

## CCR Translations

See related article by von Roemeling et al., *Clin Cancer Res*, vol. 19(9), p. 2368–80

## Stearoyl Co-A Desaturase 1 as a ccRCC Therapeutic Target: Death by Stress

Janet Y. Leung and William Y. Kim

There is a need for the discovery of novel therapeutic strategies to effectively treat advanced clear cell renal cell carcinoma (ccRCC). Inhibition of stearoyl-coA desaturase 1 (SCD1) in ccRCC reveals antitumor activity, independently and in synergy with mTOR inhibition. SCD1 may be a potential novel therapeutic target in treating ccRCC. *Clin Cancer Res*; 19(12); 3111–3. ©2013 AACR.

In a recent issue of *Clinical Cancer Research*, von Roemeling and colleagues report that inhibition of aberrant stearoyl-coA desaturase 1 (SCD1) expression attenuates cell proliferation and induces apoptosis in clear cell renal cell carcinoma (ccRCC) cells via the induction of endoplasmic reticulum stress response signaling (1). These observations implicate SCD1 as a potential therapeutic target.

The process of lipid biosynthesis is essential for metabolic energy, regulation of cell signaling, and the formation of new membranes and organelles during cell proliferation and growth. A fundamental step in lipid synthesis is the production of saturated fatty acids (SFA), which subsequently are converted to monounsaturated fatty acids (MUFA), the major fatty acid constituents in cell lipids (2). Catalytic enzymes central to this regulation of fatty acid composition are the SCDs, also known as fatty acyl-CoA delta-9 desaturases (3). The human genome contains 2 homologs of SCD (SCD1 and SCD5), among which SCD1 is the best characterized in humans.

Many cancer cells are characterized by a high rate of aerobic glycolysis resulting in an abundance of pyruvate, which generates citrate upon entry into the tricarboxylic acid (TCA) cycle. Citrate, through its sequential activation by adenosine triphosphate citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase, can serve as a building block for the *de novo* synthesis of cellular lipids (Fig. 1; ref. 2). Although the main products of glucose-derived fatty acid synthesis are SFAs, an abundance of MUFAs is also found in cancer cells and tissues, implicating an essential role for SCD1 in these cells to catalyze the conversion of SFAs into MUFAs. In support of this notion, increased expression of

SCD1 has been reported in a number of cancers and cell lines including lung and breast cancer cells, colonic and esophageal carcinomas, hepatocellular adenomas, and hepatocellular carcinomas, amongst others (3).

In a recent issue of *Clinical Cancer Research*, von Roemeling and colleagues reported that ccRCC tumors (both early stage and metastatic) express elevated mRNA and protein levels of SCD1 compared with matched normal tissues (1). Knockdown of SCD1 expression with short hairpin RNA (shRNA) resulted in a dramatic decrease in the proliferation of ccRCC cell lines but did not have a detrimental effect on several independently derived normal renal epithelial (NRE) cells. Subsequent analyses revealed that this loss in proliferation is mostly attributed to an increase in apoptosis. Furthermore, treatment of ccRCC cell lines with nanomolar concentrations of A939572, a small molecule inhibitor of SCD1 enzymatic activity, also showed a dose-dependent decrease in proliferation and an increase in apoptosis consistent with results observed from their RNAi experiments. To confirm that decreased tumor cell growth was a specific result of suppressed SCD1 activity, the authors show that a cell culture stable form of oleic acid (OA-bovine serum albumin), the principle MUFA product of SCD1-mediated SFA hydrogenation, was able to rescue the antiproliferative and apoptotic phenotypes of A939572 treatment.

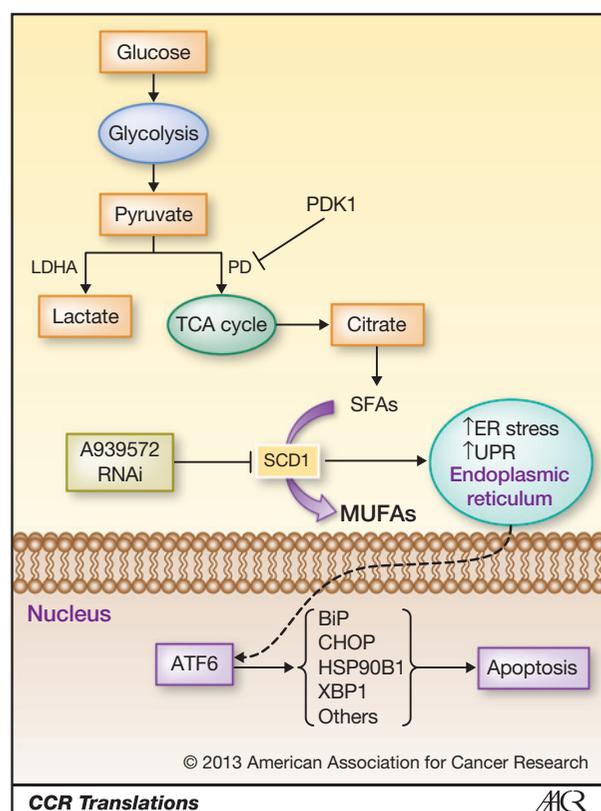
To establish the underlying mechanism responsible for the decreased proliferation and induction of cell death associated with loss of SCD1 activity, the gene expression changes induced by A939572 treatment were assessed. Pathway analysis revealed increased expression of genes associated with endoplasmic reticulum stress and the unfolded protein response (UPR), a cellular response that occurs in reaction to the buildup of misfolded and unfolded proteins in the endoplasmic reticulum (4). von Roemeling and colleagues found that mRNA expression of a number of endoplasmic reticulum stress markers (BiP, CHOP, HERPUD1, GADD45, CDBP $\beta$ ) were indeed upregulated in ccRCC cell lines treated with A939572. Moreover, ATF6, a central mediator of the UPR stress response, was also found to be activated upon inhibition of SCD1 activity (Fig. 1; ref. 4). Importantly, knockdown of ATF6 using shRNAs blocked the induction of endoplasmic reticulum stress

