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Article

Inhibiting the ERK pathway and the TRPM7 ion channel in gastric and bladder cancer cells

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SUMMARY

Gastric and bladder cancers occur when the cells start to reproduce uncontrollably, starting within the innermost layer of tissue. The transient receptor potential cation subfamily M member 7 (TRPM7) is an ion channel that plays an important role in the survival of both of these cancers. In addition, extracellular regulated kinases (ERKs) contribute to the carcinogenesis of many cancers including gastric cancer. Therefore, the treatments Ginsenoside Rd, NS8593, curcumin, and icariin that have the ability to inhibit TRPM7 and ERK could potentially be used as a treatment for these cancers. We hypothesized that these treatments would decrease proliferation and induce apoptosis by inhibiting TRPM7 and ERK in AGS gastric cancer cells and T24 bladder cancer cells; the data supported the hypothesis. Individually, the overall most effective treatment was NS8593 which reduced the proliferation by 81.1% and increased apoptosis by 38.5% in AGS cells compared to the negative control. Individually, the overall most effective treatment was NS8593 which reduced the proliferation by 88.4% and increased apoptosis by 78.0% in T24 cells compared to the negative control. Using all the treatments in combination was the most effective in reducing proliferation and increasing apoptosis in gastric cancer cells. There was also a correlation between the TRPM7, extracellular regulated kinases (ERKs), proliferation, and apoptosis levels. These results could pose in future more effective treatments for both gastric and cancer on top of other types of cancers that rely on TRPM7 and ERK.

INTRODUCTION

Gastric cancer, worldwide, is the second most common cancer, excluding non-melanoma skin cancers (1). Gastric cancer happens when cells along the lining of the stomach start proliferating uncontrollably and therefore become cancerous (1). As of now, the five-year survival rate of gastric cancer patients is between 10 and 30%. Over the last 30 years, its mortality rates have remained relatively static, and it continues to be a leading cause of cancer related death (2). Furthermore, the majority of gastric cancer symptoms such as heartburn, indigestion, and nausea arise late in its development, making it very difficult to treat (3). Similarly, bladder cancer occurs when cells in the urinary bladder start to proliferate uncontrollably beginning in the innermost layer. The five-year survival rate of metastasized bladder cancer is only 5% (4).

Recent studies have suggested a relationship between the TRPM7 channel and cancer tumor proliferation, differentiation, migration, invasion, and apoptosis (3). The transient receptor potential cation channel subfamily M member 7 (TRPM7) is a “chanzyme,” meaning it is an ion channel as well as an enzyme/kinase. The TRPM7 ion channel has a dominant role as a magnesium and calcium transporter (5). It is closely associated with cellular growth and development, also contributing to the development of several cancers, such as breast, pancreatic, and glioblastoma cancers (3, 6). In glioblastoma multiforme, TRPM7 has shown an immense inhibitory growth effect when blocked (6). This channel has additionally driven the proliferation of colon cancer, indicating that targeting this factor could potentially be an effective treatment (7).

TRPM7 could potentially play a major role in gastric cancer. Magnesium concentrations have been shown to be elevated in cancerous stomach tissue compared to levels in healthy stomach tissue (8). Furthermore, a study has demonstrated that human gastric adenocarcinoma cells have TRPM7 channels that contribute to their cell growth and survival (9). Likewise, in cancerous bladder tissue, the TRPM7 channel is overexpressed compared to the noncancerous tissues (10). This overexpression of TRPM7 in both gastric and bladder cancer relates to increased metastasis, decreased apoptosis, and is an important component in cell survival, making it a potential therapeutic target (10).

Additionally, in cancers, such as pancreatic, breast, and lung cancer, TRPM7 mediates metastasis via the mitogen-activated protein kinase (MAPK) pathway. When TRPM7 is silenced, metastasis and phosphorylation levels of MAPK are lowered (11). Moreover, the TRPM7 calcium influx is necessary for extracellular regulated kinase (ERK) activity (12). MAPK is a serine/threonine--specific protein kinase that has the ability to regulate proliferation, apoptosis, differentiation, and cell survival. The MAPK pathway usually responds to extracellular signals; when the pathway is abnormally regulated, it oftentimes becomes involved in the occurrence and progression of cancer (13). Within the MAPKs, there exist three subcategories: the extracellular signal-regulated kinases (ERKs), the c-Jun-terminal kinases (JNKs), and p38 MAPKs (14).

