The inhibition of growth and metastasis of human pancreatic ductal adenocarcinoma by MART-10

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Abstract
Previous studies demonstrated that MART-10, an analog to the active form of vitamin D3, has anti-cancer properties in anaplastic thyroid cancer (ATC). It can inhibit ATC’s metastasis by altering cadherin protein expression and preventing the EMT process. This study aims to investigate the effect of MART-10 in a different system, pancreatic ductal adenocarcinoma (PDAC), in both in vitro and in vivo conditions. Methods: The study will use two known PDAC cell lines. The cells will be treated with increasing MART-10 for various durations. In vitro metastasis will be measured by trans-well migration and Matrigel invasion assay, in vitro proliferation will be measured by CCK-8 assay, and the interaction between MART-10 and PDAC will be investigated with Western blot. Mice will be injected with tumor cells and treated with increasing MART-10; in vivo tumor growth and metastasis will be recorded weekly. The positive control for the experiments is ADH-1 (Exherin), and the negative control is PBS in DMSO. Possible results: There are three main possible results: (1) MART-10 inhibits the growth and metastasis of PDAC cells; (2) MART-10 acts as a stimulant for PDAC growth and metastasis; (3) MART-10 has no significant effect on the growth and metastasis of PDAC. Conclusion: The result of the study will provide important insight into the preclinical effectiveness of MART-10 in PDAC; it also sets the basis for future clinical studies of the drug. Future studies should investigate the mechanism underlying MART-10’s effectiveness in PDAC or search for drug combinations with MART-10 that synergize the disease.

Keywords: pancreatic ductal adenocarcinoma, MART-10, cadherin, orthotopic xenograft

1. Introduction
As one of the most aggressive solid exocrine malignancies and the fourth leading cause of cancer-related death [1], pancreatic ductal adenocarcinoma (PDAC) induces a significant and rapidly escalating incidence rate around the world. In the last decades, considerable improvements have been achieved in the screening and therapy of various types of solid cancers, drastically increasing patients’ survival and recovery rate. However, despite the advancements in research on pancreatic cancer, the mortality rate for patients with PDAC has not experienced significant improvement in the last few decades, with a one-year overall survival rate of around 24%, and a five-year survival rate of less than 9% [2].

This detrimental outcome of PDAC is associated with delayed diagnosis, which is often a consequence caused by a lack of visible and distinctive symptoms and reliable biomarkers for prognosis. Furthermore, PDAC is associated with a high potency of metastasis to adjacent organs such as the liver and gallbladder, thus escalating the difficulty of prognosis and treatment [3]. Therefore, by inhibiting its metastatic potential, a more suitable therapy for PDAC may be developed, potentially benefiting the clinical prognosis and treatment of PDAC patients.

Epithelial-to-mesenchymal transition (EMT) is a process that provides cancer cells with a metastatic phenotype and thus plays a vital role in the progression and chemoresistance of cancers [4]. For cancer cells to metastasize, they must lose their cell-cell adhesiveness and detach from the original tumor. One factor contributing to such a transition is the change in the expression of cadherins, a family of membrane glycoproteins that mediate calcium-dependent cell-cell adhesion [5,6]. E-cadherins are down-regulated during cancer EMT, usually expressed by epithelial cells. In contrast, N-cadherins, represented by mesenchymal cells, is upregulated, thus resulting in a loss of epithelial phenotypes and a de novo acquisition of mesenchymal phenotypes, rendering the cancer cells motile [7].

As a type of cancer with high metastatic potency, it is curious to investigate if inhibiting the EMT process or altering the cadherin composition of PDAC cells would inhibit PDAC growth and metastasis. Previous research has shown that a synthetic peptide ADH-1, which serves as an N-cadherin antagonist in PDAC cells, significantly reduced the progression and metastasis of PDAC [8]. The active form of vitamin D₃, calcitriol, is also shown to be effective against various types of metastatic cancers [9-11]. However, the clinical application of calcitriol to treat
cancer is still limited because the effective concentration of calcitriol to carry out its anticancer function exceeds the physiologically sustainable concentration of calcitriol, thus inducing severe side effects such as hypercalcemia [12,13].

