

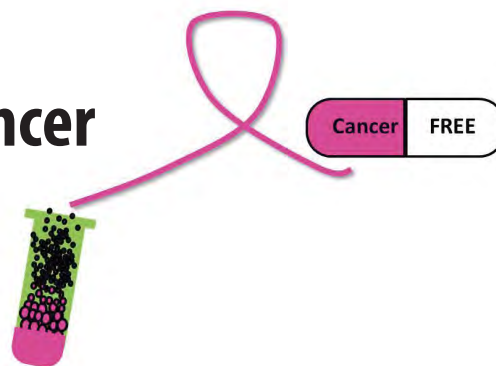
In Search of a Cure for Breast Cancer

by

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Emily was a biology major who had been accepted for a summer internship program at the University of Auckland, New Zealand. She was to join a research group whose work focussed on cancer. This disease had interested Emily ever since her grandmother passed away from breast cancer. Emily had learned that breast cancer is the most frequent cancer among American women. According to the Centers for Disease Control and Prevention (CDC), annually more than 200,000 women get breast cancer, and for more than 40,000 of them, it is the cause of cancer-related death. This disease not only affects women, but also men; each year close to 1% of breast cancer cases are detected in males. The inherited mutations in BRCA1 and BRCA2 genes are associated with an increased risk of breast cancer, but 85% of breast cancers are detected in women without a family history of this disease.

Because of her grandmother's experience, Emily had been genetically tested. The results revealed she had not inherited any of the mutations known to be responsible for familial breast cancer. It seemed to Emily that the main risks for the disease involved being a woman (increased estrogen levels) and aging, which upset her greatly. Rather than simply accepting her possible fate, she was determined to do something about it, and had decided to pursue a career in medical research. Bench work was appealing to her and she wanted to follow the steps of great women in science like Maria Sklodowska-Quire or Rosalind Franklin. The prospect of joining a real research lab seemed fantastic; perhaps it would give her an opportunity to witness the process of discovery and see what a real research laboratory looked like and how it functioned.

As you work through the four parts of this case study, you will join Emily in her journey to see that finding a cure for cancer requires incremental steps in understanding this group of diseases.

Part I – Hormones and Breast Cancer

Emily arrived at the laboratory of professor Baguley early in the morning. She was welcomed by Lena a young lab technician.

“Hi, Emily! You're the first one today! The rest of the team will show up for our weekly doughnuts lab meeting.”

“Oh, am I too early?” replied Emily.

“Not at all. I'll show you around. Put the lab coat on and follow me,” said Lena, and she led Emily to the cell culture room. “Here we keep the breast cancer cell line MCF-7. Do you know much about them?”

“Not really,” said Emily.

“No worries, Eric will teach you what you need to know. These cells are his life!” replied Lena. Just then Eric Baguley appeared in the cell culture room.

“Hi! Are you ready for some doughnuts? Our meeting will start shortly. Today we'll meet in the conference room.”

“In a minute boss, I want to show Emily the MCF-7,” said Lena.

“Oh yes,” Eric nodded. “This is our model for breast cancer. Emily, have you heard about MCF-7 cells before?”

“A little,” replied Emily.

Part II – Receptors

Eric was focusing on the estrogen receptors in his drawing. “You see Emily, this breast cancer we are trying to find a cure for depends on the activation of estrogen receptors. We know that estrogen receptors are a group of proteins found not only inside the cell, but also on the cell membrane.”

“Even though estrogen is a steroid hormone, can it also have specific membrane receptors?” asked Emily. She remembered that steroid hormones have intracellular receptors, because they can easily cross a plasma membrane.

“Yes, the membrane estrogen receptors are mostly G-protein coupled receptors,” answered Eric, drawing the G-protein coupled receptor’s activation pathway on the board.

Question

3. Draw a diagram showing the typical signaling pathway involving the G-protein coupled receptors. Share your thoughts with other groups, and discuss the differences between your diagrams. Reproduce your final diagram here and note what changes you made as a result of your discussion.