Amongst the three, it was shown that the ERK pathway

have been significant in contributing to carcinogenesis, while JNKs and p38 MAPKs contribute to signaling mechanisms that control cellular response to several types of cellular stress (15; 16). Mutated ERK/MAPK pathways exist in more than half of human cancers, indicating a relationship between the ERK/MAPK pathway and tumorigenesis (13). Moreover, the ERK/MAPK pathway regulated the epidermal growth factor receptor (EGFR), which can induce the disassembly of adhesions (17). With this change in cellular adhesiveness, cells obtain cell motility which leads to metastasis. As a result, the ERK/MAPK pathway plays a role in inducing metastasis (17).

Most chemotherapeutic drugs are cytotoxic not only to cancer cells but to non-cancer cells as well. Therefore, treatments using herbs have become a novel source of treatment for cancers. Ginseng is one of the most popular herbal remedies and contains ginsenosides, also known as steroid-like saponins known for activating antioxidant enzymes. A study has suggested that ginsenosides cause cellular injury by damaging the DNA and causing endoplasmic reticulum (ER) stress (18). More specifically, ginsenoside Rd has the ability to inhibit TRPM7 channels that were overexpressed in kidney cells and human gastric adenocarcinoma cells as well as inhibit the phosphorylation of the MAPK pathway (18). Because of its role as a magnesium transporter, TRPM7 is essential for magnesium-dependent cell survival of gastric cancer cells and is also involved in apoptosis caused by ginsenosides, steroid-like saponins in ginseng, like ginsenoside Rd (9). Therefore, ginsenosides have many pharmacologic functions and could potentially be used to increase apoptosis in gastric cancer.

Another chemical, waixenicin A, is a marine organism-derived extract from a soft coral known as Sarcothelia edmondsoni and is an inhibitor of the TRPM7 channel. This substance is unsuccessful in inhibiting similar channels, such as TRPM6, TRPM2, and TRPM4, indicating that it is a relatively specific inhibitor of the TRPM7 ion channel (19). Another similar chemical that acts as an inhibitor is NS8593. NS8593 functions similarly and acts as a negative gate modulator for TRPM7. It is specific to the TRPM7 channel in relation to other TRP channels and has been shown to interfere with the magnesium influx and thereby inhibit motility in cultured cells (20). Motility is an important factor, as it promotes metastatic spread. Therefore, inhibiting motility could prove to be a successful approach for preventing the metastasis of these cancers (20). It is a chemical that is currently not used in any cancer treatments but could aid in successfully determining whether or not the TRPM7 ion channel is an effective target for treating gastric and bladder cancer.

Yet another candidate, curcumin is a polyphenolic compound that is derived from Curcuma longa L. rhizomes. Curcumin was recently shown to inhibit proliferation, induce apoptosis, and have an anti-tumor effect on various cancers, such as lung, breast, or colon cancer (21). In colon cancer cells, curcumin has activated the p38 MAPK and JNK signaling pathways through phosphorylation, without activating ERK and has suppressed certain anti-apoptotic proteins (22). Curcumin is also generally safe for use as stated by the FDA and is not in use as treatment for cancer yet.

A final potential treatment is icariin, a flavonoid extracted from a plant called Epimedium. It is able to activate or enhance activation of the cyclic AMP/Protein Kinase A pathway, which causes a cascade of inactivation. Cyclic AMP (cAMP)-dependent protein kinase A (PKA) can block signaling by the Raf-1 kinase. The Raf-1 kinase in turn activates MEK1/2, which leads to activation of ERK1/2. Therefore, icariin could potentially inactivate ERK1/2 and reduce metastasis (23).

