Targeting MDM2 and CDK4/6 in HR+ Breast Cancer

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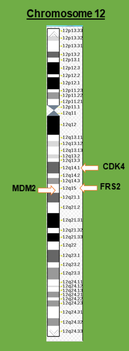
Cancer Background

Cancer is a genetic disease that occurs through unregulated cell proliferation. This can be due to a hyper-response to a growth factor ligand and/or through a mutation(s) in downstream signaling pathways. These mutations can suppress a tumor suppressor gene and/or excess an oncogene. Chemotherapy works to target proteins in affected pathways and force the cell into apoptosis.

Breast cancer is the second most common cancer in women and divides into four categories: HR+/HER2+, HR+/HER2-, HR-/HER2+, and HR-/HER2-. The positives represent overexpression, and the negatives represent the normal state. HR is a hormone receptor in the cell, and HER2 is the human epidermal growth receptor.

Research project

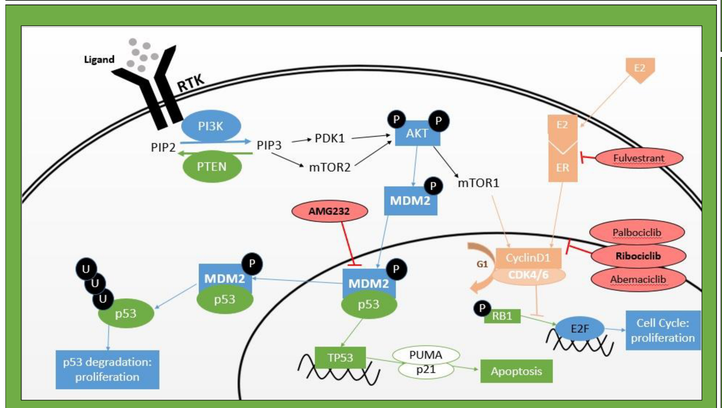
In my research, I worked with HR+/HER2- breast cancer cell lines to target oncogenes MDM2 and CDK4/6. These two oncogenes are in close proximity on chromosome 12, making them of interest.



The HR+ gives overexpression of CyclinD1, which is what binds to CDK4/6 and pushes the cell through the cell cycle by inhibiting tumor suppressor RB1.

I worked on HR+ cell lines MCF-7, which has a *PIK3CA* mutation that leads to overexpression, and ZR75-1, which has a PTEN mutation that silences its expression. These two mutations lead to an overexpression of MDM2, as well as CyclinD1. *TP53* and *Rb1* genes were both wildtype, which is important as they need to show regular expression level for treatments to work.

We used the chemotherapy drugs fulvestrant and ribociclib to inhibit the CyclinD1-CDK4/6 complex. We also used the chemotherapy drug AMG232, which inhibits MDM2, allowing p53 (tumor suppressor) to lead the cell through apoptosis.



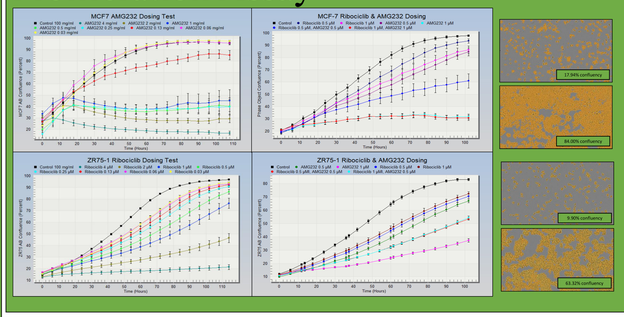
This cartoon shows the two pathways we targeted. This shows the MDM2 pathway in blue, the CyclinD1-CDK4/6 pathway in orange, the tumor suppressor genes p53 and RB1 in green, and the chemotherapy drugs that inhibit the oncogenes in red.

Hypothesis:

Inhibiting the overexpression of MDM2 and CDK4/6 will allow p53 to send the cell into apoptosis and RB1 to stop cells at the G1 phase. This will ultimately stop proliferation and tumor growth.

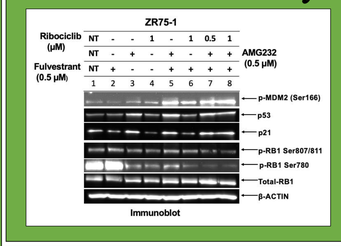
Results

Proliferation Assay



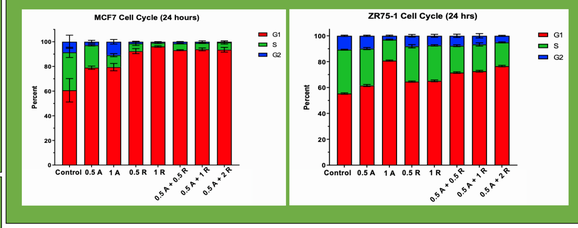
The top two graphs are testing cell line MCF7, and the bottom two are testing cell line ZR75-1. The graphs on the left are testing drug concentrations of ribociclib (MCF7) and AMG232 (ZR75-1) ranging from 4 uM to 0.03 uM concentrations. This allowed a prediction of drug concentrations. This was used for the graph on the right with drug combinations. These combinations were used later in all the experiments ran, especially the combination drugs, as the 0.5 uM AMG232 paired well with different concentrations of ribociclib.
The pictures of cells represent the cell line they are by. This shows the cell morphology. A computer program colored the cells in yellow, to measure confluency, which was used to graph.