**Authors' Affiliation:** Department of Medicine and Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

**Corresponding Authors:** Janet Y. Leung, Lineberger Comprehensive Cancer Center, University of North Carolina, CB# 7295, Chapel Hill, NC 27599. Phone: 919-966-9729; Fax: 919-966-8212; E-mail: janet\_leung@med.unc.edu; and William Y. Kim, E-mail: wykim@med.unc.edu

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**Figure 1.** Glycolysis results in the production of pyruvate, which can be converted to acetyl-CoA (not shown) by pyruvate dehydrogenase (PD). PD activity can be suppressed by PDK1 whereby pyruvate is then directed toward lactate production by lactate dehydrogenase A (LDHA). Acetyl-CoA enters the TCA cycle and is converted to citrate, a portion of which is diverted toward fatty acid synthesis. SFAs are converted to MUFAs by SCD1. SCD1 inhibition by RNAi or A939572 results in the UPR and increased endoplasmic reticulum (ER) stress resulting in the increased production of ATF6, which activates expression of genes mediating stress response pathways (BiP, CHOP, HSP90B1, and XBP1). Under prolonged ER stress, the cell responds by initiating apoptosis.

response genes in response to A939572 and rescued the phenotypes induced by A939572 treatment. These observations show that SCD1's regulation of proliferation and apoptosis is dependent upon the initiation of endoplasmic reticulum stress and the UPR.

The clinical significance and potential application of the work by von Roemeling and colleagues is most evident by the observed synergism between SCD1 inhibition with the allosteric mTOR complex (mTORC)-1 inhibitor, temsirolimus. Careful *in vitro* studies showed that A939572 inhibited proliferation synergistically with temsirolimus but not the multitargeted tyrosine kinase inhibitors, pazopanib and sunitinib. Moreover, *in vivo* combination of A939572 with temsirolimus showed enhanced tumor growth inhibition over either agent alone in a ccRCC cell line xenograft.

Several aspects of the work presented by von Roemeling and colleagues strongly support the practicality of SCD1 as a molecular target in the clinic. First, although inhibition of SCD1 decreased proliferation and induced apoptosis in

ccRCCs, no notable effects were observed in NRE cells, and only increased blinking and slight mucosal discharge from eyes were observed in immunocompromised animals treated with A939572. This makes SCD1 inhibition an ideal candidate for therapeutic intervention with possibly minimal toxicity to patients. Second, the increased expression of SCD1 in ccRCC makes SCD1, itself, an ideal potential predictive marker to identify patients who will most likely yield a response to pharmacologic inhibition of SCD1. Finally, the induction of endoplasmic reticulum stress response genes to SCD1 inhibition might serve as a pharmacodynamic marker to assess the effectiveness of anti-SCD1 therapy. Collectively, these observations strongly support SCD1 as a novel molecular target for the treatment of advanced ccRCC that warrants clinical investigation.

Nonetheless, some questions remain. For example, at what stage in tumor development does increased fatty acid synthesis become necessary for sustained tumor growth? Also, given the established role for mTORC1 in regulating lipid metabolism and sterol regulatory element-binding protein 1c (5), to what extent does the synergism between SCD1 and mTOR inhibition reflect independent effects upon endoplasmic reticulum stress or combined down-regulation of SCD1 activity? Finally, ccRCC cells deficient in the von Hippel-Lindau tumor suppressor (*VHL*) gene have constitutively elevated levels of the hypoxia-inducible factor (HIF) even under normoxia (6). HIF's ability to suppress oxidative phosphorylation, through pyruvate dehydrogenase kinase (PDK)-1, favors the production of lactate and decreases the pool of glucose-derived carbon available for lipid synthesis (7-9). Maintenance of fatty acid synthesis by *VHL*-deficient ccRCC cells is therefore mediated by the reductive carboxylation of glutamine in a glutaminase and isocitrate dehydrogenase 1-dependent manner (10). Therefore, whether glutaminase inhibition when combined with SCD1 and mTOR inhibition leads to further therapeutic gains should be explored. In summary, the observations by von Roemeling and colleagues underscore the notion that cancer cells have altered metabolic demands that can be therapeutically targeted. In addition, their findings highlight the increased recognition that tumor cells, in contrast to nontransformed cells, are dependent upon *de novo* fatty acid synthesis (rather than exogenous fatty acids) for maintenance of cellular homeostasis (2).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** W.Y. Kim, J.Y. Leung

**Writing, review, and/or revision of the manuscript:** W.Y. Kim, J.Y. Leung

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