More recently, a synthetic analog for calcitriol, MART-10 (19-nor-2α-(3-hydroxypropyl)-1α,25(OH)2D3), was discovered to be less hypercalcemia-inducing. At the same time, MART-10 can alter the expression of cadherins on cancer cells through transcriptional and translational controls, thus inhibiting cancer cell growth [14]. An experimental study by Chiang’s group investigated the efficacy of MART-10 in anaplastic thyroid cancer (ATC) [15]. With treatment of MART-10 at different concentrations, both the migration and invasion capacity of ATC cells was shown to be decreased to different extents. Furthermore, treated ATC cells had upregulated E-cadherin count and downregulated N-cadherin count, and reduced F actin formation. Chiang’s results suggest that MART-10 can inhibit the metastatic potential of ATC via the attenuation of the EMT process in cancer cells, therefore supporting the anti-cancer function of MART-10 in vitro. Additionally, MART-10 is shown to be effective at inhibiting various types of cancer growth and metastasis both in vitro and in vivo by several other studies [16-18].

However, despite being a type of cancer with a high likelihood of metastasis and a high mortality rate, the investigation of MART-10’s efficacy in PDAC remains unclear. Therefore, this study aims to elucidate the effect of MART-10 treatment in PDAC both in vitro and in vivo.

Hypothesis: Due to the similarity between PDAC and ATC, such that both types of cancers pathogenically display high potency of metastasis and elevated N-cadherin count, it is predicted that MART-10 would be effective in reducing or inhibiting metastasis of PDAC cells, just as has been seen for ATC. Further, it is also expected that the expression of E-cadherin will be increased while the expression of N-cadherin will be decreased in PDAC cells under treatment. Finally, the viability and growth of PDAC cells will be reduced both in vitro and in vivo.

2. Materials

2.1 Reagents

19-nor-2α-(3-hydroxypropyl)-1α,25(OH)2D3 (MART-10) will be synthesized and obtained via Julia olefination as described [19]. ADH-1 (Exherin) will be purchased from companies.

2.2 Cell lines

Two human PDAC cell lines (BxPC-3 and PANC-1) will be used to compare MART-10’s efficacy. BxPC-3 cells will be grown in RPMI 1640 medium, and PANC-1 cells will be produced in the DMEM medium. Both cultures will be supplemented with 2mM glutamine, 10% fetal bovine serum (FBS), 1% penicillin, and streptomycin and will be incubated at 37°C with 5% CO2 and 95% humidity; the culture medium will be changed three times per week. All required cell lines, medium, and supplemental chemicals will be purchased from companies.

2.3 Laboratory animals

40 male BALB/c Nude mice, age 4 weeks old with an average weight of 15 - 20g, will be obtained; mice will be fed with a routine rodent diet and water ad libitum. The mice will be housed in a sterile environment and allowed to acclimatize for ten days before any experiments [20]. All animal experiments and care will be by the guidelines of institutional authorities.

3. Methods

3.1 In vitro migration and invasion assays

Trans-well migration and Matrigel invasion assays will be performed using trans-well plates (24-well plates, 8μm-pore-size polycarbonate membrane); the membrane for trans-well migration assays will be left uncoated. In contrast, for Matrigel invasion assays, the membrane will be coated with a layer of Matrigel extracellular matrix proteins. BxPC-3 and PANC-1 cells will be pre-treated with MART-10 (10^-5, 10^-7, 10^-6 M), ADH-1 (0.2mg/ml), or phosphate-buffered saline (PBS) in dimethyl sulfoxide (DMSO) for two days. RPMI 1640 medium (or DMEM medium, corresponding to the cell culture) with 20% FBS will be added to the lower chamber of the plates as a chemoattractant, and 4.0×10^4 pre-treated cells suspended in the serum-free medium will be seeded to the upper section of each well. Dishes will be incubated at 37°C, 5% CO2, and 95% humidity for 24 hours in migration assay and 48 hours in invasion assay. After incubation, cells remaining in the upper chamber will be removed by sterile swap, and cells in the lower section will be fixed and stained with 0.1% crystal violet dye for 30min. Three independent visual fields from each membrane will be examined via microscopic observations, and the number of stained cells will be counted [21, 22]. Both trans-well migration and Matrigel invasion assays will be performed in triplicate.

