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Comparative Analysis of Icarrin and Curcumol as a Therapy for Pancreatic Cancer and Breast Cancer

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Abstract:

Breast cancer is the second most common type of cancer in American women. The incidence of pancreatic cancer has significantly risen over the past few decades. This study investigates whether icariin (ICA) and curcumin can show a more pronounced ability to inhibit tumor cell proliferation, induce apoptosis, and suppress cell migration and invasion in pancreatic and breast cancers. However, the specific impacts on different cancer types may vary. The objective of this research is to assess and compare the influence of ICA and curcumol on SNU-2466 pancreatic cancer cells and MCF7 breast cancer cells. The findings demonstrate that ICA and curcumol demonstrate a better effect on pancreatic cancer cells than in MCF7 breast cancer cells. Additionally, these compounds exhibit a stronger inhibitory effect on the PI3K/Akt/mTOR signaling pathway in pancreatic cancer cells compared to breast cancer cells. These results suggest that ICA and curcumol have more significant therapeutic potential in treating pancreatic cancer.

Keywords:—Breast cancer, Pancreatic cancer, Phosphatidylinositide 3-kinases signaling pathway, Traditional Chinese Medicine

1. Introduction

Cancer is one of the major causes of death worldwide and its causes are complex and usually the result of a series of triggers, including genetic factors, environmental factors, and so on. The consequences of cancer are multifaceted and serious as well. If some highly malignant cancers are diagnosed too late, the treatment effectiveness will be reduced and the mortality rate will be increased. Data for 2020 indicates that the number of new cancer cases worldwide will rise to 19.3 million, and the number of deaths is estimated to be around 10 million [1].

Breast cancer is a heterogeneous disease with one of the highest incidence rates among women worldwide [2,3]. Data from the 2020 International Agency for Research on Cancer study shows that there were more than 2.26 million new cases of breast cancer worldwide. The number of new cases in 2022 is expected to exceed 2.31 million, making breast cancer

the second most common cancer. Breast cancer is also the fourth leading cause of cancer death [4]. Figure 1 illustrates the most common cancer site among female worldwide, and breast cancer is the most common type of all cancers.

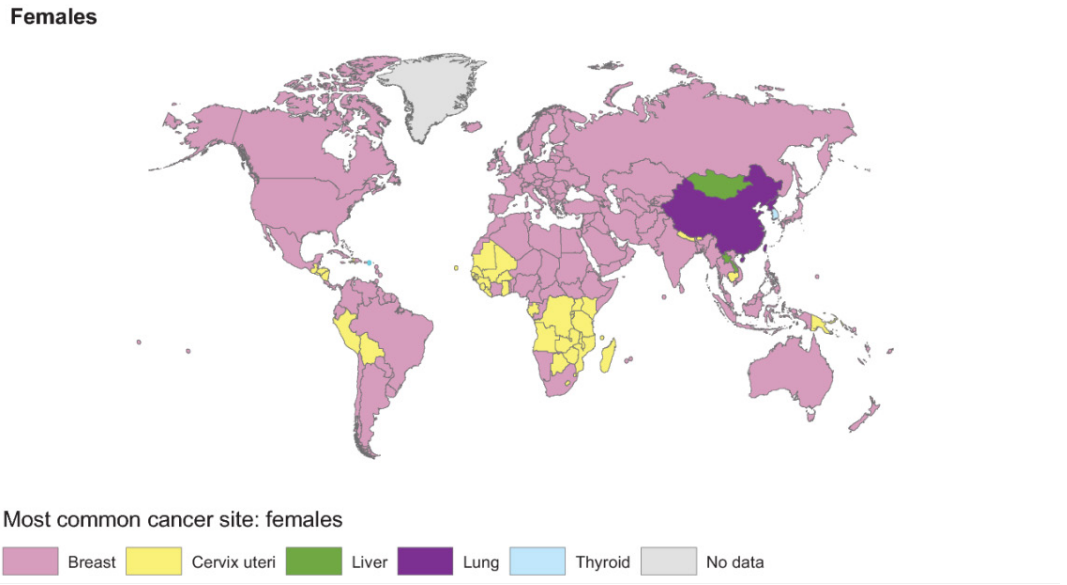

Figure 1 Most common cancer site: females [5]

