The RXR agonist, IRX4204, delays the formation of *Brca1* mutant mammary tumors via modulation of the anti-tumor immune response

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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 and others have shown that retinoid X receptor (RXR)-specific ligands (rexinoids) can prevent ER-negative breast cancers 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).

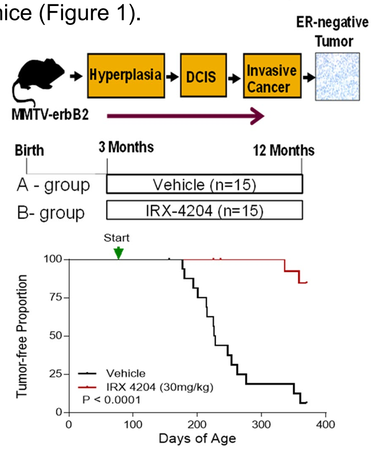

Figure 1. Prevention of ER-negative MMTV-ErbB2 tumors with an RXR agonist, IRX4204. Top: Mouse model and treatment schema; Bottom: Proportion tumor-free over time with RXR agonist, IRX4204 compared to control.

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 this 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

Brca1/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, five or three 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.

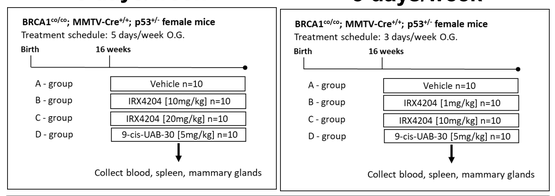

Figure 2. Experimental design and treatment schema.

Immunohistochemistry: Fixed mammary tumors from *Brca1*$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice were cut into 4uM sections, deparaffinized and mounted onto slides. Samples were blocked and probed with antibodies to ki-67, cyclin D1 cleaved-caspase 3, and CD8a before counterstaining with hematoxylin. Expression was measured as percent positivity or Allred scores and analyzed using Student's *t*-test.

In vitro cell growth: Brca1-mutant cells were seeded in 24-well plates and treated with IRX4204 [1uM] or DMSO. Cells were collected in quadruplicate, stained with Trypan Blue dye and total live cell number was calculated on days 1, 3, 5, and 7.

Oil red staining: Brca1-mutant cells were seeded in 24-well plates and treated with IRX4204 [1uM] or DMSO for 72 hours. After treatment, cells were fixed and stained with Oil Red O before imaging at 40X.

Srebf1 gene expression: Brca1-mutant cells were seeded in 6-well plates, treated with IRX4204 [1uM] and collected at 0, 1, 6 and 24 hours. Total RNA was isolated from cells and reverse transcribed into cDNA. RT-qPCR with *Srebf1* TaqMan assay was performed. Relative gene expression was determined using the comparative Ct method and normalized to cyclophilin expression (2−ΔΔCt method). Results are shown as the mean relative expression of three biologic replicates. Significance was determined with ANOVA.

Results

RXR agonists significantly delay *Brca1*-mutant tumor formation

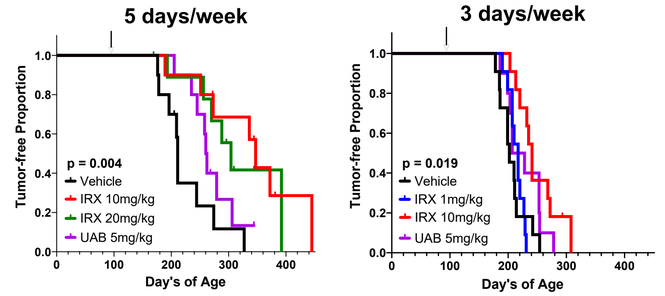

Figure 3. Tumor-free proportion of *Brca1*$^{co/co}$; MMTV-Cre; p53$^{+/-}$ mice treated with RXR agonists. Female mice were treated with vehicle or the RXR agonists: IRX4204 [low dose], IRX4204 [high dose] or UAB-30 starting at 4 months of age (arrow). Experiment 1: IRX-4204 [10mg/kg] **5 days/week** 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). Experiment 2: 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).