We hypothesized that when ginsenoside Rd, NS8593, curcumin, and icariin are administered individually and in combination into AGS (a gastric cancer cell line) and T24 (a bladder cancer cell line) cells, there would be a similar increase in apoptosis, decrease in cell proliferation, ERK levels, and TRPM7 levels between the two cell lines. Due to the inefficiency of the current treatments for these cancers, there is a need to test for better and more effective drugs. Therefore, ginsenoside Rd, NS8593, curcumin, and icariin were tested in comparison to a docetaxel, a common chemotherapeutic drug on the market to compare their potency. All four of these treatments were tested separately and in combination on the AGS cell lines while ginsenoside Rd and NS8593 were tested individually and together on the T24 cell lines. NS8593 exhibited the most successful results individually in decreasing proliferation and increasing apoptosis for both cell lines and could pose to be a potential new treatment. NS8593 reduced the proliferation by 81.1% and increased apoptosis by 38.5% in AGS cells compared to the negative control and reduced the proliferation by 88.4% and increased apoptosis by 78.0% in T24 cells compared to the negative control.

RESULTS

To test the effect of novel cancer treatments on gastric cancer cells, we treated AGS cells with different combinations of curcumin, ginsenoside Rd, icariin, and NS8593 and measured the cellular response through four assays. A proliferation assay was performed to compare the reduction in proliferation of each of the treatments to the positive and negative controls. Similarly, the apoptosis assay was conducted to compare the increase in apoptosis of each treatment to the positive and negative controls. ERK and TRPM7 ELISAs were conducted in order to test for a correlation between the activity of these two pathways and proliferation and apoptosis.

AGS gastric cancer cell lines, which express high levels of TRPM7 and ERK, were treated with ginsenoside Rd, NS8593, curcumin, and icariin individually and in combination and were compared to untreated cell lines and cell lines treated with the standard-of-care docetaxel. Combination treatments were done to test for any unknown synergistic effects and determine whether it would be advantageous to use multiple

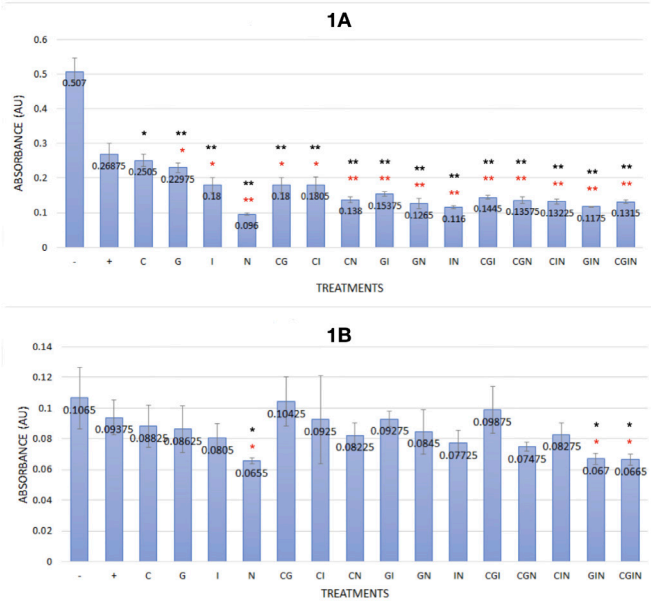

Figure 1: Proliferation and apoptosis levels after treatment in AGS cells. (A) Proliferation and (B) apoptosis of AGS cells were measured using the absorbance from WST-8 proliferation and alamarBlue apoptosis assays. Cells were treated with either curcumin (**C**) at 105 μM, ginsenoside Rd (**G**) at 3.2 μM, icariin (**I**) at 85 μM, or NS8593 (N) at 30 μM individually or in combination with each other. Measurements were compared to a negative control (media only) and a positive control (docetaxel) at 1.5 μM. Values presented represent an average of 4 trials and error bars represent the standard deviation. The black asterisks represent statistical significance compared to the negative control, while red asterisks represent statistical significance compared to the positive control. Two asterisks denote a *p*-value < 0.005, and one asterisk indicates a *p*-value < 0.05.

treatments. Following the AGS gastric cancer cells, the T24 bladder cancer cell lines, which also express high levels of TRPM7, were treated with ginsenoside Rd and NS8593 separately and in combination and compared to the cells in regular medium and those treated with docetaxel.

