

IN THE SPOTLIGHT

Through a Clear Cell, Darkly: HIF2 α /PLIN2-Maintained Fat Droplets Protect ccRCCs from ER Stress

Jingwei Sim and Randall S. Johnson

Summary: Qiu and colleagues describe how a structural component of lipid droplets is markedly induced in pseudohypoxic renal tumors, where it maintains endoplasmic reticulum (ER) homeostasis. This adaptation is indispensable in tumor cells—where growth demands and a fluctuating blood supply place unnatural stresses on ER function—and is therefore an attractive therapeutic target. *Cancer Discov*; 5(6); 584-5. ©2015 AACR.

See related article by Qiu and colleagues, p. 652 (2).

With their lethal cocktail of high metabolic demands and poor vascularization, solid tumors are regularly assailed by hypoxia and endoplasmic reticulum (ER) stress. These insults compound each other—because protein folding consumes oxygen and energy, a hypoxic cell is particularly susceptible to ER stress. Healthy cells have evolved dedicated response pathways for each insult; these favor adaptation where possible, but lead to cell death when inappropriately activated. The unfolded protein response (UPR) alleviates ER stress by inhibiting translation, expediting protein processing, ER-associated degradation, and autophagy. The hypoxic response aims to match oxygen supply to demand and is primarily mediated by transcription factors known as hypoxia-inducible factors (HIF; the most important isoforms being HIF1 α and HIF2 α). When oxygen is low, HIF availability increases dramatically because it is no longer targeted for degradation by a complex that includes the von Hippel-Lindau protein (VHL).

Clear-cell renal cell carcinomas (ccRCC) can arise from loss-of-function mutations of *VHL*—these tumors overexpress HIF, i.e., they are pseudohypoxic. The term “clear cell” alludes to the abundance of cytosolic lipid droplets in these tumors. Lipid accumulation is unsurprising, given that HIF suppresses lipolysis and β -oxidation of fatty acids while conserving energy stores in preparation for reoxygenation. However, lipid droplets have another less-recognized function—they can alleviate ER stress by sequestering toxic lipids in neutral forms and providing an ER-associated route to retrotranslocate soluble misfolded proteins (1). The current work by Qiu and colleagues (2) describes the novel convergence of HIF and ER stress response signals on lipid droplets in ccRCC.

PLIN2 is a lipid droplet-associated structural protein with well-described metabolic functions. In the current study, Qiu and colleagues first noted from ccRCC tissue samples that

PLIN2 expression increases specifically with HIF2 α expression. They went on to show that this relationship was causal, because PLIN2 levels declined as a result of HIF2 α knockdown in the 786-O and RCC4 cell lines. Furthermore, this relationship was pathologically significant. HIF2 α knockdown in 786-O cells slowed xenograft growth and depleted lipid stores, but both neutral lipid staining and tumorigenicity were restored by PLIN2 overexpression. PLIN2 loss sensitizes ccRCC cultures to oleic acid treatment and pharmacologic agents that induce proteotoxicity. Conversely, inhibition of the UPR, mTORC1, and protein synthesis rescued PLIN2-depleted ccRCCs, leading to the conclusion that ER stress drives the cytotoxicity of PLIN2 loss.

PLIN2 is a recognized HIF target that defends NADPH levels and detoxifies reactive oxygen species in cultured glioblastoma and breast cancer cells during hypoxia and reoxygenation (3). Nevertheless, the work of Qiu and colleagues explores for the first time PLIN2 deficiency and overexpression in the context of ER homeostasis and *in vivo* in renal tumorigenesis. This work adds significantly to the existing literature on interactions between the UPR and HIF pathways. HIF is known to suppress mTOR activity, an important regulator of translation, while increasing expression of ER oxidoreductases. On the other hand, the UPR suppresses translation globally, while favoring a selective translation program that includes proteins for redox homeostasis and substrate availability, improving survival under hypoxia. UPR pathways also appear to phosphorylate HIF, potentiating its activity (4). Many molecular interactions are clearly involved, but further studies such as the present work are needed to weigh the macroscopic result of these interactions on tumorigenesis.

