Targeting the RXR Pathway for the Prevention of Triple-Negative Breast Cancer

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Background

Women born with a harmful variant in *BRCA1* or *BRCA2* will have a 50-70% chance of developing breast cancer in their lifetime[1]. The ability to identify women at risk with genetic screens provides an opportunity for early intervention to prevent cancer development.

It is known that familial breast cancer associated with *BRCA1/2* mutations is more likely to be triple-negative breast cancer (TNBC)[2,3]. TNBC is often characterized by highly aggressive tumors with a poor disease prognosis, even after treatment with chemotherapy. Studies using selective estrogen receptor (ER) modulators (SERMs), and aromatase inhibitors (AIs), have shown that breast cancer prevention is feasible[4,5]; however, these drugs do not prevent ER-negative tumors, including TNBC. There remains a need for the development of effective therapies with minimal toxicity for the prevention of TNBC.

We previously demonstrated that retinoid X receptor (RXR)-specific ligands (rexinoids) can prevent ER-negative breast cancers in mice in mice. Our studies in MMTV-erbB2 mice showed that IRX-4204, a fourth generation rexinoid, prevented the development of most HER2/erbB2-positive, ER-negative tumors in these mice (Figure 1).

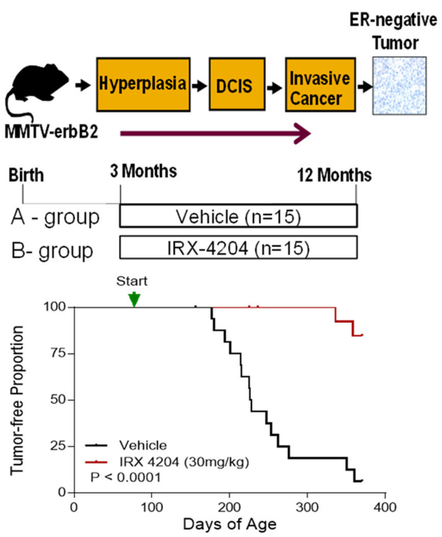

Figure 1. Prevention of ER-negative MMTV-ErbB2 tumors with an RXR agonist, IRX4204. Top: Mouse model and treatment schema; Bottom: Proportion tumor-free over time with RXR agonist, IRX4204 compared to control.

Hypothesis

We hypothesized that by targeting the RXR pathway, we can prevent the development of triple negative, BRCA1-mutant mammary tumors in mice. To test the hypothesis, we treated BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice prior to their developing tumors with IRX-4204 or UAB-30 to determine whether RXR agonists effectively prevent triple-negative breast tumors.

Methods

BRCA/p53-deficient mice: BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice (129 background) were produced by breeding BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ males with BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/+}$ females. PCR genotyping was used to select for BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ female pups. These mice all develop tumors by 35 weeks of age.

In vivo studies: Female BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ pups were separated into 4 groups: 1) sesame oil control, 2) low dose IRX-4204, 3) high dose IRX-4204 or 4) 9-cis-UAB-30. All treatments were given by oral gavage, three or five days a week from 4 months of age (Figure 2). Mice were observed daily for tumor formation, toxicity and the percentage of tumor free mice were recorded. Tumor incidence and time to tumor formation was visualized using Kaplan Meier curves and analyzed using the Log-rank test.

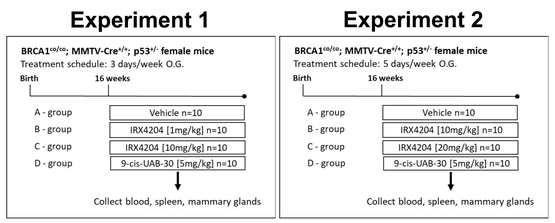

Figure 2. Experimental design and treatment schema.

Immunohistochemistry: Fixed mammary tumors from BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice (experiment 1) were cut into 4uM sections, deparaffinized and mounted onto slides. Samples were blocked and probed with antibodies to ki-67, cyclin D1 and cleaved-caspase 3 before counterstaining with hematoxylin. Expression was measured as percent positivity or Allred scores and analyzed using Student's t-test.

