

Synthesis and Testing of a Novel Chemotherapeutic Agent for Breast Cancer

Faculty:

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Project for Student Scholars:

Estrogen receptor positive (ER+) breast cancers constitute 60-65% of all breast cancers. In addition to chemotherapy, radiation and surgery, another type of treatment of ER+ breast cancers is hormonal therapy that suppresses estrogen action in breast cancer cells. A major drug for hormonal therapy is tamoxifen. This drug is a selective estrogen receptor modulator (SERM) that has antagonist and agonist effects. Tamoxifen has been used as an adjuvant therapy agent since 1978 to reduce the risk of ER+ breast cancer recurrence. The drug increases the production of breast cancer cell growth inhibitory factor TGF β and simultaneously reduces the production of breast cancer cell growth promoter TGF α . Tamoxifen also binds to the estrogen receptor and inhibits estrogen binding. In 1992 the National Cancer Institute and the National Surgical Adjuvant Breast and Bowel Project initiated the Breast Cancer Prevention Trial, also known as P-1 to test the hypothesis that tamoxifen could prevent breast cancer. This study was halted and unblinded in March 1998 when significant differences in breast cancer rates were observed in the study and control arms of the trial. For all age groups tamoxifen reduced the relative risk of invasive and noninvasive breast cancers by nearly 50%. Importantly, the study showed significant differences between the control and study groups in the rate of ER+ tumors occurrence. As expected, ER+ tumors were more frequent in the placebo-treated cases than in the drug-treated cases. However, there are complications of tamoxifen treatment including increased incidence of endometrial cancer, stroke, deep vein thrombosis, and pulmonary embolism. Lowering the dose of tamoxifen necessary for effective therapy would lower the risk of these complications.

A newer class of potential anti-cancer drugs activates p53. One of these drugs, nutlin 3a, operates by binding to endogenous inhibitors of p53—MDM2 and MDMX. Nutlin 3a may be effective in cancers where wild-type p53 has been deactivated. Breast cancers are good targets because 70% of breast cancers express wild-type p53. Nutlin 3a inhibits the growth of ER+ breast cancer cells in culture. When treated with nutlin 3a, osteosarcoma and prostate cancer cells with wild-type p53 have been shown to be eradicated in a nude mouse/human xenograft tumor model. Interestingly, the IC₅₀ of nutlin 3a for the p53/MDM2 complex is only 14 nM but the amount required to eradicate cancers in this mouse model is high, 200 mg/kg. This suggests that nutlin 3A is inefficient at targeting the cancer cells but effective once it has access to MDM2 and MDMX. If one can target nutlin 3a to breast cancer cells the amount of this drug necessary for therapeutic effect may be lowered.

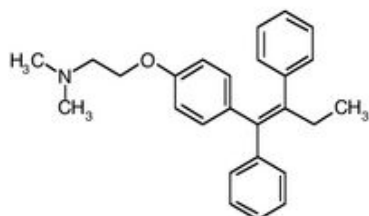
Objective/Hypothesis

We hypothesize that a bifunctional drug composed of tamoxifen and nutlin 3a covalently linked together will be more effective at preventing ER+ breast cancer cell growth than either drug alone. The rationale is that nutlin 3a has the ability to prevent breast cancer cell growth if it can be directed to breast cancer cells. Tamoxifen operates by binding to the estrogen receptor, located in the nucleus. If tamoxifen can be covalently linked to nutlin 3a without compromising the ability of the drugs from interacting with their respective protein targets then the bifunctional drug, Nut-Tam, will be a potent chemotherapeutic drug.

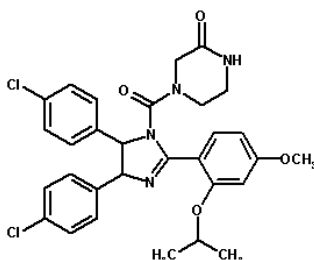
A bifunctional drug has already been created between 4-hydroxytamoxifen (4-tam) and doxorubicin, that latter being a chemotherapy agent that operates by damaging DNA. This bifunctional drug inhibits the growth of four breast cancer cell lines with 4- to 140-fold enhanced activity relative to doxorubicin. The bifunctional drug also inhibits the growth of these lines approximately 2-3-fold more than treatment of the breast cancer cells with the two drugs unattached to each other. We will perform studies with 4-tam instead of tamoxifen because the former has a lower IC_{50} in cultured cells.

Specific Aims

- 1) Demonstrate that co-treatment of four ER+ breast cancer cell lines (MCF-7, T-47D, ZR75-1 and BT474) with nutlin 3a + 4-tam lowers the IC_{50} relative to treatment with nutlin 3a or 4-tam alone.
- 2) Synthesize Nut-Tam conjugates with linkers of 1, 2, and 3 ethylene groups between the tamoxifen and nutlin 3a moieties.
- 3) Test the ability of Nut-Tam to prevent p53/MDM2 complex formation.
- 4) Test the ability of Nut-Tam to bind to the estrogen receptor on MCF7 breast cancer cells.
- 5) Test the ability of Nut-Tam to prevent MCF7 breast cancer cell growth in a xenograft/nude mouse model.



Tamoxifen



Nutlin 3a

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