Pancreatic ductal adenocarcinoma (PDAC) is the third most common cause of cancer death in the USA [6]. And there will be approximately 57,600 new cases in 2020, resulting in 47,050 deaths [7]. Therefore, it is critical to select patients who are capable of complete and curable resection. Although researchers have improved the detection and control of pancreatic cancer, only about 4 percent of patients survive five years after diagnosis. Survival rates are higher if the cancer is found only in the pancreas and nowhere else. Given the current level of medical treatment, the only chance of cure is through surgical removal [7]. However, unfortunately, 80-85% of patients are diagnosed when the malignant tumor is already impossible to remove. According to Figure 2, the incidence rate is highest in North America, Europe, and Argentina, followed by East Asia and Australia. In addition, pancreatic cancer does not respond well to most chemotherapy drugs. Therefore, it is necessary to understand the biological mechanisms of pancreatic cancer development and better treatments at each stage. This can not only reduce the side effects of treatment for patients, but also improve long-term survival [8,9].

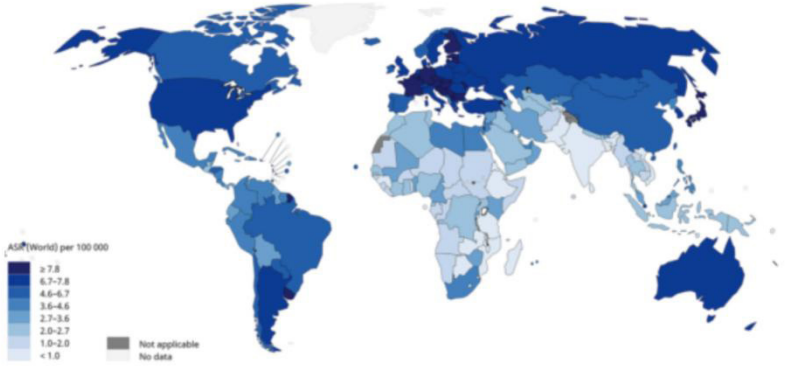

Figure 2 Age-standardized incidence rates (ASR) of pancreatic cancer across the globe in 2020. [10]

In recent years, with the rapid development of biochemistry and molecular biology, researchers have made important breakthroughs in cancer treatment. Standard treatments can improve the prognosis of people with breast cancer. However, compared to the standard treatment, Traditional Chinese Medicine(TCM) and TCM therapies are rarely mentioned in depth in the field of lung cancer. Therefore, starting from the theory of TCM, this paper

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will explore the inhibition and effect of TCM on cancer in order to promote the development of modern medicine [11]. There are increasingly numerous publications looking for the relationship between Traditional Chinese Medicine and cancer and trying to find if humans can inhibit cancer progression [12]. A study shows that the cancer cell proliferation was effectively inhibited, and apoptosis and reactive oxygen species(ROS) levels were increased. ICA and curcumol treatment successfully induced autophagy and ferroptosis in PCa cells. When the two drugs are combined, the treatment is more effective [13]. In this research, researchers are going to focus on two specific types of cancer: pancreatic cancer and breast cancer. And study the effect of TCM on the aforementioned cancers.

Figure 3 and Figure 4 show the structures of curcumol and icarrin(ICA). As we all know, the structure of a molecule determines its function and property. Curcumol has anti-cancer, antibacterial, anti-inflammatory and other pharmacological effects. These effects have been demonstrated in vitro in vivo and in clinical trials. Icariin (ICA) is a flavonoid compound that can be isolated from the Chinese herb Herba epimedium. It has biological benefits in many ways, such as treating osteoporosis and cardiovascular disease and regulating immune function and has been studied for its role in modulating various cellular processes involved in cancer progression [14]. ICA also has Anti-tumor properties and multiple mechanisms involving the regulation of lymphocyte balance and anti-inflammatory factors, and can also regulate a variety of signaling pathways, such as NF-kappaβ, Erk-p38-JNK, etc [15].

