ and KC7F2 on the U87MG cell line showed that the CI values for TMZ and KC7F2 were 0.746, so their effects were accepted as additives.

The Wnt signaling pathway is one of these important pathways that helps cell differentiation to promote tumor formation in the brain. The Wnt signaling pathway causes the proliferation and development of nerve stem cells (NSCs) [28]. The WNT system is often overactive in GBM and is one of the causes of increased cell proliferation and invasion in GBM tumors. Homologous mutation of the FAT apical cadherin (FAT1) has been observed in GBM. However, mutations in other WNT-related genes are uncommon in GBM, possibly epigenetic changes suppressing WNT inhibitors and increasing β -catenin activity [29, 30].

The Wnt signal pathway begins with ligand binding to their Frizzled (FZD1-10) and LRP5/6 receptors. This binding causes structural changes in the FZD and LRP5/6 receptors and initiates an intracellular signal [31]. When Wnt ligands increase or are located near their receptors, the levels of FZD and LRP5/6 receptors increase at the cell surface, and cell function is regulated in response to these messages [31]. The effect of KC7F2 on cell lines in our study increased FZD-8 expression and decreased FZD-7 and FZD-1 expression. The expression of FZD-8 and FZD-3 increased when the U87MG cell line was affected by TMZ. Interestingly, TMZ + KC7F2 increased the expression of FZD-8, FZD-3 LRP5, and LRP6. The variability in the Wnt signal pathway receptors appears to be due to a specific expression pattern in different tissues. However, little is known about FZD-LRP interactions, and irregularities in these interactions may cause membrane signal instability.

The large number of Wnt (19 cases) detected in humans indicates the complexity of the Wnt signal pathway and its different biological effects on cells. Temporary or constant expression of Wnt-1 can induce the β -catenin-LEF1 complex and activate the Wnt signal pathway in cancer cells. But reducing

Wnt1 can dramatically reduce the migration and invasion of cancer cells [32]. One study found that Wnt-1 expression inhibits cancer treatment and suggests that Wnt-1 may exert its oncogenic potential through an anti-apoptotic mechanism [33]. When we exposed the U87MG cell lines to TMZ, we found that Wnt-1 expression was increased. This increase may be one of the causes of resistance to TMZ treatment. But when we tested TMZ + KC7F2, we found that Wnt-1 expression decreased, and this result can be promising. Wnt2 is an oncogene that can activate normal Wnt signaling during tumor formation. High levels of Wnt2 expression are seen in colon cancer and liver metastasis [32]. The results of a study showed that the concentration of Wnt3A secreted in the conditioned medium from normal tissues or tumors of people with colon cancer is much higher than the tissues of healthy people [34]. However, Wnt3A has been shown to inhibit the proliferation and migration of human colon myofibroblasts. In other words, increasing the expression of Wnt3A prevents the progression of cancer [35]. In our study, TMZ and KC7F2 decreased Wnt2 expression. In fact, in the U87MG cell line, Wnt2 stimulates the Wnt signal pathway as an oncogene. However, Wnt3A has different expression changes in different cancers. The results of our study showed that Wnt3A in glioblastoma has a low level of expression. TMZ and TMZ + KC7F2 increase Wnt3A expression. According to previous studies, this increase may inhibit metastasis in this cell line, although more detailed studies are needed to elucidate this.

β -catenin acts as a key gene in the Wnt signal pathway. In fact, increasing the amount of β -catenin stimulates transcription factors and decreasing it inhibits transcription factors. The APC/AXIN1 mutation and the β -catenin activating mutation are among the most common genetic changes that activate the Wnt signaling pathway in various cancers [36, 37]. The results of our study showed that KC7F2, TMZ, and TMZ + KC7F2 increases the APC gene in U87MG cell lines. This increase can decrease β -catenin and thus inhibit cancer cell proliferation. Our study showed that APC, CCND1, CXXC4, DAAM1, FBXW11, FRZB, LEF1, PYGO1, RHOA, RHOU, RUVBL1, VANGL2, and WIF1 genes, have an oncogenic role, and NKD1, PRICKLE1, DKK1 genes act as tumor suppressor genes in U87MG cell lines.

Conclusion

The Wnt signaling pathway is essential for regulating embryogenesis and tissue homeostasis. However, Wnt signaling pathway disorder is the pathological basis of many human malignancies. Therefore, modifying this pathway can be a hotspot in the treatment of tumors. The use of combination drugs is recommended to reduce cytotoxicity and increase the effectiveness of treatment in cancers. We suggest that the effect of TMZ + KC7F2 on the Wnt signal pathway and its association with the cell cycle, and apoptosis, be considered in future studies. However, the TMZ and KC7F2 combination is well tolerated and can induce Wnt signal pathway genes in U87MG cell lines.

