CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)
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Project Number
S1520

Project Title
Transformation of Herceptin-Sensitive Breast Tumor Cells into Resistant Cells by PI3K/Akt Pathway Activated by t-Darpp

Abstract

Objectives/Goals
This study aimed to confirm that t-Darpp protein can cause Herceptin-resistance in previously Herceptin-sensitive Her-2 positive breast cancer cells by activating the anti-apoptotic PI3K/Akt pathway.

Methods/Materials
SKBR3 breast tumor cells transfected (experimental clones) and not transfected (control clones) with t-Darpp were used. Sulforhodamine B (SRB) Assay determined the cells' total protein biomass to indicate cell growth in the presence of different Herceptin concentrations, including 0uM. A cell counting assay directly measured cell growth in the presence of 0.2uM Herceptin. Western analysis determined the cells' expression levels (also in the presence of 0.2uM Herceptin) of the anti-apoptotic proteins Akt and pAkt, as well as t-Darpp to confirm that transfection with t-Darpp in experimental clones was successful.

Results
Cell counting assays showed that experimental clone cells grew in Herceptin's presence whereas control clone cells died. On day 4, the Control Clone NVo cells treated with 0.2uM Herceptin had an average cell count of 6.00E+04 cells, which decreased to 2.00E+04 cells on day 10. On the other hand, on day 4, the average cell count of Experimental Clone A cells treated with 0.2uM Herceptin was 9.33E+04 cells, which increased to 2.20E+05 cells on day 10. SRB assay showed similar results. The control clone cells that were treated with 0.03uM Herceptin had an average protein biomass of 0.1964 after 7 days and 0.1401 after 10 days, indicating that the cells died in the drug's presence. On the other hand, the experimental clone cells that were treated with 0.03uM Herceptin had an average biomass of 0.4231 after 7 days and 0.7580 after 10 days, indicating that the cells continued to grow in Herceptin's presence. Western analysis showed that control and experimental clones expressed Akt before and after Herceptin treatment. On the other hand, control clones expressed pAkt only before drug treatment while experimental clones expressed both pAkt before and after treatment.

Conclusions/Discussion
This is the first study that shows that Herceptin-sensitive breast cancer cells become resistant through transfection with t-Darpp that possibly causes this resistance by activating the anti-apoptotic PI3K/Akt/pAkt pathway. Understanding t-Darpp's drug-resistant mechanisms in breast tumor cells will facilitate in blocking these pathways and promoting sensitivity to Herceptin in breast tumor patients.

Summary Statement
The protein t-Darpp can activate the anti-apoptotic PI3K/Akt/pAkt cellular pathway to make once Herceptin-sensitive SKBR3 breast tumor cells become resistant to Herceptin.

Help Received
Used lab equipment at Beckman Research Institute at City of Hope under the supervision of Dr. Susan Kane and Dr. Long Gu.