Does the Glandular Tolerant to McL-1 Inhibitor S63845?

Xuming Chang

Abstract

MCL-1 is an anti-apoptotic protein that regulates cell apoptosis and has been observed in over-expression in many cancer. It makes the MCL-1 an available target. S63845 is introduced to treat cancer, an inhibitor with a high affinity to MCL-1. However, the tolerability of the human body to the treatment remains unanswered. Therefore in this paper, we designed the experiment to test the tolerability of regular issue glandular with cancerous individuals of the same cell line, the apoptosis will be tested by the MTT theory, and the direct production of inhibition --BAX will be measured by western blot. The result is predicted, and this work will provide meaningful information for future cancer treatment. Future studies should focus on cooperation with other BCL-2 inhibitors and the impact of the treatment.

Keywords: MCL-1 BCL-2 s63845 BAX CRC Glandular

1. Introduction

1.1. *CRC*

Colorectal cancer (CRC) is currently the third most common cancer in both men and women [1]. About 95% of colorectal cancers develop in glandular cells that make up the lining of the colon and rectum [1]. Incidence and mortality rates have been declining since the 1980s, partially due to improvements in screening and prevention [1]. In 2021, the American Cancer Society estimated that approximately 104,270 new cases would be diagnosed and 52,980 deaths due to colorectal cancer in the United States [1]. With current medical treatments, cancer can only be cured entirely by resection, with a risk of recurrence. Other medical treatments, such as chemotherapy and physiotherapy, can only inhibit or slow cancer growth in most cases. The recent study on cancer cell apoptotic leads us to a potential target protein MCL-1.

1.2. *MCL-1*

Myeloid leukemia 1 (MCL-1) is an antiapoptotic protein of the BCL-2 family [2]. The BCL-2 family divide into 3 groups: anti-apoptotic proteins(BCL-2, MCL-1, BCL-xl) ; multi-domain pro-apoptotic proteins(BAX,BAK) and pro-apoptotic BH3-only proteins (Puma, Bad) [2]. Normally in cells, anti-apoptotic proteins behave as an inhibitor of the Multi-domain pro-apoptotic proteins by binding with them. In the apoptosis model, BH3-only proteins will be overexpression and act as an inhibitor by binding to the anti-apoptotic so the multi-domain pro-apoptotic proteins will have a chance to attach to the membrane of mitochondria and release cytochrome c, which launches the apoptosis of the cancer cell. However, in cancer cells, overexpression of MCL-1 is frequently observed in many tumor types and is closely associated with tumorigenesis, poor prognosis, and drug resistance [2]. The presence of vary amounts of MCL-1 proteins gives the cancer cells a strong resistance to apoptosis by itself, so the mitochondrial apoptotic pathway is largely regulated by MCL-1, making it a desirable target for cancer treatment [2]. Therefore, further research led to the discovery of MCL-1 inhibitor s63845.

1.3. *S63845*

S63845 is a small molecule that binds with MCL-1 to inhibit it [3]. S63845 binds human MCL1 with a KD of 0.19nM (surface plasmon resonance) and mouse MCL1 with an around 6-fold lower affinity, with no appreciable binding to BCL-2 or BCL-XL [4]. The s63845 behaves similarly to a BH3-only protein with high affinity to the MCL-1 proteins. After s63845 is released and bound to MCL-1 just like the normal apoptosis, the proapoptosis protein BAK/BAX will attach to the membrane of mitochondria and initiate the apoptosis of the cancer cell, which changes s63845 into a potential molecule for curing cancer that has primarily depended on MCL-1.

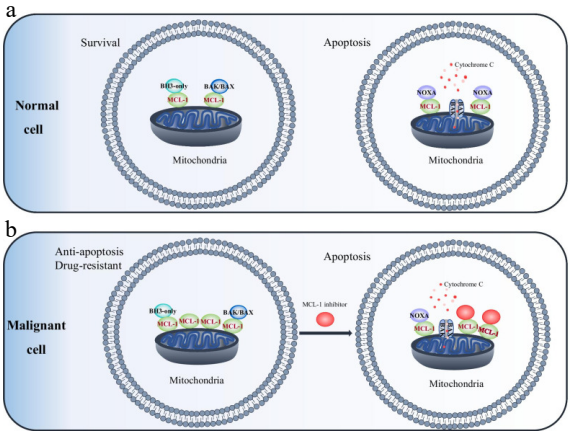

Figure 1. Cells apoptosis by inhibit MCL-1

However, in glandular which are the original cells of CRC, MCL-1 binds with BAX/BAK proteins making them unavailable to initiate apoptosis [5]. Moreover, conditional gene knockout studies have shown that MCL1 is essential for the survival of hematopoietic stem cells, cardiomyocytes, and several other critical cell types, raising the tolerability issue when targeting MCL1 [4]. Furthermore, there were no similar drugs like MCL-1 inhibitors. Therefore, the critical question is the tolerability, which means that reduction in MCL-1 levels does not negatively impact the general health of normal tissues, in this case, glandular cells, to the inhibitor s63845. The answer to this question remains blank.