Declarations

Author contributions

CBA, **HK** and **BK** Study conception and design; **BKG**, **LST**, **MST**, **ZA**, **NPO** and **BS** Methodology and Data curation; **BS** Writing - original draft; **CBA** Writing - review & editing.

Data availability

The data and materials used in this study are available.

Conflict of interest

The authors declare no conflict of interest.

References

1. Oh SJ, Yang JI, Kim O, Ahn EJ, Kang WD, Lee JH, Moon KS, Lee KH, Cho D. Human U87 glioblastoma cells with stemness features display enhanced sensitivity to natural killer cell cytotoxicity through altered expression of NKG2D ligand. *Cancer cell international*. 2017;17(1):1-9.
2. Ostrom QT, Bauchet L, Davis FG, Deltour I, Fisher JL, Langer CE, Pekmezci M, Schwartzbaum JA, Turner MC, Walsh KM, Wrensch MR. The epidemiology of glioma in adults: a “state of the science” review. *Neuro-oncology*. 2014;16(7):896-913.
3. Ng LF, Kaur P, Bunnag N, Suresh J, Sung IC, Tan QH, Gruber J, Tolwinski NS. WNT signaling in disease. *Cells*. 2019; 8(8):826.
4. Yang K, Wang X, Zhang H, Wang Z, Nan G, Li Y, Zhang F, Mohammed MK, Haydon RC, Luu HH, Bi Y. The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies. *Laboratory investigation*. 2016; 96(2):116-36.
5. Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017; 36(11):1461-73.
6. Hua Y, Yang Y, Li Q, He X, Zhu W, Wang J, Gan X. Oligomerization of frizzled and LRP5/6 protein initiates intracellular signaling for the canonical WNT/ β -catenin pathway. *Journal of Biological Chemistry*. 2018; 293(51):19710-24.
7. Cheng X, Xu X, Chen D, Zhao F, Wang W. Therapeutic potential of targeting the Wnt/ β -catenin signaling pathway in colorectal cancer. *Biomedicine & Pharmacotherapy*. 2019; 110:473-81.
8. Valenta T, Hausmann G, Basler K. The many faces and functions of β -catenin. *The EMBO journal*. 2012; 31(12):2714-36.
9. Silva-García O, Valdez-Alarcón JJ, Baizabal-Aguirre VM. Wnt/ β -catenin signaling as a molecular target by pathogenic bacteria. *Frontiers in immunology*. 2019; 10:2135.
10. Gasior K, Hauck M, Wilson A, Bhattacharya S. A theoretical model of the Wnt signaling pathway in the epithelial mesenchymal transition. *Theoretical Biology and Medical Modelling*. 2017; 14(1):19.
11. Doumpas N, Lampart F, Robinson MD, Lentini A, Nestor CE, Cantù C, Basler K. TCF/LEF dependent and independent transcriptional regulation of Wnt/ β -catenin target genes. *The EMBO journal*. 2019; 38(2):e98873.

