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### Title

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### Permalink

<https://escholarship.org/uc/item/84d0k5b4>

### Journal

Genes & Diseases, 10(1)

### ISSN

2352-3042

### Authors

Jiang, Nian

Xie, Bowen

Fan, Ming

et al.

### Publication Date

2023

### DOI

10.1016/j.gendis.2022.07.019

Peer reviewed



RESEARCH HIGHLIGHT

# Does burning fat make tumor immune hot? Discovery of CD47 overexpression by radiation induced fatty acid oxidation



Nian Jiang <sup>a,b</sup>, Bowen Xie <sup>a,c</sup>, Ming Fan <sup>a</sup>, Jian Jian Li <sup>a,d,\*</sup>

<sup>a</sup> Department of Radiation Oncology, University of California Davis School of Medicine, Sacramento, CA 95817, USA

<sup>b</sup> Department of Neurosurgery, National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China

<sup>c</sup> Institute for Immunology and School of Medicine, Tsinghua University, Beijing 100084, China

<sup>d</sup> NCI-Designated Comprehensive Cancer Center, University of California Davis, Sacramento, CA 95817, USA

Received 23 July 2022; accepted 26 July 2022

Available online 13 August 2022

## KEYWORDS

CD47;  
Immune checkpoint;  
Immunotherapy;  
Metabolic rewiring;  
Radiation therapy;  
Tumor acquired  
resistance

**Abstract** Although extensively studied, it is unknown what is the major cellular energy driving tumor metastasis after anti-cancer radiotherapy. Metabolic reprogramming is one of the fundamental hallmarks in carcinogenesis and tumor progression featured with the increased glycolysis in solid tumors. However, accumulating evidence indicates that in addition to the rudimentary glycolytic pathway, tumor cells are capable of reactivating mitochondrial OXPHOS under genotoxic stress condition to meet the increasing cellular fuel demand for repairing and surviving anti-cancer radiation. Such dynamic metabolic rewiring may play a key role in cancer therapy resistance and metastasis. Interestingly, data from our group and others have demonstrated that cancer cells can re-activate mitochondrial oxidative respiration to boost an annexing energy to meet the increasing cellular fuel demand for tumor cells surviving genotoxic anti-cancer therapy with metastatic potential.

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\* Corresponding author. University of California Davis, Sacramento, CA 95817, USA.

E-mail address: [jjli@ucdavis.edu](mailto:jjli@ucdavis.edu) (J.J. Li).

Peer review under responsibility of Chongqing Medical University.