The results of the proliferation assay on AGS cells showed that all test treatments, with the exception of curcumin alone, significantly reduced proliferation compared to the positive control, docetaxel. Overall, the most effective treatment for reducing proliferation was NS8593 alone. Moreover, most of the treatments showed a significant reduction in proliferation compared to docetaxel, the positive control, and the negative control, cells treated with regular medium **(Figure 1A)**. All treatments individually and in combination had a significant difference when compared to the negative control; on the other hand, all but curcumin had a significant difference when compared to the positive control. NS8593 was most effective when used alone, but when used in combination with other treatments it became less effective. When compared to the positive control, for example, NS8593 had its effect reduced when put in the combination with all treatments (p = 0.00178 vs. p = 0.002271).

Similar to the proliferation results, the apoptosis assay showed the greatest induction of apoptosis for cells treated with either NS8593 or the combination treatment of all the chemicals. The results of the apoptosis assay on AGS cells showed that only NS8593 and the combination of ginsenoside, icariin, and NS8593 compared to the positive control, significantly increased apoptosis. Overall, the most effective treatment for increasing proliferation was NS8593 alone **(Figure 1B)**. We have found a significant difference between the positive control and NS8593 (p = 0.012571), the combination of ginsenoside, icariin, and NS8593 (p = 0.0303490, and the combination of all treatments (p = 0.008813). Likewise, when comparing to the negative control, we have found a significant difference between with NS8593 (p = 0.020874), the combination of icariin and NS8593 (p = 0.03456), and the combination of ginsenoside, icariin, and NS8593 (p = 0.035997), and the combination of all treatments (0.026487).

For the T24 cells, the proliferation assay showed that all three treatments compared to both the positive and negative

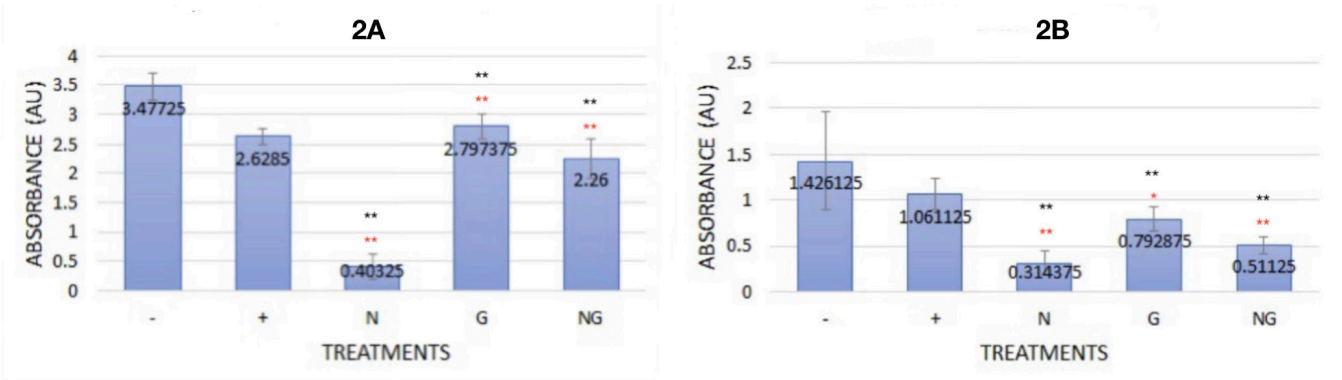

Figure 2: Proliferation and apoptosis levels after treatment in T24 cells. (A) Proliferation and (B) apoptosis of T24 cells were measured using the absorbance from WST-8 proliferation and alamarBlue apoptosis assays. Cells were treated with either ginsenoside Rd (**G**) at 3.2 μM or NS8593 (**N**) at 30 μM individually or in combination with each other. Measurements were compared to a negative control (media only) and a positive control (docetaxel) at 1.5 μM. Values presented represent an average of 8 trials and error bars represent the standard deviation. The black asterisks represent statistical significance compared to the negative control, while red asterisks represent statistical significance compared to the positive control. Two asterisks denote a *p*-value < 0.005, and one asterisk indicates a *p*-value < 0.05.