12. Mao CD, Byers SW. Cell-context dependent TCF/LEF expression and function: alternative tales of repression, de-repression and activation potentials. *Critical Reviews™ in Eukaryotic Gene Expression*. 2011; 21(3).
13. Yang K, Wang X, Zhang H, Wang Z, Nan G, Li Y, Zhang F, Mohammed MK, Haydon RC, Luu HH, Bi Y. The evolving roles of canonical WNT signaling in stem cells and tumorigenesis: implications in targeted cancer therapies. *Laboratory investigation*. 2016; 96(2):116-36.
14. Hao YH, Lafita-Navarro MC, Zacharias L, Borenstein-Auerbach N, Kim M, Barnes S, Kim J, Shay J, DeBerardinis RJ, Conacci-Sorrell M. Induction of LEF1 by MYC activates the WNT pathway and maintains cell proliferation. *Cell Communication and Signaling*. 2019; 17(1):129.
15. Kim YM, Kahn M. The role of the Wnt signaling pathway in cancer stem cells: prospects for drug development. *Research and reports in biochemistry*. 2014; 4:1.
16. Cheung-Ong K, Giaever G, Nislow C. DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology. *Chemistry & biology*. 2013; 20(5):648-59.
17. Oliver L, Lalier L, Salaud C, Heymann D, Cartron PF, Vallette F. Drug resistance in glioblastoma: are persisters the key to therapy?. *Cancer Drug Resistance*. 2020; 3:Online-ahead.
18. Strobel H, Baisch T, Fitzel R, Schilberg K, Siegelin MD, Karpel-Massler G, Debatin KM, Westhoff MA. Temozolomide and other alkylating agents in glioblastoma therapy. *Biomedicines*. 2019; 7(3):69.
19. Tan C, de Noronha RG, Roecker AJ, Pyrzynska B, Khwaja F, Zhang Z, Zhang H, Teng Q, Nicholson AC, Giannakakou P, Zhou W. Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. *Cancer research*. 2005; 65(2):605-12.
20. Liao D, Johnson RS. Hypoxia: a key regulator of angiogenesis in cancer. *Cancer and Metastasis Reviews*. 2007; 26(2):281-90.
21. Abbaszade Z, Bagca BG, Avci CB. Molecular biological investigation of temozolomide and KC7F2 combination in U87MG glioma cell line. *Gene*. 2021 Apr 15;776:145445.
22. Alves AL, Gomes IN, Carloni AC, Rosa MN, da Silva LS, Evangelista AF, Reis RM, Silva VA. Role of glioblastoma stem cells in cancer therapeutic resistance: a perspective on antineoplastic agents from natural sources and chemical derivatives. *Stem Cell Research & Therapy*. 2021; 12(1):1-22.
23. Fernandes C, Costa A, Osório L, Lago RC, Linhares P, Carvalho B, Caeiro C. Current standards of care in glioblastoma therapy. *Exon Publications*. 2017 Sep 20:197-241.
24. Tamimi AF, Juweid M. Epidemiology and outcome of glioblastoma. *Exon Publications*. 2017 Sep 20:143-53.
25. Ozates NP, Soğutlu F, Lerminoglu F, Demir B, Gunduz C, Shademan B, Avci CB. Effects of rapamycin and AZD3463 combination on apoptosis, autophagy, and cell cycle for resistance control in breast cancer. *Life Sciences*. 2021; 264:118643.
26. Ozdemir Kutbay N, Biray Avci C, Sarer Yurekli B, Caliskan Kurt C, Shademan B, Gunduz C, Erdogan M. Effects of metformin and pioglitazone combination on apoptosis and AMPK/mTOR signaling pathway in human anaplastic thyroid cancer cells. *Journal of Biochemical and Molecular Toxicology*. 2020; 34(10):e22547.

27. Jadid MF, Shademan B, Chavoshi R, Seyyedsani N, Aghaei E, Taheri E, Goleij P, Hajazimian S, Karamad V, Behroozi J, Sabet MN. Enhanced anticancer potency of hydroxytyrosol and curcumin by PLGA-PAA nano-encapsulation on PANC-1 pancreatic cancer cell line. *Environmental Toxicology*. 2021; 36(6):1043-51.
28. Lee Y, Lee JK, Ahn SH, Lee J, Nam DH. WNT signaling in glioblastoma and therapeutic opportunities. *Laboratory investigation*. 2016; 96(2):137-50.
29. Rheinbay E, Suvà ML, Gillespie SM, Wakimoto H, Patel AP, Shahid M, Oksuz O, Rabkin SD, Martuza RL, Rivera MN, Louis DN. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. *Cell reports*. 2013; 3(5):1567-79.
30. Zhang N, Wei P, Gong A, Chiu WT, Lee HT, Colman H, Huang H, Xue J, Liu M, Wang Y, Sawaya R. FoxM1 promotes β -catenin nuclear localization and controls Wnt target-gene expression and glioma tumorigenesis. *Cancer cell*. 2011; 20(4):427-42.
31. DeBruine ZJ, Xu HE, Melcher K. Assembly and architecture of the Wnt/ β -catenin signalosome at the membrane. *British journal of pharmacology*. 2017; 174(24):4564-74.
32. Nie X, Liu H, Liu L, Wang YD, Chen WD. Emerging roles of WNT ligands in human colorectal cancer. *Frontiers in Oncology*. 2020;10.
33. Chen S, Guttridge DC, You Z, Zhang Z, Fribley A, Mayo MW, Kitajewski J, Wang CY. Wnt-1 signaling inhibits apoptosis by activating β -catenin/T cell factor-mediated transcription. *The Journal of cell biology*. 2001;152(1):87-96.
34. Schinzari V, Timperi E, Pecora G, Palmucci F, Gallerano D, Grimaldi A, Covino DA, Guglielmo N, Melandro F, Manzi E, Sagnotta A. Wnt3a/ β -catenin signaling conditions differentiation of partially exhausted T-effector cells in human cancers. *Cancer immunology research*. 2018; 6(8):941-52.
35. Ferrer-Mayorga G, Niell N, Cantero R, González-Sancho JM, Del Peso L, Muñoz A, Larriba MJ. Vitamin D and Wnt3A have additive and partially overlapping modulatory effects on gene expression and phenotype in human colon fibroblasts. *Scientific reports*. 2019 May 30;9(1):1-3.
36. Daa T, Kashima K, Kaku N, Suzuki M, Yokoyama S. Mutations in components of the Wnt signaling pathway in adenoid cystic carcinoma. *Modern pathology*. 2004; 17(12):1475-82.
37. Wang X, Goode EL, Fredericksen ZS, Vierkant RA, Pankratz VS, Liu-Mares W, Rider DN, Vachon CM, Cerhan JR, Olson JE, Couch FJ. Association of genetic variation in genes implicated in the β -catenin destruction complex with risk of breast cancer. *Cancer Epidemiology and Prevention Biomarkers*. 2008; 17(8):2101-8.