“Well,” said Emily, “If the survival of the cancer cell line MCF-7 (ER+) depends heavily on the presence of estrogens in the environment, can deprivation of estrogens induce a cell cycle arrest and eventually lead to cell death?”

“You’re on the right track, Emily!” replied Eric.

Question

4. What are some possible mechanisms that could be used to target breast cancer with ER+ phenotype?

“Emily, have you heard of tamoxifen?” asked Eric.

“Yes, but I don’t see the connection between tamoxifen and breast cancer.”

“Well, initially this drug was used in contraceptive pills. Currently it is used in the treatment of ER+ breast cancer, as a competitive inhibitor for estrogen receptors.”

“OK guys,” interrupted Lena, “it’s time for our lab meeting!”

“OK, let’s go,” replied Eric. “Dr. Mendoza promised to show up for our meeting, so we’d better not be late!”

Question

5. Tamoxifen is used in treatment of ER+ breast cancer as a competitive inhibitor for estrogen receptors. What is competitive inhibition?

Part III – Generating TamC3 and TamR3 Cell Lines

As Emily, Eric, and Lena entered the conference room, they found Dr. Mendoza helping himself to coffee and a doughnut, seated at a conference table with a couple of graduate students.

“Dr. Mendoza, I would like you to meet Emily, our internship student from Alfred, New York. Dr. Mendoza is an oncologist, and he works at the clinic. We collaborate,” said Eric.

“Hi Emily! Nice to meet you. Would you like a cup of coffee?”

“Hello Dr. Mendoza, yes. Thank you.”

“You’ve already met the rest of the group. I gather they took you out last night. Did you guys have fun?” Eric smiled as he looked at Mike and Jen, the graduate students in his lab.

“Yes boss! We need more time off like this,” replied Mike.

“OK, who is presenting first today?” asked Eric.

“I’m up,” said Mike coming up to the white screen, where the PowerPoint presentation with the experimental results would be displayed. Lena dimmed the lights and Mike started his presentation.

“Some breast cancer cells can develop resistance to the tamoxifen treatment and can survive the estrogen deprivation conditions. The mechanisms of the drug and hormone deprivation resistance are not known. To investigate this phenomenon, we took the MCF-7 cells into the laboratory”

“Did you try to make the cell resistant to tamoxifen?” asked Dr. Mendoza.

“Yes. We divided them into two groups. One batch of the cells was exposed to tamoxifen for some period of time, which we did to mimic the clinical condition for the tamoxifen treatment in patients. Some of the cells died during the course of the experiment, but those that survived were selected and cultivated further.”

“Wow!” interrupted Eric. “So in this way you generated a new cell line. What did you call it?”

“We’re calling it TamC3. These cells were resistant to tamoxifen. The other batch of the MCF-7 cells was subjected to conditions without estrogen.”

“Very clever!” this time Dr. Mendoza interrupted. “That’s mimicking the condition in patients subjected to hormone deprivation therapy. And what happened?”

“The cells that survived the estrogen deprivation established another cell line, TamR3. Those cells are estrogen independent. Look at this graph,” replied Mike (Figure 1).

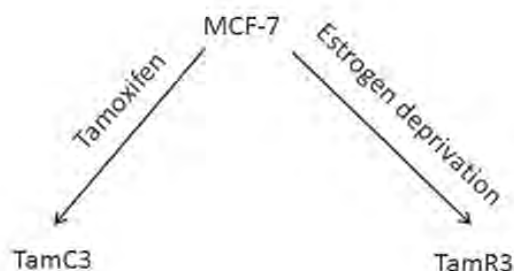


Figure 1. Generation of tamoxifen resistant (TamC3) and estrogen-independent (TamR3) cell lines.

Question

- The conditions employed in laboratory settings could mimic the outcomes of prolonged usage of tamoxifen and hormone deprivation therapy. We don’t know much about the phenotypic profile of these newly emerged cancer cells. How might you explain the lack of response of TamC3 to tamoxifen treatment and TamR3 to estrogen deprivation?