Results

RXR agonists significantly delay *BRCA1*-mutant tumor formation

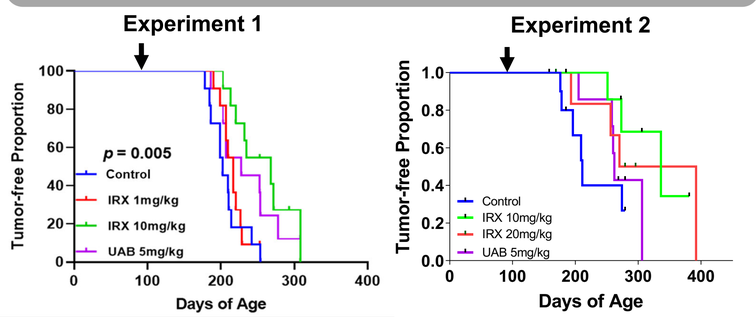

Figure 3. Tumor-free proportion of BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice treated with RXR agonists. Female mice were treated with sesame oil control or the RXR agonists: IRX4204 [low dose], IRX4204 [high dose] or UAB-30 starting at 4 months of age (arrow). Experiment 1: IRX-4204 [10mg/kg] 3 days/week reduced tumor incidence the most and was associated with a significant increase in median tumor formation from 200 days to 268 days (p=0.001). Experiment 2: IRX-4204 [10mg/kg] 5 days/week also reduced tumor incidence with a significant increase in median tumor formation from 209 days to 336 days (p=0.005). Similarly, the RXR agonist 9-cis-UAB-30 also significantly delayed tumor formation (p=0.04).

RXR agonists decrease proliferation of *BRCA1*-mutant tumors

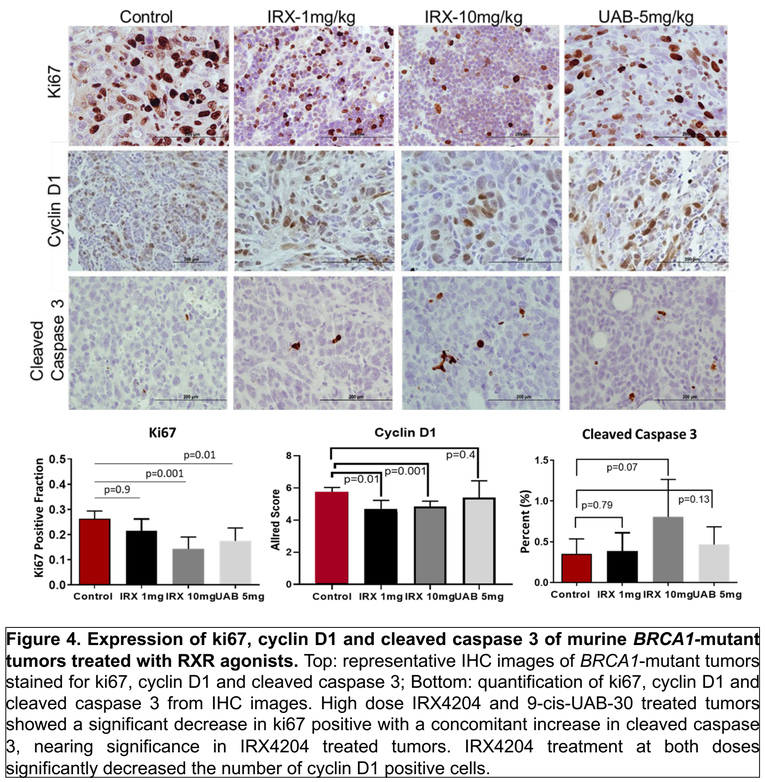

Figure 4. Expression of ki67, cyclin D1 and cleaved caspase 3 of murine *BRCA1*-mutant tumors treated with RXR agonists. Top: representative IHC images of *BRCA1*-mutant tumors stained for ki67, cyclin D1 and cleaved caspase 3; Bottom: quantification of ki67, cyclin D1 and cleaved caspase 3 from IHC images. High dose IRX4204 and 9-cis-UAB-30 treated tumors showed a significant decrease in ki67 positive with a concomitant increase in cleaved caspase 3, nearing significance in IRX4204 treated tumors. IRX4204 treatment at both doses significantly decreased the number of cyclin D1 positive cells.

Conclusions

RXR agonists, IRX4204 and 9-cis-UAB-30, delayed ER-negative mammary tumor formation in BRCA1$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice without notable toxicities.

Both RXR agonists significantly decreased ki67 expression in *BRCA1* mutant mammary tumors. In addition, IRX4204 decreased Cyclin D1 expression.

Targeting the RXR pathway should be considered for the prevention of breast cancer in high-risk patients, alone or in combination with other preventative therapies.

Future Directions

- Test the effects of RXR agonists on the prevention of other mouse models of TNBC and ER-negative breast cancer
- Combine RXR agonists with other breast cancer prevention therapies (i.e., PARP inhibitors)
- Investigate the mechanism of RXR agonists in the prevention of TNBC

References

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