Research question: Since inhibiting MCL-1 can lead to apoptosis of a Colorectal cancer cell, will normal cells be affected or apoptosis by the treatment? In normal cells, MCL-1 sequesters the BH3-only activators BIM, BID, and PUMA or neutralizes the effector proteins BAX and BAK, thereby antagonizing apoptosis. Therefore, the treatment of inhibiting MCL-1 will deal equal damage to both cancer cells and normal cells.

Hypothesis: I predict CRC cells have a relative higher sensitivity to MCL-1 inhibition than the glandular cells because CRC has a higher expression and dependency on MCL-1. Measure cell death by MTT assay, and measure the product of MCL1 inhibition by western blot. The positive control is Taxol and the negative control is PBS/DMSO.

2. Methods

2.1. *Materials*

This experiment will use section samples of CRC and glandular cells, MCL-1 inhibitor s63845, and Taxol.

All groups will be cultured in 5mM solution and placed for 4 hours under the constant temperature of 37 degrees with a constant pH of 7.4 and 5% of CO2 [4,6]. The samples will be divided into four groups. Each group will have a sample of CRC cells and a sample of glandular cells. 3 of 4 groups will use two negative controls and one positive control. In negative control A, nothing will be changed to ensure the effect of DMSO. In the negative control B, both samples will be added DMSO, and in the positive group, Taxol will be added to ensure the most significant possible effect of the s63845. The remaining group will add the s63845 solution, which is the experiment itself. The experiment will repeat three times to confirm the result.

2.2. *MTT Cell Viability Assay*

MTT colorimetry was used to assess cell viability. For the duration of the experiment, control (untreated) cells were maintained in an exponential growth phase by being injected at a density of 96 well microplates [7]. The cells were exposed to the substance for 48 hours, followed by 4 hours of incubation with 1 mg/ml 37°C MTT. At 540 nm, the absorbance was measured 18 hours after adding the cracking buffer (20 percent SDS) [7]. At least three times, each of the tests was repeated. Take the average of the percentages of live cells in each well. Percentage Growth was estimated as (OD treated cells / OD control cells) / 100, where IC50 was the concentration and represented the optical density decrease of 50% determined by linear regression. On the dose-response curve's linear portion [7].

2.3. *Immunodetection.*

Immunodetection, known as western blot. It is used to detect the product of MCL-1 inhibition, which are the BAX proteins that remain in the cell. Cells were treated with the aforementioned substances for 6 hours after being seeded, then collected in lysis buffer (10mM HEPES pH 7.4, 142.5mM KCl, 5mM MgCl2, 1mM EDTA, 1 percent NP40, protease, and phosphatase inhibitor cocktails (Calbiochem)) 24 hours later [8]. Using the MSD apoptosis panel whole cell lysate kit (MSD) in 96-well plates as directed by the manufacturer, cleared lysates (5 g protein) were produced for immunodetection of cleaved PARP (a marker of apoptosis).

Table 1. possible result list

Possible observation	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6	Result 7	Result 8
S63845 releases more BAX proteins in CRC than glandular cells?	+	-	+	-	+	-	+	-
s63845 increases MTT apoptosis in CRC?	+	+	-	-	+	+	-	-
s63845 increases MTT apoptosis in glandular cells?	+	+	+	+	-	-	-	-
Support Hypothesis	partly	no	no	no	yes	yes	partly	no

Note. "+" represents a significant decrease in cell proliferation. "-" represent not significantly different from negative control

All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The P value less than 0.05 was considered statistically significant [10].