control significantly decreased the proliferation **(Figure 2A)**. When comparing to the positive control, there is a significant difference with NS8593 (p = 4.654E-08), ginsenoside Rd (p = 3.59E-080, and the combination of NS8593 and ginsenoside Rd (p = 0.000272). Similarly, we have found a significant difference between the negative control and NS8593 (p = 2.309E-080), ginsenoside Rd (p = 0.000505), and the combination of NS8593 and ginsenoside Rd (p = 1.74E-05). Similarly, in the apoptosis assay, all three treatments had significantly increased apoptosis **(Figure 2B)**. Additionally, there is a significant difference between the positive control and NS8593 (p = 2.3089E-05), ginsenoside Rd (p = 0.017224), and the combination of NS8593 and ginsenoside Rd (p = 2.41E-05). We also have found a significant difference between the negative control and NS8593 (p = 0.000165), ginsenoside Rd (p = 0.006192), and the combination of NS8593 and ginsenoside Rd (p = 0.001769).

As the ERK antigen levels decrease, the apoptosis levels increase **(Figure 3A)**. There is a general, weak negative cubic correlation between apoptosis and ERK antigen levels with an R^2 = 0.3936. As the TRPM7 antigen levels decreased, so did the proliferation levels **(Figure 3B)**. There is a strong positive quadratic correlation shown in the graph with R^2 = 0.8058. Similarly, as the TRPM7 antigen levels decreased, the apoptosis levels increased (Figure 3C). There is a weak positive quadratic correlation shown with R^2 = 0.3111. Similarly, as the ERK antigen levels decrease, so did the cell proliferation level **(Figure 3D)**. There is a moderately strong quadratic correlation between proliferation and ERK with an R^2 = 0.5596.

DISCUSSION

The overall most effective treatment in decreasing the proliferation and increasing apoptosis was the NS8593 in AGS cells. Additionally, when there were lower levels of TRPM7 and ERK, there were also lower levels of proliferation and higher levels of apoptosis. Following the AGS gastric cancer cells, the T24 bladder cancer cells, which also express high levels of TRPM7, were treated with ginsenoside Rd and NS9503 separately and in combination and compared to positive and negative controls. The most effective treatment in decreasing proliferation levels was NS8593, while the most effective in increasing apoptosis was ginsenoside Rd in T24 cells with a correlation to lower TRPM7 levels as well. The difference in effects of the treatments on the gastric cancer cell lines versus the bladder cancer cell lines may be due to different pathways and functions that cause the cells to react differently.

The observed decreases in cell proliferation in the T24 cells may have been partly due to the increase in apoptosis as well as the effects of inhibiting the two pathways. The rate of cell division may have been decreased due to the functions of TRPM7 such as its role as a magnesium and calcium transporter limiting the magnesium dependent survival of gastric and bladder cancer or due to ERK no longer regulating the cell survival and division. The results supported that inhibiting the ERK and TRPM7 pathway was effective in reducing proliferation and increasing apoptosis in the cancers related to these pathways. Similarly, to the reasons as to why cell proliferation decreased, apoptosis had increased due to the dependency of these cells on the TRPM7 and/or ERK and no longer having those functions.