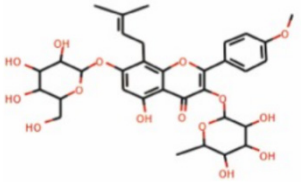

Figure 3 ICA Structural Formulae [16]

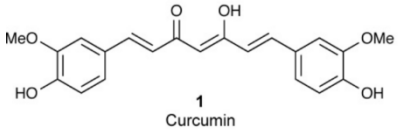

Figure 4 Curcumin Structural Formulae [16]

The PI3K/AKT/mTOR (PAM) signaling pathway is a highly conserved signal transduction network in eukaryotic cells that promotes cell survival, cell growth, and cell cycle progression. Growth factor signaling to transcription factors in the PAM axis is highly regulated by multiple cross-interactions with several other signaling pathways, and regulation of signal transduction can predispose to cancer development. The PAM axis is the most frequently activated signaling pathway in human cancer and is often implicated in resistance to anticancer therapies [16].

The SNU-2466 pancreatic cell line and the MCF7 breast cancer cell line have been intensively investigated recently [17]. Based on the characteristics of the PI3K signaling pathway and the two types of cell lines, plus with the above discussion, ICA and curcumol influence the cell proliferation, cell migration, cell invasion, and apoptosis of SNU-2466 pancreatic cancer cells and MCF7 breast cancer cells through up-regulating the expression of pi3k signaling pathway, and then control the downstream signaling pathway.

Researchers have studied the role of ICA and curcumin products in the prevention and treatment of various cancers, including colorectal cancer and liver cancer. However, researchers do not yet know the effects of ICA and curcumin products on the treatment of breast and pancreatic cancer [18]. This article aims to provide a deeper understanding of the therapeutic effects of ICA and curcumol on pancreatic and breast cancers. Insights gained from this research could potentially lead to the development of novel and more effective treatment strategies, improving the prognosis and living quality for patients suffering from these cancers.

2. Hypothesis

The combined effect of icarrin and curcumol has a remarkable effect on prostate cancer. I predict that icarrin(ICA) and curcumol compared to no treatment reduce cell migration and invasion, cell growth, and PI3K/Akt/mTOR signaling more in SNU-2466 pancreatic cancer than in MCF7 breast cancer cells.

3. Material and Method

Measure the viability, cell migration, and phospho-AKT of each type of cancer cell to compare the differences in the treatment effects of ICA and curcumin on pancreatic cancer and breast cancer. Expression of PI3K signaling was assessed by p-AKT western blots. Cell migration was measured by Boyden chamber assay. Cell growth was detected by MTT assay. The concentration of ICA and curcumol was measured by two-dimensional titration. The bioavailability of curcumin is relatively low, so higher concentrations are required, and the ratio of ICA and curcumin is 1:5. Therefore, the concentration range of ICA is 3-10 micromolars, and that of curcumin is 15-50 micromolars. This concentration will be used for all of

the experiments. Additionally, in the experiment, the positive control is taxioland and the negative control is PBS/DMSO. The control groups will be applied in all of the experiments. The treatment duration will be discussed in each method.

3.1 Western blot

This research use western blot to detect the PI3K pathway. Pancreatic cancer cells and breast cancer cell samples are needed, and researchers also need electrophoresis equipment, membrane transfer equipment, specific primary antibodies (for PI3K path-related proteins), secondary antibodies, etc.[19] First, extract the total protein of cells or tissues, then SDS-PAGE electrophoresis was performed to isolate the proteins. Transfer the proteins to a PVDF or NC membrane and seal the membrane with skim milk. Next, incubate the primary antibody at 4℃ overnight. After washing the film, incubate the second antibody at room temperature. Finally, wash the film again for color reaction [19]. Considering the difference in protein expression and regulation rate, researchers set the treatment duration at 3, 6, and 9 days. At the same time, it can be compared with the positive control group in order to understand the treatment effect at different time points [20,21]. The experiment will be repeated 5 times [22].