RXR agonists decrease proliferation of *Brca1*-mutant tumors

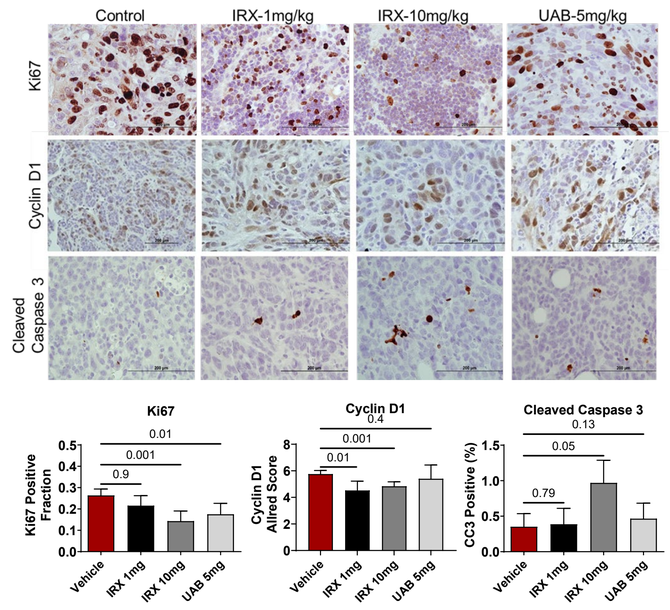

Figure 4. Expression of ki67, cyclin D1 and cleaved caspase 3 of murine *BRCA1*-mutant tumors treated with RXR agonists. Top: representative IHC images of *BRCA1*-mutant tumors stained for ki67, cyclin D1 and cleaved caspase 3; Bottom: quantification of ki67, cyclin D1 and cleaved caspase 3 from IHC images (n=5 tumors per treatment group). 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. IRX4204 treatment at both doses significantly decreased the number of cyclin D1 positive cells.

RXR agonists alter lipid metabolism in *Brca1*-mutant cells

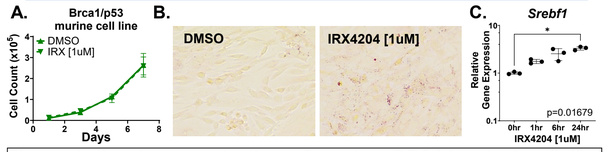

Figure 5. *In vitro* examination of murine *BRCA1*-mutant cell line treated with IRX4204. A) *in vitro* growth of *Brca1*-mutant cell line derived from *Brca1*/p53 mammary tumors treated with DMSO or IRX4204 **B)** representative images of the *Brca1*-mutant cell line stained for lipids with Oil Red O. **C)** gene expression of *Srebf1,* a key transcription factor that regulates lipid metabolism. IRX4204 treated cells show a significant increase in lipid accumulation and *Srebf1* expression.

IRX4204 treatment increases CD8+ T-cells in *Brca1*-mutant tumors

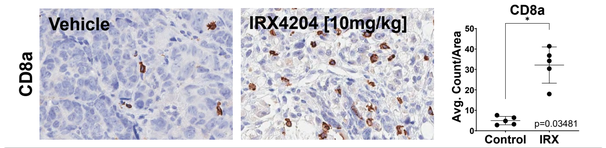

Figure 6. Expression of CD8a in murine *BRCA1*-mutant tumors treated with IRX4204. Left: representative IHC images of a *BRCA1*-mutant tumor stained for CD8a. Right: quantification of CD8a from IHC images (n=5 tumors). High dose IRX4204 treated tumors showed a significant increase in the infiltration of CD8-positive T-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. IRX4204 10mg/kg treatment led to a significant decrease in tumor ki67 and cyclin D1 expression, with an increase in cleaved caspase 3 expression.

IRX4204 has no effect on *Brca1*-mutant cell growth *in vitro* but does increase lipid droplet accumulation and *Srebf1* expression.

IRX4204 treatment increases the infiltration of CD8-positive T cells into *Brca1*-mutant tumors.

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 and ER-negative breast cancer

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References

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