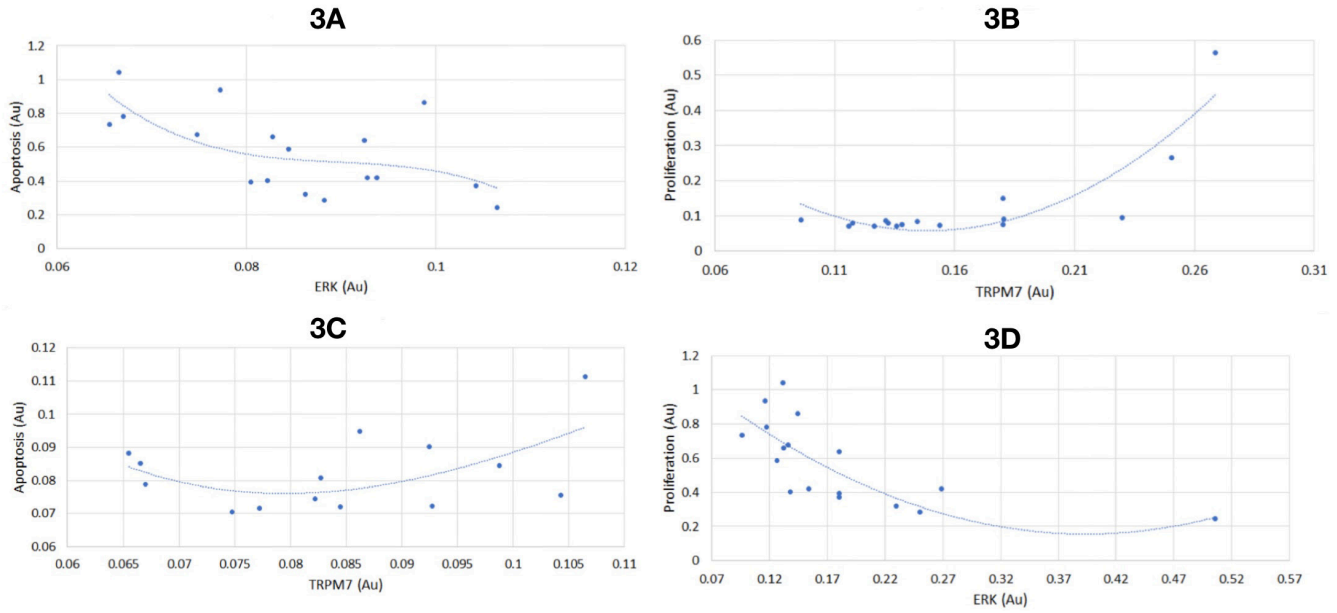

Figure 3: Relationship between apoptosis and proliferation with ERK and TRPM7 antigen levels. (A) Apoptosis versus ERK antigen levels (B) proliferation versus TRPM7 antigen levels (C) apoptosis versus TRPM7 antigen levels (D) proliferation versus ERK antigen levels. The data show a general correlation between the pathway levels and apoptosis and proliferation; as the pathways' levels decrease, the apoptosis levels increase, and proliferation decreases.

This study showed that ginsenoside Rd, NS8593, curcumin, and icariin are effective in reducing proliferation and inducing apoptosis in both gastric and bladder cancer cell lines through the TRPM7 and ERK pathway. These treatments could pose a more effective treatment for bladder and gastric cancer compared to the harsh chemotherapeutics. Many of the chemotherapeutics are cytotoxic to normal cells in addition to cancerous ones, therefore finding more natural and plant derived extracts could be a possible solution to these issues. With further research onto the side effects that NS8593 and the plant extracts might cause, a more effective and less painful treatment than current chemotherapeutic drugs could be developed.

The results from this experiment could aid in discovering treatments for more than just gastric and bladder cancer. In addition to bladder and gastric cancer, the TRPM7 ion channel is overexpressed in cancers such as pancreatic, breast, prostate, glioblastoma, and ovarian cancers (11). ERK pathway is overexpressed in cancers including prostate and gallbladder cancer (23).

Further research that would provide more information about the treatments' effect on the cancer cells could be additionally conducting a migration assay to measure their efficiency in treating metastasis. Moreover, in order to see the consequences or side effects these treatments may cause, testing on fibroblasts and seeing their effect on proliferation and apoptosis may be an option. It would also be beneficial to conduct these tests on other noncancerous stomach and bladder cells and any other cells that surround these areas; this would test if the treatments are cytotoxic to normal cells. As well as beginning to test on animal models and seeing their effect and any fatal consequences in vivo.

Additionally, future research that could be conducted would be testing on more cell lines that have overexpressed TRPM7 and ERK levels to further see if the treatments are effective across all cancers containing these pathways.

There were many limitations present in this study. Due to limitations such as a time constraint and a limited budget, more trials and assays such as a migration assay could not be conducted. The culture conditions proved challenging, and lack of accurate equipment made exact calculations difficult. Additionally, each well had an approximation of the same number of cells although they did not begin or end with exactly the same number.

In conclusion, these treatments could yield a potential new alternative to treat gastric and bladder cancer than current existing treatments. With further testing, searching for treatments that are derived from plants may pose to be a possible solution to the current harmful effects of existing drugs. Moreover, these results could aid in finding treatments for other cancers that also are dependent on these two pathways for their survival.