Although this work has addressed many pressing questions, others remain (3). What is the downstream pathway by which PLIN2 alleviates ER stress? Is ER stress relieved simply because of the presence of lipid droplets, whose formation requires PLIN2, or does PLIN2 itself interact in novel ways with other signaling molecules (5)? Outcomes of lipid storage and the UPR are dose dependent. Deficient and excess lipid storage are both disadvantageous to cells, causing lipotoxicity and energetic inefficiency, respectively. Both an underactive and an overactive UPR can lead to cell death. If PLIN2 is a key determinant of tumor cell survival via its roles in ER stress

Physiological Laboratory, University of Cambridge, Cambridge, United Kingdom.

Corresponding Author: Randall S. Johnson, University of Cambridge, Physiology Building, Downing Site, Cambridge CB2 3EG, United Kingdom. Phone: 44-01223-765931; Fax: 1-858-534-5831; E-mail: rsj33@cam.ac.uk

doi: 10.1158/2159-8290.CD-15-0480

©2015 American Association for Cancer Research.

and lipid storage, is its precise dosage important? This is an important therapeutic consideration (6). Lipid droplets are dynamic structures that interact with many other organelles. Is there any role for a HIF2 α -PLIN2 axis in organelle cross-talk with, say, mitochondria (4)? PLIN2 overexpression in ccRCCs is attributable to VHL/HIF2 α dysregulation, but PLIN2 is an oxygen-sensitive transcript in its own right. How is pseudohypoxic PLIN2 activity modified by real-time fluctuations in oxygen within the tumor microenvironment?

In the same vein, it is interesting that PLIN2's expression and tumorigenic effects in ccRCC were found to be driven by HIF2 α activity, but antagonized by HIF1 α . This is reminiscent of the "sibling rivalry" between HIF1 α and HIF2 α documented across various ccRCC models (7). Similar results were seen in a mouse colon tumor model, where tumor proliferation was PLIN2- and lipid droplet-dependent, whereas the HIF1 α target FOXO3 induced tumor death and PLIN2 and lipid depletion (8). However, interactions between HIF isoforms are often cell type specific. PLIN2 is HIF1 α dependent but not HIF2 α dependent in glioblastoma and breast cancer cell lines (3). HIF1 α -HIF2 α interactions in tumor lipid droplet-ER homeostasis are likely complex and merit separate investigation.

The role of the HIF2 α -PLIN2 axis in ccRCC progression could be practically useful for diagnosis and staging of ccRCCs. Elevated PLIN2 levels in urine are now established as a specific marker of ccRCCs, distinguishable from other renal pathologies (6). In contrast, one study correlated increased PLIN2 gene transcription with a lower tumor grade and improved clinical outcomes (9). This was an interesting result, but could be more clearly interpreted if tumors had been stratified by HIF1 α /HIF2 α predominance, in addition to VHL status.

The current study has significant therapeutic applications, having shown that a surprisingly large portion of HIF2 α 's tumorigenicity depends on PLIN2 activity. ER stress is therefore an Achilles' heel of tumor cells that can be exploited therapeutically when HIF2 α activity is inhibited. Conversely, PLIN2 and HIF2 α status may predict resistance of certain ccRCCs to anticancer agents that work by inducing ER stress. PLIN2 is likely a versatile drug target because it can be manipulated through a variety of pathways in addition to HIF-PPAR γ , PPAR α , inflammatory, and metabolic pathways. PLIN2 is also attractive for its selectivity. It is important for the survival of ccRCC cells, but not for healthy, nontransformed cells. Why is this so? The anabolic drive in ccRCC cells is much higher, placing them at greater risk of ER stress. Their PLIN2 levels are raised without reciprocal changes in other perilipins, unlike in regular cell models reported elsewhere. Thus, ccRCCs may be pushed into PLIN2 "addiction" specifically to stave off ER stress.