Possible Result 1: According to the negative control group, s63845 lead to an increase in apoptosis in both CRC cells and glandular cells, and CRC cells increase more than glandular cells in BAX proteins.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 2: According to the negative control group, s63845 lead to an increase in apoptosis in both CRC cells and glandular cells, and glandular have a greater increase of BAX proteins.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 3: According to the negative control group, s63845 lead glandular cells to have a greater increase of BAX proteins, and increase of apoptosis only in glandular cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 4: According to the negative control group, s63845 increases the releases of BAX proteins in CRC cells. s63845 increased the apoptosis in the glandular cells, but not a significant change in CRC cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 5: According to the control group, s63845 increases the releases of BAX proteins in CRC cells, and only increases apoptosis in CRC cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 6: According to the negative control group, s63845 increased BAX remains in the glandular cells, but the apoptosis increased in CRC cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 7: According to the negative control group, s63845 increased BAX remains in both CRC cells and glandular cells, but no change occurs in apoptosis of both cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

Possible Result 8: According to the negative control group, s63845 increased the BAX remaining in glandular cells more than in CRC cells, but no change occurred in the apoptosis of both glandular cells and CRC cells.

Calculated the percentage of the viable cells by the following formula: (%) = [(sample abs)/ (control abs)×100]. All values obtained were represented as mean±standard error (SE) of at least three independent experiments. The result was considered statistically significant when P value was lower than 0.05.

3. Discussion

Previous studies report is meanly focused on the effectiveness of the s63845 on one or more specific cancer. Little was done about the effect on the surrounding cells with a structure similar to cancer cells. Wei AH and his team have investigated the use of MCL-1 inhibitors in curing specific cancer and the potential when cooperating with BCL-2 inhibitors [9]. Xiang Wang and his team are focused on the theory of the BCL-2 family and the effect of the MCL-1 in the cancer cell [5], but both researches leaves a gap in the effect of the normal issues when inhibiting MCL-1. In this study, normal issue glandular are treated in the same condition as CRC. By acquiring measurements we could know the change that occurred in the cell..

Possible result 1: the result partly supports the hypothesis of glandular tolerability in both CRC and glandular cells, by the inhibition of MCL-1 s63845 bind to MCL-1 and release BAX proteins, but by the expression of BCL-2 family glandular receive lease negative impact in the absent of MCL-1 protein, more BAX proteins are released

and bind to the mitochondria and initiate the apoptosis of CRC cells. Therefore the CRC cells are sensitive of the CRC treatment.

Possible result 2: the result contradicts the hypothesis. Glandular cells have a great er increase in pro-apoptotic protein BAX, but both CRC and glandular cells increase apoptosis in the MTT result. The release of BAX protein in CRC cells binds with other BCL-2 families. Therefore glandular cells are sensitive under the CRC treatment

Possible result 3 : the result contradicts the hypothesis. s63845 increases the release of BAX proteins greater in CRC cells, but the MTT result apoptosis does not change in the CRC cells. It may cause by other members in the BCL-2 family that could bind with the BAX proteins. Therefore, the CRC cells have higher tolerability of s63845 than the glandular cells.

Possible result 4: the result contradicts the hypothesis of glandular tolerability, s63845 increases BAX protein in glandular cells over the CRC, and the MTT only results increase in glandular cells. The result may cause by the expressions of other BCL-2 anti-apoptotic in the CRC cells. Therefore CRC may have resistant to the s63845.

Possible result 5: the result supports the hypothesis. s63845 increases BAX proteins in CRC greater than in glandular cells, but the apoptosis only occurs in CRC cells. It may cause by other members in the BCL-2 family that could bind to inhibit the BAX proteins. Therefore, glandular have greater tolerability to s63845.

Possible result 6: the result supports the hypothesis. s63845 increase BAX proteins in glandular greater than CRC cells, but the apoptosis only occur in CRC cells. It may cause by other members in the BCL-2 family that could bind to inhibit the BAX proteins. Therefore, glandular may have resistant to s63845.

Possible result 7: the result partly supports the hypothesis. s63845 increases BAX proteins more in glandular cells than CRC cells, but no changes in apoptosis, which means that both CRC and glandular cells have other BCL-2 family members and glandular have resistant to s63845, but CRC is only tolerant to s63845.

Possible result 8: the result contradicts the hypothesis. s63845 increases BAX proteins more in CRC cells than glandular cells, but no changes in apoptosis, which means that both CRC and glandular cells have other BCL-2 family members, and CRC cells have resistant to s63845, but glandular cells are only tolerant to s63845.

4. Conclusion

In conclusion, this study explores the effect of s63845 on CRC and glandular. The experiment result would indicate whether the apoptosis of normal cells in the body will be affected by the inhibition of MCL-1. It also provides an idea of the impact when s63845 is used in cooperation with other BCL-2 inhibitors. The observed inhibition effect would provide a suitable indication for future clinical and pharmaceutical development against the negative impact on the human body and accelerate the treatment applied to cancer curing. Future research should focus on cooperation with other BCL-2 inhibitors in cancer treatment, and the negative impact should be tested.

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