MATERIALS AND METHODS

RPMI 1640, a medium for cells, supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (P/S) was used to culture the gastric cancer (AGS) cells. Meanwhile the McCoy's 5A medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (P/S) was used to culture the bladder cancer (T24) cells. The treatments were dissolved in dimethyl sulfoxide (DMSO) and diluted in the respective culture medium. For the negative control, only medium was used without adding DMSO. Ginsenoside Rd (MedChem Express, New Jersey), NS8593 (Tocris, Minnesota), curcumin (MedChem Express, New Jersey), icariin (MedChem Express, New Jersey), and docetaxel (MedChem Express, New Jersey) were diluted to 3.2 μM, 30 μM, 105 μM, 85 μM, and 1.5 μM solutions respectively. These concentrations are commonly used in literature about these chemicals.

A WST-8 cell proliferation assay was conducted on the AGS and T24 cells according to manufacturer instructions using the WST-8 Cell Proliferation Assay (Cayman Chemicals, Michigan). Afterwards, an alamarBlue apoptosis assay (ThermoFisher Scientific, Massachusetts) was performed on the AGS and T24 cells according to the manufacturer instructions. Next, a TRPM7 ELISA assay (Biobool, Hong Kong) was conducted on the two cell lines according to manufacturer instructions. The last assay conducted was an ERK ELISA assay (ExpressBio, Maryland) following the manufacturer instructions on the AGS cells. The WST-8 cell proliferation assay is used to study the inhibition of cell proliferation in in vitro models. It is based on NADH causing the extracellular reduction of WST-8 in the mitochondria that yields a water-soluble formazan that dissolves in culture medium. Absorbance is then measured, the higher the absorbance, the higher the cell count. The alamarBlue cell viability reagent is a resazurin-based solution that can quantitatively measure viability. Resazurin is a non-toxic compound and can easily enter the cell. It is initially blue, and when entering living cells, it is reduced to resorufin which is red in color. These changes were measured using absorbance. The lower the absorbance, the more apoptosis. In the TRPM7 ELISA, the lower the absorbance, the lower the antigen levels. In the ERK ELISA, the higher the absorbance, the lower the antigen levels.

Using Excel, a 1-way ANOVA was also performed as a general comparison amongst all the groups per assay; Tukey's post hoc test was used to compare the treatments to the positive and negative controls. Values less than 0.05 were given one black asterisk and values below 0.005 were given two black asterisks when compared to the negative control and one red asterisk for *p*-values below 0.05 and two red asterisks for *p*-values below 0.005 when compared to the positive control.