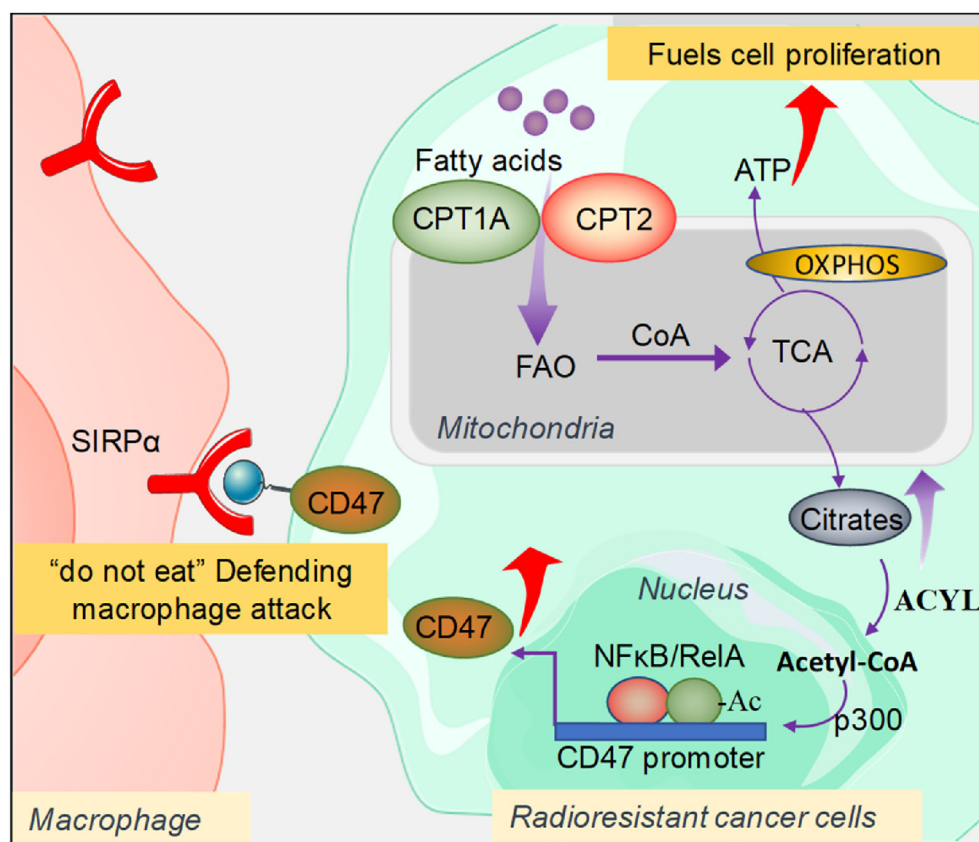
Although extensively studied, it is unknown what is the major cellular energy driving tumor metastasis after anti-cancer radiotherapy. Metabolic reprogramming is one of the fundamental hallmarks in carcinogenesis and tumor progression featured with the increased glycolysis in solid tumors. However, accumulating evidence indicates that in addition to the rudimentary glycolytic pathway, tumor cells are capable of reactivating mitochondrial OXPHOS under genotoxic stress condition to meet the increasing cellular fuel demand for repairing and surviving anti-cancer radiation. Such dynamic metabolic rewiring may play a key role in cancer therapy resistance and metastasis. Interestingly, data from our group and others have demonstrated that cancer cells can re-activate mitochondrial oxidative respiration to boost an annexing energy to meet the increasing cellular fuel demand for tumor cells surviving genotoxic anti-cancer therapy with metastatic potential.

Our previous studies have revealed that mitochondrial metabolic rewiring is required for cell cycle progression and radiation induced nuclear DNA repair. We found that the mitochondrial OXPHOS are shown to be dynamically adjusted to supply cellular energy required for cell cycle progression and DNA repair.<sup>1,2</sup> Such stress adaptive mitochondrial metabolism in normal cells are further identified and characterized in an array of human cancer cells and tumors with radiation. Radiation-surviving breast cancer

cells that are found to be with a more aggressive tripism due to a glycolysis-to-fatty acid oxidation (FAO) metabolic shifting.<sup>3</sup> The proliferative potential in the radioresistant breast cancer (BC) is driven by dual overexpression of HER2 and CD47, a fundamental immune checkpoint protein signaling “do not eat me” against macrophage phagocytosis.<sup>4</sup>

However, we wonder if such FAO-associated metabolic rewiring may change the immune landscape. Recently our group demonstrated that radioresistant glioblastoma (GBM) cells can rewire FAO which not only fuels the aggressive proliferation but also protects cancer cells from macrophage-mediated phagocytosis by FAO-derived citrates, which mediated NF- $\kappa$ B/RelA acetylation in CD47 transactivation. This harmonic pro-survival coordination between metabolic dynamics and the anti-phagocytotic function represents a highly flexible adaptive capacity of resistant cancer cells under genotoxic condition, also highlighting a vital role of metabolic rewiring in the immune-escaping ability of tumor cells.

Through IHC analysis of 46 high-grade gliomas (HGG) with primary tumor paired recurrent disease following treatment of radio- and/or chemotherapy after initial surgery, we found CD47 is co-enhanced with FAO enzymes (CPT1A, CPT2 and ACAD9) in recurrent GBM tumor. This phenomenon is also demonstrated in radioresistant GBM



**Figure 1** Immunosuppressive status are induced in radioresistant breast cancer and in recurrent GBM milieu after RT via radiation induced metabolic rewiring of fatty acid oxidation (FAO). FAO provides the ATP for the energy demands of tumor cell proliferation whereas FAO metabolite acetyl-CoA upregulates CD47 gene overexpression, leading to aggressive growth with immune evasion.