3.2 MTT assay

MTT assays were conducted to assess cell proliferation [22]. Firstly, the cell suspension is prepared and inoculated on 96-researchers plates. ICA and curcumin solution was added. After culturing for 24 hours, MTT solution is added for further incubation that will last for 4 hours at 37℃, then supernatant is removed, and DMSO is added for dissolution and crystallization. The absorbance is determined by enzymolysis assay for the next seven days to get a trend of cell growth [23]. The treatment durations are 3 hours, 6 hours, 12 hours and 24 hours. Repeat the process 3 times to obtain reliable data.

3.3 Boyden chamber assay

Prepare the Boyden cells and treat the membrane pores. Use sterile phosphate-buffered saline (PBS) to wash the cells and add appropriate chemokines to the lower chamber. Cultivate for 4 hours at 37℃ and then stop the reaction. Stain the cells that pass through the membrane pores. Count the stained cells using a cell counter [24]. The treatment duration is 3 hours, 6 hours, 12 hours, and 24 hours. This experiment will be repeated 3 times to ensure the reliability and accuracy of the data [25,26].

3.4 Two-dimensional titration

This method aims to determine the optimal concentration of ICA and curcumin, respectively, in a mixture of drugs to make sure each drug would have a 50% effect. Prepare the solution of ICA and curcumin of known concentration. researchers need to fix ICA at a certain concentration and titrate curcumin solution. By varying the amount of the two reagents, multiple combinations of reaction results can be obtained. Then, the concentration of ICA was increased ten-fold, and curcumin was titrated.[28] The results are reflected in a two-dimensional matrix, where the rows and columns correspond to the different amounts of the two reagents added, and each cell records the corresponding reaction result. Then, using methods such as multiple regression to analyze the data in the matrix, the concentration of the two components can be determined simultaneously. This method can improve the accuracy and efficiency of measurement. This experiment will be performed 3 times [27].

To make the process easier, researchers can also use another method to find the ratio of these two drugs that need to be used. First, prepare ICA and curcumol solution. Next, slowly increase the amount of ICA and curcumin and find the volume of each when killing cancer cells and calculate the ratio of dosages and determine the concentration of each drug. Then researchers can alter their concentrations together to find the optimal concentration. For each test, its concentration will be lower concentrated than the previous test concentration.

3.5 Statistical Analysis

Each experiment will be conducted at least three times, and the data was reported as "± standard deviation" . researchers need to use GraphPad Prism 6.0 to test P-value. There would be a significant difference between the two sets of data if the P-value is lower than 0.05.

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4. Possible Results

Table 1 A comparison of the effects of ICA and curcumol on signaling pathways, cell migration, and cell activity in pancreatic and breast cancer

Combination Result # (CR#)				
	TCM decrease viability more in SNU-2466 than MCF7 by MTT?	TCM decrease migration more in SNU-2466 than MCF7 by Boyden assay?	TCM decrease phospho-AKT more in SNU-2466 than MCF7 by Western blot?	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	-	Partial
4	-	+	+	Partial
5	-	-	+	Partial
6	-	+	-	Partial
7	+	-	+	Partial
8	-	-	-	Fullly contradicted

"+" indicates that the result performs similarly or better than the positive control and is consistent with the hypothesis . "-"indicates the result performs similarly or worse than the negative control and is not consistent with the hypothesis.

Table 1 demonstrates the possible results of each experiment. And the description for each result are shown below.