3.2 Western blot

After two days of pre-treatment with MART-10, ADH-1, or PBS, cells will be collected, rinsed with PBS once, and lysed with RIPA lysis buffer. Cell lysates will then be centrifuged at 12000rpm for 10min at 4°C. The
concentration of the protein content in the resulting supernatant will be quantified using BCA Protein Assay Kit to ensure equal loading of samples. Proteins will then be electrophoresed through sodium dodecyl sulfate/polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. After incubation with 4% non-fat milk in Tris-buffered saline and Tween-20 for 1 hour at room temperature to block non-specific binding, the membrane will be rinsed and incubated with rabbit monoclonal primary antibodies against E-cadherin, N-cadherin, and tubulin overnight at 4°C with agitation. The membrane will then be washed with TBST 3 times for 5min each and incubated with HRP-conjugated anti-rabbit secondary antibody for 1 hour at room temperature. Bands will be visualized through the ECL system after 3 washes with TBST for 5min each [15, 23]. The expression of targeted proteins relative to tubulin (as the loading control) will be calculated.

3.3 CCK-8 cell proliferation assay

BxPC-3 and PANC-1 cell viability and proliferation under MART-10’s effect will be assessed with CCK-8 assay. After two days of pre-treatment, cells will be played at a 96-well plate with a density of 2.0×10^3 cells/well. After culturing for 24, 48, 72, and 96 hours, a complete medium containing 10% CCK-8 reagents will be added to respective wells at the indicated time points. After 1 hour of incubation in the dark at 37°C, the optical density of the corresponding wells will be measured at 450nm absorbance using a microplate reader [24]. The experiment will be performed in triplicate.

3.4 Animal studies

The mice will be randomly assigned into two groups of 20; each group will receive an orthotopic injection of a different tumor cell line. BxPC-3 and PANC-1 cells will be trypsinized and suspended in PBS. The cell suspension will then be washed and incubated with rabbit monoclonal primary antibodies against E-cadherin, N-cadherin, and tubulin overnight at 4°C with agitation. The membrane will then be washed with TBST 3 times for 5min each and incubated with HRP-conjugated anti-rabbit secondary antibody for 1 hour at room temperature. Bands will be visualized through the ECL system after 3 washes with TBST for 5min each [15, 23]. The expression of targeted proteins relative to tubulin (as the loading control) will be calculated.

3.5 Flow cytometry

Cell density will be measured before the corresponding experiment to ensure an accurate number of cells used in each of the above experiments using flow cytometry. Tumor cells will be trypsinized and then washed and suspended in PBS. The cell suspension will then be incubated with APC anti-mouse CD274 monoclonal antibody for 30min at 4°C. Flow cytometry analyses will then be performed on BD FACSCalibur [28].

3.6 Statistical analysis

The statistical significance of all numerical data acquired from migration/invasion assays, western blot, CCK-8 assay, and animal studies will be analyzed using the student’s T-test, with the level of significance set to p < 0.05.

4. Possible Results

4.1 Description of each combination

Combination of possible results 1 (CR1): There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

Combination of possible results 2 (CR2): There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

Combination of possible results 3 (CR3): There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. On the western blot, the band of E-cadherin is lighter. At the same time, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no
significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 4 (CR4):** There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is lighter, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 5 (CR5):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 6 (CR6):** There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 7 (CR7):** There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is thicker, while the band of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

**Combination of possible results 8 (CR8):** There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is lighter. At the same time, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 9 (CR9):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is lighter. In comparison, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 10 (CR10):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is thinner, while the band of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 11 (CR11):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is thicker, while the crew of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The
growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

**Combination of possible results 12 (CR12):** There are fewer cells in the lower chamber after the invasion and migration assay for the MART-10 treated groups than for the negative control group. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is lighter. In comparison, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

**Combination of possible results 13 (CR13):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is lower than that for the negative control group. On the western blot, the band of E-cadherin is lighter. In comparison, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

**Combination of possible results 14 (CR14):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is thicker, while the band of N-cadherin is lighter for the MART-10 treated groups than for the negative control group. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups.

**Combination of possible results 15 (CR15):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is lighter. At the same time, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is lower than in negative control mice.