“Now it’s time to show what I got from these cells,” said Jen as she replaced Mike. “I compared the response of MCF-7, TamC3, and TamR3 cells to tamoxifen. Look at the results.” (Figure 2.)

“Oh yes, we do see resistance of these cell lines to tamoxifen,” said Eric. “It is known that MCF-7 cells are sensitive to inhibitors of mTOR (mammalian target of rapamycin); rapamycin. The incubation with rapamycin is toxic to MCF-7 cells. But how will TamC3 and TamR3 respond to that drug?” asked Eric.

“Hold on,” interrupted Dr. Mendoza: “I don’t understand it... why rapamycin?” Emily was glad this question had been asked; she was wondering the same thing.

“Well,” started Eric and grabbed the marker as he approached the white board to draw, “the mTOR is an evolutionary conserved transduction pathway, which conveys signals from epidermal growth factor receptors (EGFR) on the cell membrane into the cell through phosphatidylinositol 3-kinase (PI3K) pathway and Akt/protein kinase B. Did you guys check the phosphorylation status of signaling molecules?” Eric asked his graduate students.

“Yes, we did a western blot to check the key proteins’ phosphorylation status,” replied Jen, and she continued showing her new results. “The phosphorylation level of Akt kinase and its downstream protein p70S6K is illustrated in the picture (Figure 3).”

“Good job! But now, the question is how the cells will respond to rapamycin? What do you think?”

Question

- Look at the data provided and predict how the TamC3 and TamR3 would respond to the rapamycin treatment. Predict the results of the cells’ growth rate in the presence and absence of rapamycin for all three cell lines: MCF-7, TamC3 and TamR3. Draw a graph and share it with other groups. Do your predictions correlate? Write an explanation of your graphical prediction using knowledge about mTOR pathway. What does the phosphorylation of kinases mean for the cell signaling pathways?

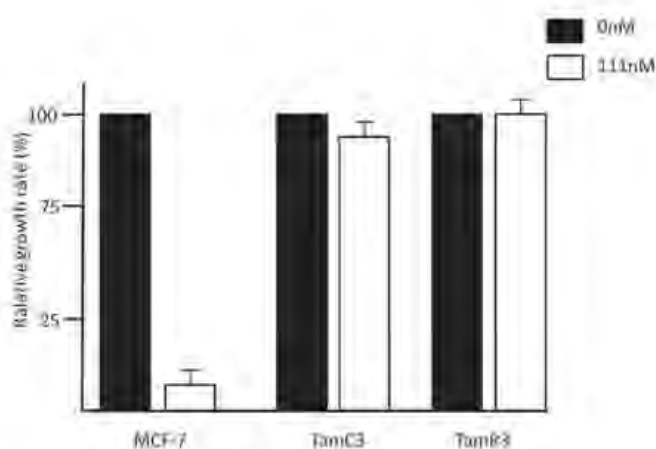


Figure 2. Effects of tamoxifen on cell growth. The cells were exposed to 0nM or 111nM concentration of tamoxifen for four days, and the growth rate was estimated with thymidine incorporation assay. (Adapted from: Leung, E. *et al.*, 2014, *Frontiers in Oncology* 4, CC BY 3.0, <<https://doi.org/10.3389/fonc.2014.00221>>.)