CR1: SNU2466 is much lower active than MCF7, has fewer cell migrations, and SN
U-2466 is more inhibited than MCF7 in phospho-AKT expression. This supports the hypothesis.
CR2: SNU2466 is much lower active than MCF7, has fewer cell migrations, and SNU-2466 is lower inhibited than MCF7 in phospho-AKT expression.
CR3:SNU2466 is much lower active than MCF7, has more cell migrations, and SNU-2466 is lower inhibited than MCF7 in phospho-AKT expression.
CR4:SNU2466 is much more active than MCF7, has fewer cell migrations, and SNU-2466 is more inhibited than MCF7 in phospho-AKT expression.
CR5:SNU2466 is much more active than MCF7, has more cell migrations, and SNU-2466 is more inhibited than MCF7 in phospho-AKT expression.
CR6:SNU2466 is much more active than MCF7, has fewer cell migrations, and SNU-2466 is lower inhibited than MCF7 in phospho-AKT expression.
CR7:SNU2466 is much lower active than MCF7, has more cell migrations, and SNU-2466 is more inhibited than MCF7 in phospho-AKT expression.
CR8:SNU2466 is much more active than MCF7, has more cell migrations, and SNU-2466 is lower inhibited than MCF7 in phospho-AKT expression. This completely contradicts the hypothesis.

5. Discussion

The experimental findings of the test groups from the inhibition of ICA and curcumol in pancreatic cancer and breast cancer cells present a complex and nuanced understanding of the role of ICA and curcumol in the progression of the two types of cancer. The results from CR1 indicate a more significant reduction of the viability and migration of SNU-2466 than MCF7, leading to a corresponding decrease in cell proliferation. This result is consistent with the initial hypothesis, which suggests that TCM has a stronger inhibitory effect on SNU-2466 cells than that on MCF7 cells.
From CR2—CR8, some of the experimental results do not agree with the hypothesis. The possible explanations of the inconsistent results might be diverse, including differences in concentration and reaction time. For example, if researchers increase the treatment duration in some trials, the results may change as a longer time may be needed to see the expected results. However, the guesses about the reasons need to be tested further.
For CR2, SNU-2466 shows a lower decrease in phospho-AKT levels compared to MCF7. The PI3K/Akt/

mTOR pathway might have alternative activation mechanisms in SNU-2466 that are not as effectively targeted by ICA and curcumol. For example, there might be a mutation in the PI3K/Akt/mTOR pathway that can lead to constitutive activation of these proteins and keeps the pathway active regardless of upstream signaling and inhibition. Also, the overexpression or heightened sensitivity of receptor tyrosine kinases (RTKs) can drive some pathways to change, such as the overexpression of EGFR or HER2 [28]. Future experiments can include alternative pathway analysis. researchers can explore other signaling pathways that might be compensating for the PI3K/Akt/mTOR pathway inhibition, including JAK/STAT, NF-kB, or Wnt/β-catenin pathways and measure phospho-Akt levels and cell response to determine if these pathways are contributing to resistance [29,30].

According to CR3, SNU-2466 shows a lower decrease in migration and a lower decrease in phospho-AKT levels compared to MCF7. The explanation is similar to CR2. Migration and PI3K/Akt/mTOR signaling might be regulated by different pathways in SNU-2466 that are not as sensitive to ICA and curcumol treatment [31]. And researchers can conduct targeted pathway inhibiting studies for the future experiment.

The possible reasons for CR4 SNU-2466 might be inappropriate concentration and exposure time duration. So, researchers ought to ensure that the concentration and treatment duration of ICA and curcumol are sufficient to affect cell viability. To determine the optimal concentration, a dose-response study with these two drugs is needed [32]. And the optimal exposure duration can be identified with time-course experiments. In general, the resulting concentration and time will increase compared to that used in the original experiment. The new concentration might be 10 micromolar for ICA and 50 micromolar for curcumin. With the new concentration and duration. And the most likely result is that with increasing concentration and duration, the more consistent the observations will be with the hypothesis. The reason why researchers cannot see the expected result at first may be the inadequate concentration and duration [33]. Finally, researchers can measure cell viability at various concentrations and time points using the MTT assay.