Figures

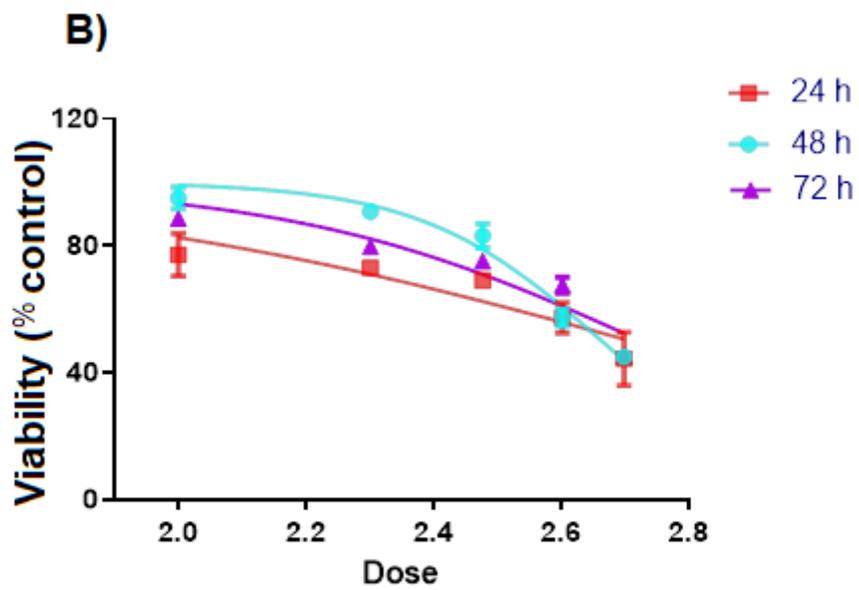
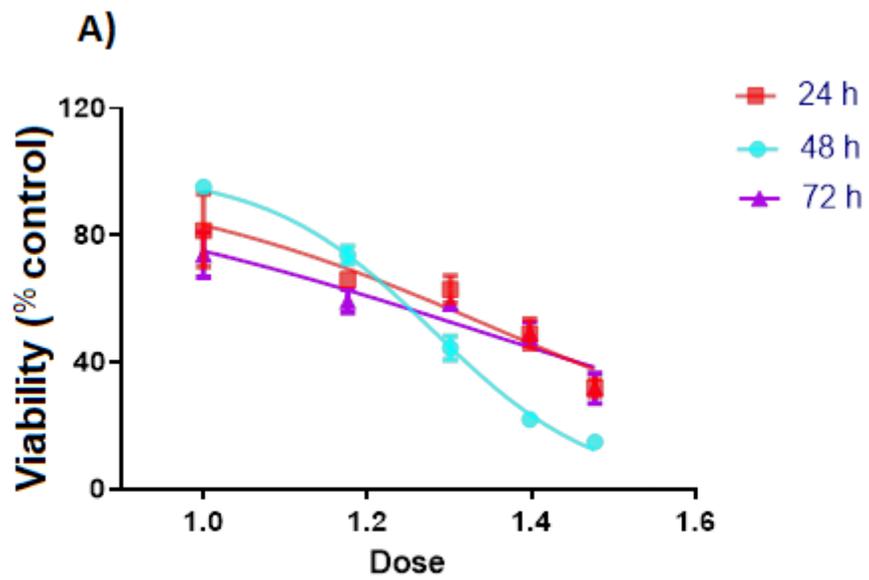


Figure 1

IC50 doses of (A) KC7F2 and (B) TMZ on U87MG cell lines.