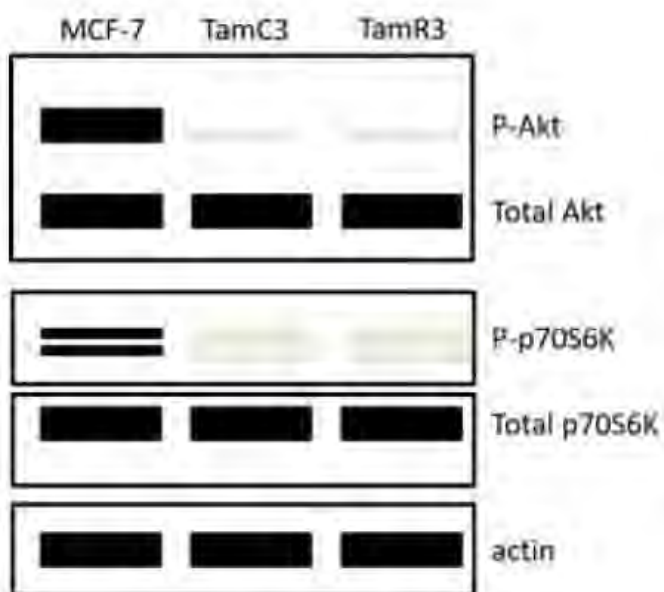


Figure 3. The relative level of phosphorylated Akt and p70S6K in MCF-7, in TamC3 and TamR3 cells. Actin is shown as a loading control. (Adapted from: Leung, E. *et al.*, 2014, *Frontiers in Oncology* 4, CC BY 3.0, <<https://doi.org/10.3389/fonc.2014.00221>>.)

Part IV – Killing TamC3 and TamR3 Cell Lines

Time passed quickly as the members of the research team discussed their interesting results, and it was soon time for lunch. Dr. Mendoza turned to Emily and asked, “After lunch would you like to see our clinic?”

“Yes!” Emily responded.

“Good, I want to show you how we take care of our cancer patients who take chemo. We administer to them a cocktail of drugs like camptothecin, doxorubicin, cisplatin, and fluorouracil.”

The meeting was over. The whole group went to the cafeteria to grab a bite. After lunch Emily went with Dr. Mendoza to see the oncology patients. She learned that some of them suffered from side effects of chemotherapy.

The following weeks at Eric’s lab were very productive. Emily learned new techniques, and performed experiments with some help from Lena, Jen and Chris. They exposed the MCF-7 and TamC3 to different chemotherapeutic agents.

Question

8. Look at the data provided in Figure 4 and describe the effects of drugs and oxidative stress on the cell proliferation (growth) inhibition. What are the implications of the data for patient treatment?

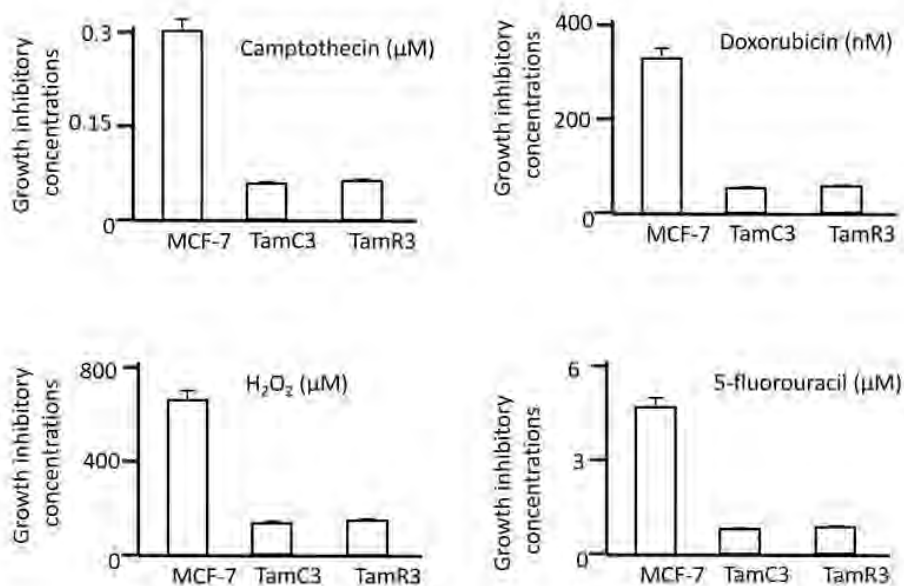


Figure 4. The effects of camptothecin, doxorubicin, H_2O_2 and 5-fluorouracil on MCF-7, TamC3 and TamR3 growth inhibition. The data shows the drugs’ concentrations at which 50% of the cells subjected to treatment stopped growing as measured by a cell proliferation assay. (Adapted from: Leung, E. *et al.*, 2014, *Frontiers in Oncology* 4, CC BY 3.0, <<https://doi.org/10.3389/fonc.2014.00221>>.)

