Title

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Journal

Molecular & Cellular Oncology, 7(2)

ISSN

2372-3548

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Publication Date

2020-03-03

DOI

10.1080/23723556.2019.1701362

Peer reviewed

MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 2, e1701362 (3 pages)
https://doi.org/10.1080/23723556.2019.1701362

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AUTHOR'S VIEWS

A master regulator of cholesterol biosynthesis constitutes a therapeutic liability of triple negative breast cancer

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ABSTRACT

Lipid and cholesterol reprogramming are often observed in specific cancer subtypes. We find that triple-negative breast cancers (TNBCs), but not estrogen receptor-positive (ER+) ones, adopt nuclear receptor RAR-related orphan receptor γ (RORγ) as their new master activator of cholesterol biosynthesis program. Its dominant role over sterol regulatory element-binding protein 2 (SREBP2) renders TNBC highly vulnerable to RORγ inhibitors alone or in combination with statins.

ARTICLE HISTORY
Received 20 November 2019
Revised 27 November 2019
Accepted 3 December 2019

KEYWORDS
TNBC; cholesterol homeostasis; statins; ER-positive breast cancer; RORγ; SREBP2; chromatin; therapy

TNBC patients with advanced disease generally have worse prognosis than patients with ER+ breast cancer. Trials of immune therapies for TNBC patients have not provided markedly improved outcomes.[1] Breast cancer tumors likely display context-dependent metabolic reprogramming.[2] However, clear distinctions between the subtypes have not been documented. We interrogated the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) datasets with close to 2000 samples for potential differences between ER+ and TNBC tumors in the activities of major metabolic pathways. Interestingly, our analysis revealed that most of the TNBC tumors displayed higher activity in cholesterol biosynthesis (CB) and efflux pathways than the majority of ER+ tumors.[3] Our metabolomics study of an independent cohort of tumors also showed a significantly higher tumor cholesterol content in TNBCs than in ER+ tumors. To search for regulators that contribute to the distinct expression of CB program in TNBC, we pharmacologically perturbed the function of sterol regulatory element-binding proteins (SREBPs) and members of the nuclear receptor (NR) family that are known controller of lipid and/or cholesterol metabolism. We found that two antagonists (GSK805 and XY018) of RORγ[4] consistently reduced the expression of CB genes in TNBC cells but not in ER+ cells. Surprisingly, modulators of the SREBPs did not show any distinct effect.[3]

RORγ, a member of the RAR-related orphan receptor (ROR) subfamily of NRs, plays important roles in control of mammalian metabolism and circadian rhythm. RORγt, a T cell-specific isoform, is well known for its role in the induction of pro-inflammatory cytokines and has been an attractive target for autoimmune diseases.[5] However, RORγ or RORγt was not known to directly control the CB pathway. We thus perturbed the expression of RORγ and found that ectopic RORγ or its knockdown strongly increased or suppressed, respectively, the expression of most of the CB genes in TNBC cells. Our RNA-seq profiling of TNBC cells treated with the RORγ antagonists clearly showed that CB program was most significantly down-regulated. Inhibition of RORγ also strongly reduced the cholesterol content and CB rate in TNBC cells and tumors. In contrast, in ER+ cells the RORγ antagonist increased expression of the CB program.[3] Thus, we demonstrated for the first time that indeed RORγ controls CB program and that it does so in a cancer subtype-specific manner.

SREBP2 is widely conceived as the master regulator of CB in normal tissues or tumors. Several major oncogenic signaling pathways including that of phosphoinositide-3-kinase–protein kinase B (PI3K-Akt), mammalian target of rapamycin (mTORC), and c-myc proto-oncogene protein (Myc) converge at SREBP2 to reprogram cholesterol homeostasis.[6] That was why it came as a surprise when our further studies revealed that RORγ played a dominant role over SREBP2. We first found that the SREBP2-mediated induction of CB genes was effectively mitigated by the RORγ antagonist. In contrast, RORγ-mediated induction of them was not affected by SREBP2 silencing. Secondly, RORγ inhibition completely negated statin-induced, SREBP2-mediated feedback activation of CB genes. More interestingly, our chromatin immunoprecipitation sequencing (ChIP-seq) and other analyses revealed that RORγ interacts with SREBP2 and that SREBP2 binding to its chromatin targets requires RORγ function,[3] thus providing a mechanistic insight to the dominant role played by RORγ (Figure 1).

The rationale for why TNBCs co-opt RORγ as their master regulator of CB program is currently unclear. The high proliferation rate of TNBC tumors may necessitate a hyper-activated CB program that is unrestrained by the SREBP2-mediated feedback. It is possible that RORγ can be