Finally, it is tempting to speculate that the importance of this HIF2 α -PLIN2 relationship extends to metabolic and ischemic pathologies where, as in the tumor microenvironment, hypoxic insults and ER stress often converge.

Conditions of interest might include diabetic nephropathy, atherosclerosis, and organ infarcts, where PLIN2 abundance and lipid droplets have been documented (10). Fatty liver disease is particularly relevant, as PLIN2 is the most abundant of all liver perilipins. Global deletion of PLIN2 specifically protects high-fat diet-fed mice from hepatic steatosis (5), with less consistent effects on other tissues. Conversely, forced HIF2 α overexpression leads to metabolic derangements in the liver (11). Perhaps a role for HIF contributions to ER stress and cell survival could bridge these sets of findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online June 2, 2015.

REFERENCES

1. Rutkowski DT, Wu J, Back S-H, Callaghan MU, Ferris SP, Iqbal J, et al. UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators. *Dev Cell* 2008;15:829-40.
2. Qiu B, Ackerman D, Sanchez DJ, Li B, Ochocki JD, Grazioli A, et al. HIF2 α -dependent lipid storage promotes endoplasmic reticulum homeostasis in clear-cell renal cell carcinoma. *Cancer Discov* 2015;5:652-67.
3. Bensaad K, Favaro E, Lewis CA, Peck B, Lord S, Collins JM, et al. Fatty acid uptake and lipid storage induced by HIF-1 α contribute to cell growth and survival after hypoxia-reoxygenation. *Cell Rep* 2014;9:349-65.
4. Pereira ER, Frudd K, Awad W, Hendershot LM. Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). *J Biol Chem* 2014;289:3352-64.
5. Chang BH-J, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, et al. Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. *Mol Cell Biol* 2006;26:1063-76.
6. Morrissey JJ, Mobley J, Figenshau RS, Vetter J, Bhayani S, Kharasch ED. Urine aquaporin 1 and perilipin 2 differentiate renal carcinomas from other imaged renal masses and bladder and prostate cancer. *Mayo Clin Proc* 2015;90:35-42.
7. Raval RR, Lau KW, Tran MGB, Sowter HM, Mandriota SJ, Li J-L, et al. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 2005;25:5675-86.
8. Qi W, Fitch PS, Cornwell ML, Greenberg J, Cabe M, Weber CR, et al. FOXO3 growth inhibition of colonic cells is dependent on intraepithelial lipid droplet density. *J Biol Chem* 2013;288:16274-81.
9. Yao M, Huang Y, Shioi K, Hattori K, Murakami T, Nakaigawa N, et al. Expression of adipose differentiation-related protein: a predictor of cancer-specific survival in clear cell renal carcinoma. *Clin Cancer Res* 2007;13:152-60.
10. Straub BK, Gyoengyoesi B, Koenig M, Hashani M, Pawella LM, Herpel E, et al. Adipophilin/perilipin-2 as a lipid droplet-specific marker for metabolically active cells and diseases associated with metabolic dysregulation. *Histopathology* 2013;62:617-31.
11. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, et al. Hypoxia-inducible factor 2 regulates hepatic lipid metabolism. *Mol Cell Biol* 2009;29:4527-38.

CANCER DISCOVERY

Through a Clear Cell, Darkly: HIF2 α /PLIN2–Maintained Fat Droplets Protect ccRCCs from ER Stress

Jingwei Sim and Randall S. Johnson

Cancer Discovery 2015;5:584-585.

Updated version Access the most recent version of this article at:
<http://cancerdiscovery.aacrjournals.org/content/5/6/584>

Cited articles This article cites 11 articles, 7 of which you can access for free at:
<http://cancerdiscovery.aacrjournals.org/content/5/6/584.full#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://cancerdiscovery.aacrjournals.org/content/5/6/584>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.