Western Blot Assay



This is a Western Blot, which looks at the protein amount before and after treatment of drugs. I used different combinations of the drugs, as well as the drugs individually. I worked with the cell line ZR75-1.
With AMG232 treated cells, we saw the amount of phosphorylated MDM2 increase, meaning it did not get bound to p53 and degrade it. We also see higher concentrations of p53 and p21, a downstream protein that results from the p53 acting as a transcription factor.
With the CDK4/6 pathway, we see a decrease of phosphorylated RB1, indicating that the CDK4/6-CyclinD compound did not inhibit RB1, suggesting RB1 was allowed to stop the cell cycle.

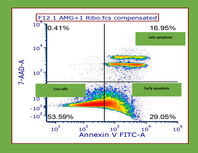
Cell Cycle Assay

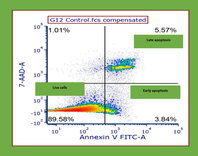


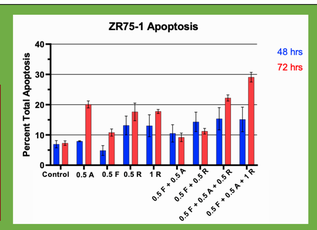
This assay measured the percentage of cells in each stage of cell division. The cells were treated with drugs in concentrations between 0.5-2 uM of drugs AMG232 (A) and Ribociclib (R). We saw that with treatment of ribociclib, there was an increase of cells in G1, indicating that there were more cells undergoing cell division. This would make sense, as ribociclib targets the CDK4/6-Cyclind1 complex, which induces the cell cycle. AMG232 affects the pathway that induces apoptosis of the cell.

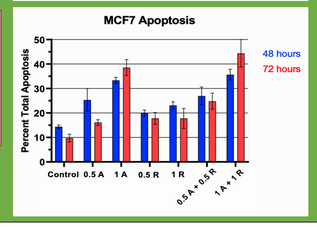
Apoptosis Assay

This assay measured the percentage of cells going through apoptosis. The cells were treated with drugs in concentrations between 0.5-1 uM of drugs AMG232 (A) and Ribociclib (R), and Fulvestrant (F) was only used in the ZR75-1 cell line. The cells were measured after 48 hours and 72 hours of treatment. The charts next to the graphs are examples of how we measured cell apoptosis, adding late and early apoptotic cells. With treatment of AMG232, we found that there was an increase of apoptosis in cells. We did not see much affect with Fulvestrant on the ZR75-1 cell line.





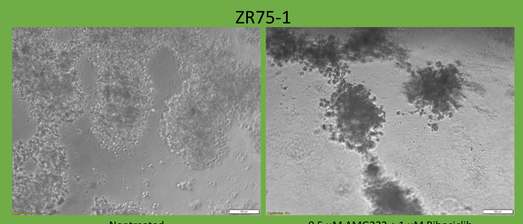




Abstract

Breast cancer is the second most common cancer in women, making research over the different types of breast cancer a major concern. We decided to target two oncogenes located on the same chromosome, MDM2 and CDK4/6. We targeted MDM2 with AMG232, and CDK4/6 with ribociclib and measured different outcomes. We used cell lines ZR75-1 and MCF7 cell lines, as they show an overexpression of these targeted proteins. With this data, we were able to conclude that ribociclib and AMG232 work to decrease cell proliferation in ZR75-1 and MCF7 HR+ breast cancer cell lines.

3D Image



I grew the ZR75-1 cell line on a nitrogenous gel over a span of a few days. The nontreated image was a cell line with just media. The treated cell line was treated with AMG232 and ribociclib. The visual shows the effect treatment has, which shows darkened, dead cells.

Conclusion

The proliferation assay gave us the ideal drug combination to decrease cell proliferation. The Western blot assay proved the drugs target the intended proteins.

The cell cycle assay proved that ribociclib stopped cells in the G1 phase. The apoptosis assay proved that AMG232 sent cells into apoptosis. Both assays showed that the combinations worked well together.
The 3D image visually showed the effect the drug combination had on ZR75-1.

Overall, the combination of AMG232 and ribociclib effected $HR^{+}/HER2^{-}$ breast cancer with overexpression of CDK4/6 and MDM2.

Acknowledgements:

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Targeting Cell Cycle in Breast Cancer: CDK4/6 inhibitors