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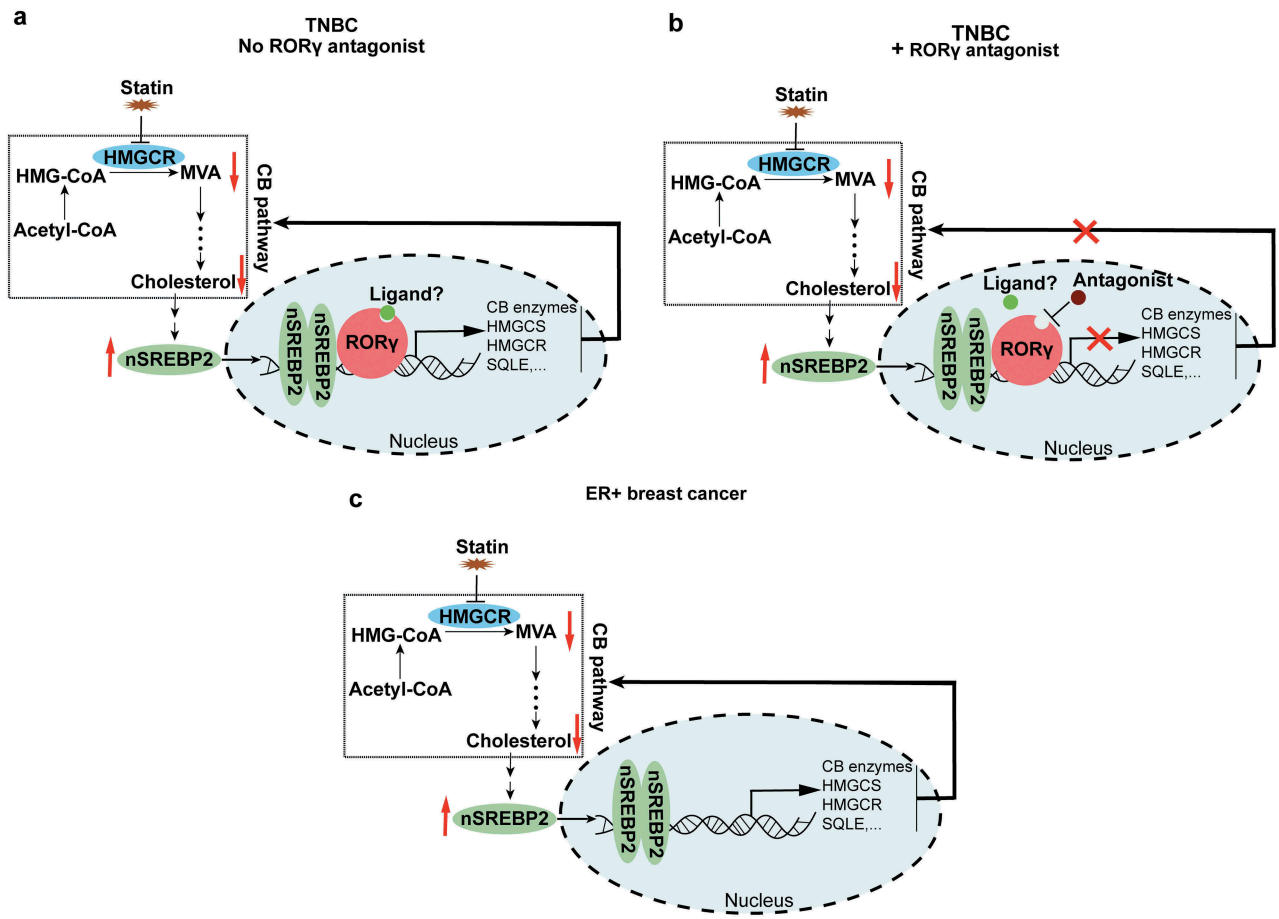

Figure 1. Role of RORγ in cholesterol biosynthesis in TNBC cells. In TNBC and ER+ breast cancers (panel a and c), statin treatment initially results in a reduction of MVA and cholesterol production, which then triggers the processing of full-length, primary form of SREBP2 protein to its transcriptionally active nSREBP2. nSREBP2 activates the expression of CB enzyme genes. In TNBC, such feedback activation may also involve RORγ which might be bound with an endogenous ligand that can further activate RORγ (panel a). Treatment of TNBC tumors with a RORγ antagonist can effectively block the feedback activation. TNBC, triple-negative breast cancer; RORγ, RAR-related orphan receptor γ; Acetyl-CoA, acetyl coenzyme A; HMGCR, hydroxy-3-methylglutaryl-CoA reductase; MVA, mevalonate; nSREBP2, nuclear sterol regulatory element-binding protein; CB, cholesterol biosynthesis; HMGCS, hydroxymethylglutaryl-CoA synthase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; SQLE, squalene epoxidase; ER+, estrogen receptor-positive.

activated by products of CB program,[7] thus constituting a positive feedback loop. Future studies are warranted to examine whether CB intermediates or metabolites can activate RORγ specifically in TNBC and whether RORγ plays a similar, dominant function in other cancer types or subtypes.

CB program is crucial for cell proliferation and oncogenic signaling and is a long-sought-after pathway for cancer therapeutic targeting. Use of statins, a group of drugs that inhibit the enzymatic activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in CB for control of hypercholesterolemia, is significantly associated with decreased mortality.[8] However, clinical trials with statins in the adjuvant setting have largely failed to yield significant benefit to cancer patients. One major observation is that tumors display a heightened CB feedback response to statin.[9] Different from targeting the CB pathway per se, inhibition of RORγ does not invoke any of the feedback, which may explain the high anti-TNBC tumor efficacy of the RORγ antagonists we observed when they were administered at relatively low doses. Our finding that low dose combinations of RORγ antagonists and statins elicited a strong anti-tumor synergy in different patient-derived xenograft (PDX) models underscores the dominant role of RORγ and the effectiveness of eliminating statin-induced CB rebound by targeting RORγ. The effectiveness in blocking tumor metastasis by targeting RORγ and the overall safe profile are also worth noting. To facilitate the translation, identification of biomarkers that predict tumor response to the treatment will be an important next step. Moreover, given that RORγt-dependent interleukin-17 (IL-17)-producing γδT cells and T helper 17 (Th17) cells can recruit neutrophils, myeloid-derived suppressor cells (MDSCs) and regulatory T (Treg) cells to promote metastasis and immune suppression,[10] it is also important to examine the pro-tumorigenic role of RORγt in tumor immune microenvironment and the contribution of RORγt inhibition to the overall anti-tumor efficacy. In summary, our study revealed a novel function of RORγ co-opted by a tumor subtype and offers a new therapeutic strategy of targeting tumor metabolic reprogramming.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

Our work is supported by grants from NIH [R01CA224900 and R01CA206222].

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