cell and regrown mouse syngeneic tumors after radiation. By analyzing The Chinese Cancer Genome Atlas data, we found co-enhancement of the FAO enzymes and CD47 is linked with a poor prognosis in GBM patients.<sup>5</sup> Result above strongly suggested that the rewired FAO enhancement may defend the radioresistant (RR) tumor cells against extrinsic immune surveillance via CD47 mediated immune suppression. To further explore the possibility of FAO mediated immunosuppression, wildtype (WT) and RR tumor cells were incubated with THP1 cells and a remarkably lowered phagocytosis rate was observed in RR cells than that in the WT counterparts. Then this remarked phagocytosis inhibition also present in regrown orthotopic mouse tumor after radiation therapy.

To explore the mechanisms underlying the coordination between metabolic dynamics and the anti-phagocytotic function, qPCR and Western blot assays were conducted which found CD47 mRNA was decreased obviously in RR U251 and RR U87 cells with CPT1A inhibition. In luciferase reporter assay, NF- $\kappa$ B reporter activity was reduced more than half in *CPT1A*<sup>-/-</sup>, *CPT2*<sup>-/-</sup> and *ACAD9*<sup>-/-</sup> cells, and reporters controlled by CD47 promoter containing NF- $\kappa$ B motif was suppressed in the *CPT1A*<sup>-/-</sup>, *CPT2*<sup>-/-</sup>, or *ACAD9*<sup>-/-</sup> cells, and such CD47-promoter activity was blocked when the NF- $\kappa$ B motif was deleted. Next, we tested that FAO-enhanced cytoplasmic citrates may function to bridge the FA metabolism and the immune checkpoint expression. We found the citrate and acetyl levels were remarkably elevated in RR cells and reduced by ET treatment or in *CPT1A*<sup>-/-</sup>, *CPT2*<sup>-/-</sup>, and *ACAD9*<sup>-/-</sup> cells. Luciferase reporter assay showed CD47 promoter-driven luciferase activity was enhanced by citrate treatment whereas no obvious enhancement was observed in the absence of NF- $\kappa$ B binding motif. By applying SB204990, a specific inhibitor of ACLY, citrate-enhanced CD47 mRNA levels and NF- $\kappa$ B activation was reversed, as well as citrate-enhanced RelA acetylation and CD47 protein levels, demonstrating that citrate-mediated RelA acetylation is actively involved in CD47 transcription.

To evaluate the potential synergetic effects of inhibiting both FAO and CD47, the regrown model of mouse orthotopic GL261 tumors expressing with GFP/Luciferase was generated allowing *in vivo* monitoring tumor growth after radiation. Treatments were administered when tumors were detected after shrinkage. Result showed that although tumor sizes were variable within each group, a noteworthy reduction of the regrown tumor volume was detected in mice treated by dual blockage with ET and CD47 antibody compared to ET or anti-CD47 alone. In consistence with the *in vivo* tumor imaging, the animal survival rate was remarkably enhanced in the group of dual blockades of FAO

and CD47 compared to single treatment with one mouse actually surviving by Day 54.

## Conclusion

As shown in [Figure 1](#), our findings suggest that FAO is boosted in cancer cells surviving radiotherapy which provide cellular fuel for aggressive cell proliferation and also boost immune checkpoint gene overexpression causing resistant cancer the aggressive growth with immune evasion (a situation as a breaking-room robbery with house monitor masked). Inhibition of FAO by specific therapeutic targets in FAO enzymes or FAO metabolites may enhance immune therapy by activating the cold microenvironment to eliminate such radioresistant-immunosuppressive tumor cells, thus improve outcome of patients with recurrent and metastatic diseases.

## Author contributions

N.J. drafted the main text. B.X. S.X., M.F., and J.J.L. made the figure and drafted the figure legend. J.J.L. edited the paper.

## Funding

J.J.L. is supported by a US National Institutes of Health (NIH) grant (RO1 CA213830).

## Conflict of interests

The authors declare no conflict of interests.

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