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REFERENCES

1. National Cancer Institute. "What Is Cancer?" *National Cancer Institute*, 9 Feb. 2015, www.cancer.gov/about-cancer/understanding/what-is-cancer.
2. Dicken, Bryan J., *et al*. "Gastric Adenocarcinoma." *Annals of Surgery*, vol. 241, no. 1, Jan. 2005, DOI:10.1097/01.sla.0000149300.28588.23.
3. Kim, Byung Joo, and Chansik Hong. "Role of transient receptor potential melastatin type 7 channel in gastric cancer." *Integrative Medicine Research*, vol. 5, no. 2, June 2016, pp. 124-30, doi:10.1016/j.imr.2016.04.004.
4. American Cancer Society. "Bladder Cancer." *cancer.org*, 2019.
5. Dhennin-Duthille, Isabelle, *et al.* "TRPM7 involvement in cancer: a potential prognostic factor." *Magnesium Research*, vol. 27, no. 3, 2014.
6. Sanders, Philip, *et al.* "Vacquinol-1 inducible cell death in glioblastoma multiforme is counter regulated by TRPM7 activity induced by exogenous ATP." *Oncology*, vol. 8, no. 21, 30 Mar. 2017, doi:10.18632/oncotarget.16703.
7. Huang, Junhao. "Inhibition of TRPM7 suppresses cell proliferation of colon adenocarcinoma in vitro and induces hypomagnesemia in vivo without affecting azoxymethaneinduced early colon cancer in mice." *Cell Communication and Signaling*, vol. 15, no. 30, 2017, DOI10.1186/s12964-017-0187-9.
8. Pasternak, K., and W. Przyszlak. "Magnesium in stomach cancer." *Magnesium Research*, 1999.
9. Kim, Byung Joo, *et al.* "Suppression of transient receptor potential melastatin 7 channel induces cell death in gastric cancer." *Cancer Science*, vol. 99, no. 12, 3 Dec. 2008, doi:10.1111/j.1349-7006.2008.00982.x.
10. Gao. "TRPM7 is overexpressed in bladder cancer and promotes proliferation, migration, invasion and tumor growth." *Oncology Reports*, vol. 38, no. 4, Oct. 2017, doi:10.3892/or.2017.5883.
11. Meng, Xiaojing, *et al.* "TRPM7 mediates breast cancer cell migration and invasion through the MAPK pathway." *Cancer Letters*, vol. 333, no. 1, 1 June 2013, pp. 96-102, doi:10.1016/j.canlet.2013.01.031.
12. Takahashi, Kiriko, *et al.* "TRPM7-mediated spontaneous Ca^{2+} entry regulates the proliferation and differentiation of human leukemia cell line K562." *Physiological Reports*, vol. 6, no. 14, July 2018, doi:10.14814/phy2.13796.
13. Yang, Mei, and Chang-Zhi Huang. "Mitogen-activated protein kinase signaling pathway and invasion and metastasis of gastric cancer." *World Journal of Gastroenterology*, 7 Nov. 2015, doi:10.3748/wjg.v21.i41.11673.
14. Liu, Minghua, *et al.* "Development of Certain Protein Kinase Inhibitors with the Components from Traditional Chinese Medicine." *Frontier in Pharmacoloft*, 9 Jan. 2017, doi:10.3389/fphar.2016.00523.
15. Dhanasekaran, D. N., and Reddy, E. P. (2008). JNK signaling in apoptosis. *Oncogene* 27, 6245-6251. doi. 10.1038/onc.2008.301
16. Nakao, Yoichi, and Nobuhiro Fusetani. "Enzyme Inhibitors from Marine Invertebrates." *Journal of Natural Products*, 16 Mar. 2007, pp. 689-710, doi:10.1021/np060600x.
17. Xie H, Pallero MA, Gupta K, Chang P, Ware MF, Witke W, Kwiatkowski DJ, Lauffenburger DA, Murphy-Ullrich JE, Wells A. EGF receptor regulation of cell motility: EGF induces disassembly of focal adhesions independently of the motility-associated PLCgamma signaling pathway. *Journal of Cell Science*, 1998;111(Pt 5):615–624.
18. Kim, Byung Joo. "The role of ginseng total saponin in transient receptor potential melastatin type 7 channels." *Animal Cells and Systems*, vol. 16, no. 5, 27 Apr. 2012, doi:10.1080/19768354.2012.680495.
19. Zierler, Susanna. "Waixenicin A Inhibits Cell Proliferation through Magnesium-dependent Block of Transient Receptor Potential Melastatin 7 (TRPM7) Channels." *The Journal of Biological Chemistry*, 16 Sept. 2011, doi:10.1074/jbc.M111.264341.
20. Chubanov. "Natural and synthetic modulators of SK (Kca2) potassium channels inhibit magnesium-dependent activity of the kinase-coupled cation channel TRPM7." *British Journal of Pharmacology*, vol. 166, no. 4, June 2012, doi:10.1111/j.1476-5381.2012.01855.x.
21. Banerjee, M., Singh, P., and Panda, D. (2010). Curcumin suppresses the dynamic instability of microtubules, activates the mitotic checkpoint and induces apoptosis in MCF-7 cells. *The FEBS Journal*, 277, 3437–3448. doi: 10.1111/j.1742-4658.2010.07750.x
22. Collett, Gavin P., and Frederick Charles Campbell. "Curcumin induces c-jun N-terminal kinase-dependent apoptosis in HCT116 human colon cancer cells." *Carcinogenesis*, vol. 25, no. 11, 1 Nov. 2004, pp. 2183-89, doi:10.1093/carcin/bgh233.
23. Guegan, Jean-Philippe, *et al.* "The MAPK MEK1/2-ERK1/2 Pathway and Its Implication in Hepatocyte Cell Cycle Control." *International Journal of Hepatology*, 24 Oct. 2012, doi:10.1155/2012/328372.