**Combination of possible results 16 (CR16):** The number of cells in the lower chamber after the invasion and migration assay is significantly higher for the MART-10 groups than for negative control, or there is no significant difference between the two groups. The optical density measured for MART-10 treated groups in the CCK-8 assay is significantly higher than that for the negative control group, or there is no significant difference between the two groups. On the western blot, the band of E-cadherin is lighter. At the same time, the crew of N-cadherin is thicker for the MART-10 treated groups than for the negative control group, or there is no significant difference between the two groups. The growth rate of the orthotopic tumor in MART-10 treated mice is higher than in negative control mice, or there is no significant difference between the two groups (Table 1).

**Table 1: Table of a combination of possible results**

<table>
<thead>
<tr>
<th>Variety of possible outcomes (CR)</th>
<th>Decrease in vitro metastasis?</th>
<th>Decrease in vitro growth?</th>
<th>Increase the E to N cadherin ratio.</th>
<th>Decrease in vivo growth?</th>
<th>Does it support the hypothesis?</th>
</tr>
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<tr>
<td>CR1</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>CR2</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
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<td>+</td>
<td>Partially</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>Partially</td>
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<td>CR5</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>Partially</td>
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<tr>
<td>CR6</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>Partially</td>
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</table>
5. Discussion

5.1 Discussion about differential effects in two cell lines

The two cell lines that were originally chosen (BxPC-3 and PANC-1) have different responses when treated with MART-10 in any of the experiments conducted. This may be due to the different mechanistic nature of causing PDAC in the two tumor cell lines, or perhaps the two cell lines innately have different chemoresistance against drugs like MART-10. Any following experiments demonstrating such differential results would only partially support the hypothesis, and the experiment should be repeated with other PDAC cell lines to confirm MART-10’s efficacy.

5.2 Discussion about combinations of possible results

Previous studies frequently demonstrated that MART-10 exhibits anti-cancer properties in various metastatic cancers [15-18]. However, little is known about the effect of MART-10 in pancreatic cancer systems. Therefore, to test the therapeutic preclinical impact of MART-10 in PDAC systems, this study applies MART-10 treatment to two well-studied PDAC cell lines to establish an in vitro model and use orthotopically transplanted mice to establish an in vivo PDAC model under MART-10 treatment.

A combination of Possible results (CR) 1 is consistent with the findings of previous studies investigating the in vitro effect of MART-10 in ATC [15]. There are fewer cells present after the invasion and migration assay for the MART-10 treated groups than for the negative control groups, indicating that the metastatic potential of PDAC cells is reduced in vitro by MART-10. Similarly, a lower optical density as observed through the CCK-8 assay for the MART-10 groups than for the negative control groups indicate fewer MART-10-treated cells survived after the assay, therefore suggesting MART-10 also inhibits PDAC growth in vitro. Through western blot, an increase in the E- to N- cadherin ratio in the MART-10 treated groups suggest MART-10 upregulated the expression of E-cadherin and downregulated the expression of N-cadherin and as a result, potentially participates in the attenuation of EMT process in PDAC. Growth of PDAC is also reduced in vivo as shown by the animal experiments, the size of the orthotopic tumor would be growing at a lower rate for the MART-10 groups than for the negative control groups. Taken together, the results from CR1 would fully support the hypothesis and support an anti-metastasis and anti-proliferative role of MART-10 in PDAC.

In CR2, MART-10 has a complete in vitro effect; it can inhibit both growth and metastasis of PDAC cells in vitro and increase the E- to N- cadherin ratio, as demonstrated by the western blot. The in vivo effect of MART-10, however, is not observed. This could be explained if MART-10 indeed does not have any in vivo effect for PDAC, or this could be due to the dosage given to mice being too low for MART-10 to carry out its anti-cancer function. The animal experiment should be repeated with mice given higher dosages of MART-10. Meanwhile, raising MART-10 concentration would also increase the possibility of overdose toxicity; the mice treated with higher MART-10 dosages should thus be monitored extra carefully for any signs of side effects, such as hypercalcemia. Similar to CR2, in CR7 MART-10 does not support the hypothesis.