Through CR5, SNU-2466 shows a lower decrease in cell viability and a lower decrease in migration compared to MCF7. One possible reason is that both cell growth and migration in SNU-2466 might involve compensatory pathways that are not present or not as active in MCF7. The other is the insufficient drug concentration and exposure time to impact viability and migration. To improve this experiment, researchers can either perform optimization of drug concentration and exposure time that researchers have discussed in CR4 or use combination therapy with migration agents to enhance the anti-migration effect of ICA and curcumin. Combine ICA and curcumol with migration-inhibiting agents such as Rho kinase inhibitors (e.g.Y-27632) or MMP inhibitors (e.g. marimastat) and use Boyden assay and wound healing assay to estimate the combined effect on cell migration [34,35].

The part of CR6 that is different from the hypothesis is SNU-2466 which shows a lower decrease in cell viability and in phospho-AKT levels compared to MCF7. Since the combination of ICA and curcumin might not effectively target the viability and the PI3K/Akt/mTOR pathway in SNU-2466, there may be a compensatory alternative pathway in SNU-2466. To identify and inhibit the specific pathway, researchers need to combine ICA and curcumol with inhibitors targeting specific isoforms of PI3K/Akt/mTOR (e.g. BYL719 for PI3K, MK-2206 for pan-Akt, Torin1 for mTOR). And evaluate the combined effects on phospho-AKT levels, cell viability, and migration.

Reasons that may cause the phenomenon presented in CR7 may be additional factors or pathways that can affect the migration in SNU-2466 and different drug sensitivities. This way, even if the concentration and treatment duration are close to perfect, the observed results are not what is expected. To identify genetic and transcriptional differences between SNU-2466 and MCF7 that may result in differential drug sensitivity, researchers can use transcriptomic and genomic analysis. First, Perform RNA-seq and whole-genome sequencing on both cell lines before and after ICA and curcumol treatment [36]. Next, identify differentially expressed genes and genetic mutations associated with drug resistance. Finally, use PCR to test the hypothesis.

For CR8, the most likely reason for this result is the insufficient drug concentration and reaction time. As a result, researchers can optimize the drug concentration and exposure time that researchers have discussed in CR4 and CR5.

In general, when researchers reconsider these negative results, the explanation is the inadequate duration and concentration. The easiest future experiment to perform is conducting longer-term and high-concentration experiments to observe whether the negative results are transient and if prolonged treatment leads to different outcomes. If the results become more and more supportive of the hypothesis, it means that higher concentrations and longer reaction times can make the drug more effective.

Researchers do not rule out the possibility of the emergence of alternative pathways, and it seems very likely. Therefore, researchers need to figure out whether it is the compensatory effect of the pathway that is causing the results that are not consistent with the hypothesis. Gene

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knockdown studies will be applied. It can help determine if the observed resistance is due to alternative pathways. For instance, use siRNA or CRISPR-Cas9 to knock down specific genes in SNU-2466 and observe changes in response to ICA and curcumin treatment [37]. If the knockdown of specific survival genes results in decreased cell viability when treated with ICA and curcumin, then this outcome would suggest that the targeted genes play a crucial part in the survival of SNU-2466 cells. The observed resistance is likely due to these genes compensating for the effects of ICA and curcumol. However, if there is no significant change in cell viability, then there might be other pathways or factors contributing to the resistance, or the targeted genes are not the primary drivers of survival in these conditions. And researchers should perform broader pathway analyses to identify other potential survival pathways or consider targeting multiple pathways simultaneously.

In future experiments, once the researchers have accurate and reliable data interpretation, it will be possible to further explore the role of ICA and curcumol in the two cancers.

6. Conclusion

The data suggest that compared to no treatment, ICA and curcumol have a more significant effect on treating SNU-2466 pancreatic cancer cells than on MCF7 breast cancer cells. By conducting future experiments, it may be possible to gain a deeper understanding of the mechanisms behind the differential responses of SNU-2466 pancreatic cancer and MCF7 breast cancer to ICA and curcumol, potentially leading to more effective treatment strategies and can provide insights for future studies of using Traditional Chinese Medicine to treat a broader range of cancers with similar apoptosis pathways.

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