Elaborating the Mechanism of Cell Killing of a Novel Chemotherapeutic Drug Targeting Breast Cancer Cells

Phat Do\textsuperscript{a}, Sridhar Varadarajan\textsuperscript{b}, Giridhar Akkaraju\textsuperscript{a}  
\textsuperscript{a}Department of Biology, Texas Christian University, Fort Worth, TX 76129, USA  
\textsuperscript{b}Department of Chemistry and Biochemistry, University of North Carolina Wilmington, Wilmington, NC 28403-5932, USA

Abstract

Breast cancer (BC) is the second most commonly diagnosed cancer among American women after skin cancer. Traditional treatments of BC include surgery, radiation, and chemotherapy therapy; however, these treatments are non-specific and potentially kill peripheral, healthy cells. There emerges a need for more specific treatments, most notably to develop chemotherapy agents that target a unique feature of the cancer cells. Interestingly, 70% of BC cells upregulate estradiol-dependent pathway, a characteristic essential for rapid cell growth. Current BC drugs, such as Herceptin and Tamoxifen, have targeted this pathway to preferentially kill BC cells. However, most women relapse within 15 years due to drug-resistance. Thus, there is a need for new chemotherapeutic drugs. Our research group studies a novel estrogen-receptor targeting drug: Est-n-Melex. This compound has the estradiol molecule linked to a DNA alkylating agent, Melex. We hypothesize that Est-n-Melex enters the cancer cells via an interaction between the estradiol moiety and the estrogen receptor alpha (ER-alpha). ER-alpha then enters the nucleus and binds to Estrogen Response Elements on the DNA. This movement positions the Melex moiety on the DNA and allows the transfer of a methyl group to the N3 adenine on the DNA. In this project, we test the hypothesized mechanism of action of our compound. Since Est-n-Melex has a DNA methylation component (Melex) conjugated to estrogen, our hypothesis is that after the drug binds to the estrogen receptor in the cytosol, it translocates to the nucleus, specifically methylates the N3-region of adenine bases, eventually triggering cell death.

Hypothesis

Conjugating Melex to estradiol will result in increased absorption in cancer cells which will result in increased 3Me-Adenine and apoptosis.

Mode of Action

Theoretical simulation modelling shows Est-n-Melex binds to the minor groove of DNA. To prove the theoretical model, DNA minor-groove binding agent, Hoechst, is used to compete with the DNA binding of Est-n-Melex. Since Est-n-Melex is cytotoxic and invisible under the microscope, its movement is tracked via a non-cytotoxic fluorescent analogue, Est-n-NBD. Without Hoechst treatment, NBD activates ER-a and the complex moves primarily into the nucleus. Hoechst pretreatment competes with NBD, preventing nuclear localization of NBD. Thus more NBD is observed in the cytoplasm. Immunofluorescence study is used to track ER-a localization upon Est-n-Melex treatment. Without the drug, inactive ER-a remains cytosolic. Est-n-Melex treatment activates ER-a and causes ER-a to translocate into the nucleus.

Discussion

• Est-n-NBD, the non-toxic fluorescent analogue of Est-n-Melex binds to the minor groove of DNA  
• Est-n-Melex induces nuclear localization of ER-a

Conclusions

• Clarify the mode of killing of Est-n-Melex  
• Elaborate estradiol-dependent gene expression upon Est-n-Melex treatment

Future Directions

Abstract

Breast cancer (BC) is the second most commonly diagnosed cancer among American women after skin cancer. Traditional treatments of BC include surgery, radiation, and chemotherapy therapy; however, these treatments are non-specific and potentially kill peripheral, healthy cells. There emerges a need for more specific treatments, most notably to develop chemotherapy agents that target a unique feature of the cancer cells. Interestingly, 70% of BC cells upregulate estradiol-dependent pathway, a characteristic essential for rapid cell growth. Current BC drugs, such as Herceptin and Tamoxifen, have targeted this pathway to preferentially kill BC cells. However, most women relapse within 15 years due to drug-resistance. Thus, there is a need for new chemotherapeutic drugs. Our research group studies a novel estrogen-receptor targeting drug: Est-n-Melex. This compound has the estradiol molecule linked to a DNA alkylating agent, Melex. We hypothesize that Est-n-Melex enters the cancer cells via an interaction between the estradiol moiety and the estrogen receptor alpha (ER-alpha). ER-alpha then enters the nucleus and binds to Estrogen Response Elements on the DNA. This movement positions the Melex moiety on the DNA and allows the transfer of a methyl group to the N3 adenine on the DNA. In this project, we test the hypothesized mechanism of action of our compound. Since Est-n-Melex has a DNA methylation component (Melex) conjugated to estrogen, our hypothesis is that after the drug binds to the estrogen receptor in the cytosol, it translocates to the nucleus, specifically methylates the N3-region of adenine bases, eventually triggering cell death.

Hypothesis

Conjugating Melex to estradiol will result in increased absorption in cancer cells which will result in increased 3Me-Adenine and apoptosis.

Mode of Action

Theoretical simulation modelling shows Est-n-Melex binds to the minor groove of DNA. To prove the theoretical model, DNA minor-groove binding agent, Hoechst, is used to compete with the DNA binding of Est-n-Melex. Since Est-n-Melex is cytotoxic and invisible under the microscope, its movement is tracked via a non-cytotoxic fluorescent analogue, Est-n-NBD. Without Hoechst treatment, NBD activates ER-a and the complex moves primarily into the nucleus. Hoechst pretreatment competes with NBD, preventing nuclear localization of NBD. Thus more NBD is observed in the cytoplasm. Immunofluorescence study is used to track ER-a localization upon Est-n-Melex treatment. Without the drug, inactive ER-a remains cytosolic. Est-n-Melex treatment activates ER-a and causes ER-a to translocate into the nucleus.

Discussion

• Est-n-NBD, the non-toxic fluorescent analogue of Est-n-Melex binds to the minor groove of DNA  
• Est-n-Melex induces nuclear localization of ER-a

Conclusions

• Clarify the mode of killing of Est-n-Melex  
• Elaborate estradiol-dependent gene expression upon Est-n-Melex treatment

Future Directions