<table>
<thead>
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<th>Variety of possible outcomes (CR)</th>
<th>Decrease in vitro metastasis?</th>
<th>Decrease in vitro growth?</th>
<th>Increase the E to N cadherin ratio.</th>
<th>Decrease in vivo growth?</th>
<th>Does it support the hypothesis?</th>
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<td>-</td>
<td>-</td>
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</table>

Note: “+” represents the result of the experiment conducted supports the hypothesis, “-” represents the result contradicts the hypothesis.
not inhibit the growth of PDAC either in vitro or in vivo, this could be explained as MART-10 having no anti-proliferative effect against PDAC, it only serves to inhibit the metastasis of PDAC cells.

CR3 demonstrates that MART-10 is capable of inhibiting both the growth and metastasis of PDAC cells in vitro, as well as inhibiting the growth of PDAC tumors in vivo, however, the expected increase in E- to N- cadherin ratio is not observed in this combination. In principle, performing a western blot against E- and N- cadherins in this study aims to investigate whether the treatment of MART-10 can induce a reversal of the cadherin switch in PDAC cells, as seen in previous studies on ATC cells [15]. The results from this western blot could also serve as an indirect measure of the attenuation in the EMT process. In this case, although the results from the western blot contradict the hypothesis, the rest of the results in this combination still supports the hypothesis. Thus, the EMT process in PDAC is likely attenuated but through a different mechanism than expected.

On the contrary, while the result of the western blot in CR6, CR9, and CR12 contradicts the hypothesis, there are other parts of the developments in those combinations that also reject the idea; for instance, in CR6, MART-10 does not inhibit PDAC tumor growth in vivo, in CR9, MART-10 does not inhibit PDC cell metastasis in vitro, and in CR12, MART-10 does not inhibit PDAC cell growth in vitro. Those combinations not only do not support that MART-10 is not causing a cadherin switch, but also do not support an attenuation in the EMT process. As a result, those combinations can only partially support the hypothesis.

In CR4, it is curious to observe that when MART-10 does not inhibit PDAC growth in vitro, it does inhibit PDAC tumor growth in vivo. This pattern is also observed in various other combinations of results, such as CR8, CR10, and CR15. Such observations where an effect is not seen in vitro but seen in vivo are not expected as it opposes the common knowledge, and therefore contradict the hypothesis. One explanation for such results would be that there are errors in performing the animal experiment, one probability is that the number of tumor cells injected into each mouse is not equalized, leading to different tumor sizes in the first place. In this case, the experiment should be repeated with the same setup and extra caution.

In CR5, MART-10 does not inhibit metastasis of PDAC cells in vitro, while it is still capable of inhibiting the growth of PDAC cells both in vitro and in vivo. Similarly, CR5, CR11, and CR14 also show no in vitro anti-metastatic effect. However, in this case, it is not able to conclude that MART-10 has no anti-metastatic effect for PDAC in general because whether MART-10 can still inhibit PDAC tumor metastasis in vivo is not being tested in this study. As a result, CR4, CR5, CR11, and CR14 can only partially support the hypothesis as their data on the anti-metastatic ability of MART-10 on PDAC is inconclusive.

CR16 is the most minor expected outcome in all the possible combinations, it demonstrates that the results of every experiment conducted contradict the hypothesis. It would suggest that although the anti-cancer effect of MART-10 is supported by various evidence in other systems, MART-10 does not have any effect in treating PDAC.

6. Conclusion

In general, this study explores the effect of MART-10 in two different PDAC cell lines and xenografted mice. The results of this study will indicate whether or not the treatment with MART-10 is capable of inhibiting the proliferation and metastasis of pancreatic cancer in vitro and in vivo, and whether or not a change in the expression of cadherin membrane proteins mediates this inhibition. Such a favorable therapeutic effect of MART-10 would thus prepare the basis for its transition to clinical trials. The results of this study would also provide insights into the direction of future studies in the same field. The mechanism of interaction between MART-10 and PDAC can be further investigated, thus providing insights into the molecular pathways involved, and potentially revealing more therapeutic targets along the way. The combination of MART-10 with other anti-cancer drugs for PDAC, such as Taxol or ADH-1, can also be examined for potential synergistic effects between the drugs.

References