9. Eric and his research group were trying to understand why daughter cell lines TamC3 and TamR3 have “different” responses to chemotherapeutic agents and oxidative stress relative to the parental cell line (MCF-7). Indicate the relative response of the cell lines (more/less sensitive or resistant) to the drugs/conditions listed:

	<i>MCF-7</i>	<i>TamC3 and TamR3</i>
Tamoxifen		
Rapamycin		
Doxorubicin, fluorouracil, camptothecin		
Oxidative stress		

From that point on, Emily was very busy. The metabolic profile in the three cell lines was investigated. The uptake of glucose, the level of reactive oxygen species (DCFDA fluorescence) and production of lactate acid was measured in MCF-7, TamC3 and TamR3 cells. The mitochondrial activity was also assessed with the alamar blue fluorescence assay. Emily was pleased. She had obtained good results to report during the next lab meeting (Figure 5)!

Question

10. Based on the results (Figure 5), what can we conclude about the metabolic profile of these three cell lines? Explain why the level of lactic acid was measured. Support your answer with an equation/diagram of the appropriate chemical reaction.

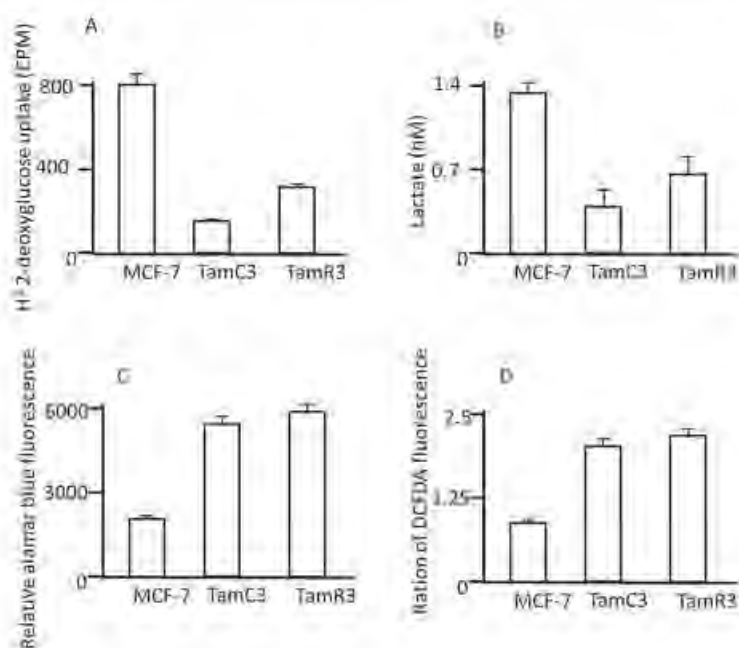


Figure 5. The H3 2-deoxyglucose uptake (A), lactate production (B), mitochondrial activity (C), and reactive oxygen species generation (D) in MCF-7, TamC3 and TamR3 cell lines. (Adapted from: Leung, E. *et al.*, 2014, *Frontiers in Oncology* 4, cc by 3.0, <<https://doi.org/10.3389/fonc.2014.00221>>.)

Short Essay

Based on the data presented in this case study, what conclusion can be drawn about sub-lines of the MCF-7 breast cancer cell lines, TamC3 and TamR3? What is the molecular mechanism of their phenotypic profile? How could their resistance to rapamycin and sensitivity to chemotherapeutic drugs and oxidative stress be explained? Discuss your thoughts with the data provided. Your response should be at least 250 words and be modelled on the format of the “Discussion” section of a standard scientific research paper.