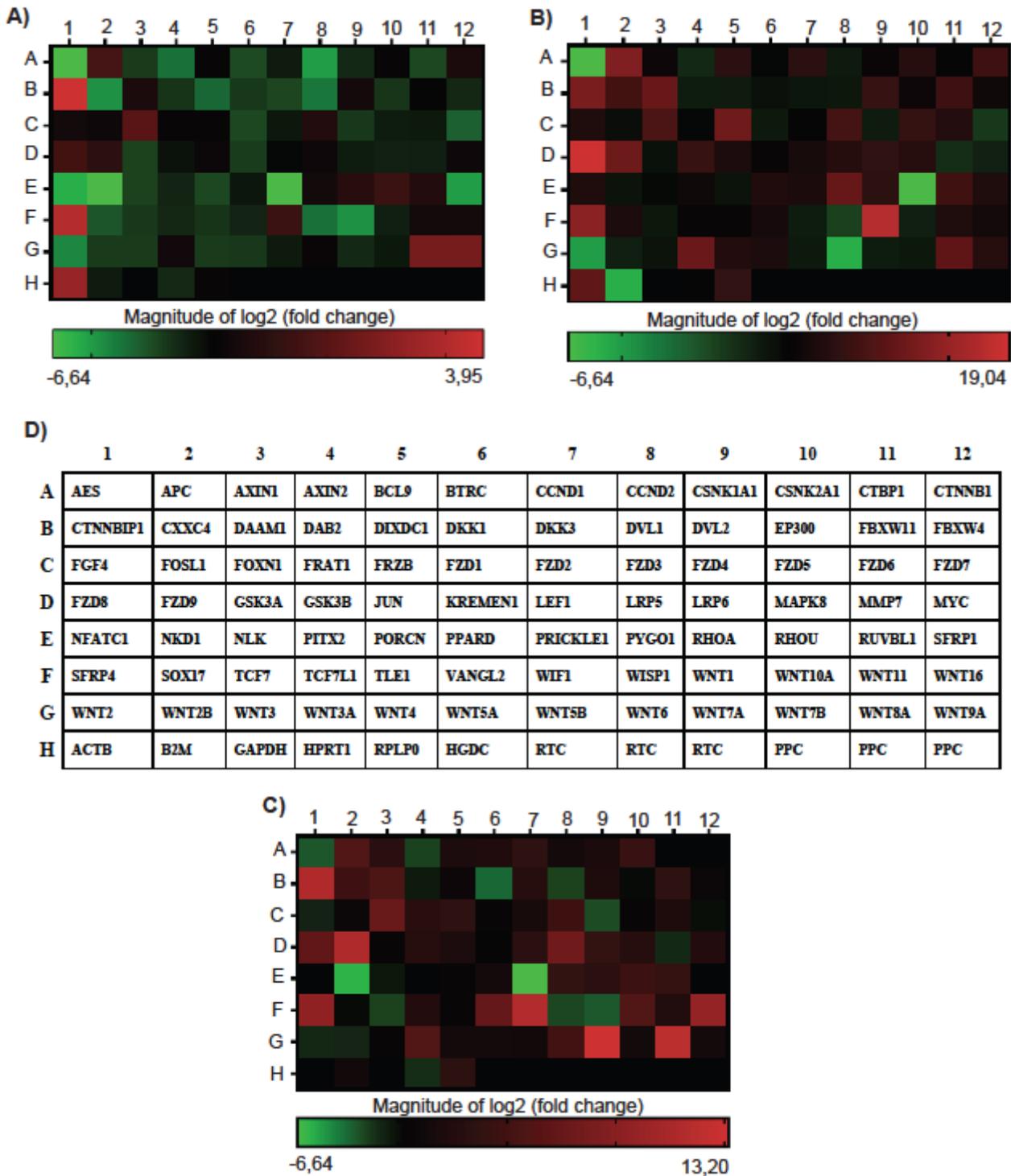


Figure 2

The heat map of the changes expression of Wnt signal pathway genes under the influence of (A) KC7F2, (B) TMZ, and (C) TMZ + KC7F2 in the U87MG cell lines. (D) Wnt signal pathway genes whose expression changes were examined.