Stearoyl-CoA desaturase 1 is a novel molecular therapeutic target for clear cell renal cell carcinoma

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ABSTRACT

Purpose: Identify SCD1 as a novel molecular target in clear cell renal cell carcinoma (ccRCC) and examine its role in tumor cell growth and viability *in vitro* and *in vivo* independently as well as in combination with current FDA approved regimens.

Experimental Design: Patient normal and ccRCC tissue samples and cell lines were examined for *SCD1* expression. Models of genetic knockdown and targeted inhibition of SCD1 through use of a small molecule inhibitor, A939572, were analyzed for growth, apoptosis, and alterations in gene expression using gene array analysis. A therapeutic model of synergy was evaluated by combining A939572 with the mTOR inhibitor temsirolimus.

Results: Our studies identify increased *SCD1* expression in all stages of ccRCC. Both genetic knockdown and pharmacologic inhibition of SCD1 decreased tumor cell proliferation and induced apoptosis *in vitro* and *in vivo*. Further analysis of A939572 treated or SCD1 lentiviral knockdown samples demonstrated induction of endoplasmic reticulum (ER) stress response signaling, providing mechanistic insight for SCD1 activity in ccRCC. Furthermore, combinatorial application of A939572 with temsirolimus synergistically inhibited tumor growth *in vitro* and *in vivo*.

Conclusions: Increased SCD1 expression supports ccRCC viability and therefore we propose it as a novel molecular target for therapy either independently or in combination with an mTOR inhibitor for patients whose disease cannot be remedied with surgical intervention, such as in cases of advanced or metastatic disease.
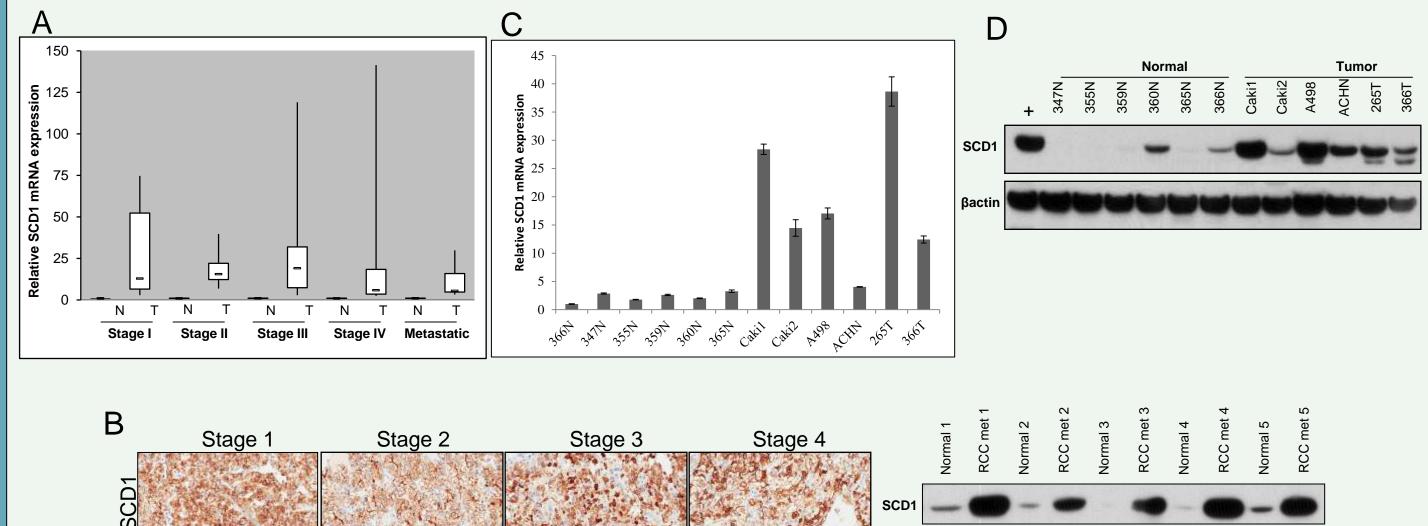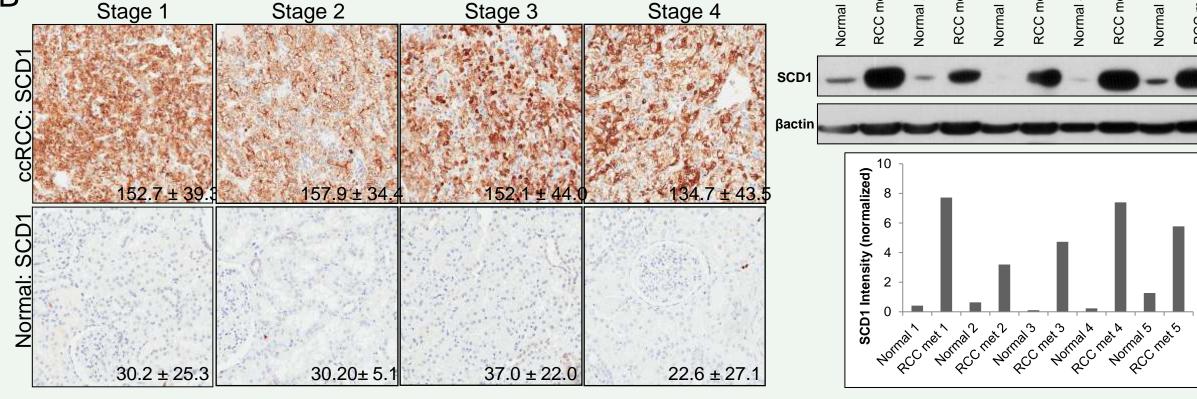
BACKGROUND

- Renal cell carcinoma (RCC) is the third most prevalent urological cancer, the 10th most common cause of cancer death in men and the 9th most common cause in women.

- clear cell carcinoma (ccRCC) is the most common subtype of RCC accounting for ~80% of all renal cancers.

- Standard of care for patients presenting with localized ccRCC is partial or whole nephrectomy, however ~30% of these patients go on to develop metastatic ccRCC.

- For individuals presenting with advanced disease, treatment options are limited with no current drug therapy leading to long term survival with the exception of 6-7% of patients who respond to interleukin-2.

- Currently there are very few hallmark genetic features which are known to contribute to ccRCC development and progression which can be specifically targeted as an anti-tumor treatment strategy

- Our group has identified that SCD1 is overexpressed in ccRCC at all stages of disease.

- SCD1 is an iron-containing enzyme belonging to a family of fatty acyl desaturases, whose role is to catalyze the biosynthesis of Δ9 monounsaturated fatty acids (MUFA), oleic and palmitoleic acid, from the saturated fatty acids (SFA) stearic and palmitic acid. It is a critical enzyme in the fatty acid metabolism pathway and is a rate limiting step in MUFA synthesis.

- MUFAs are involved in many biological processes and are a major constituent of biological structures such as membranes, and can also function as or modify signaling molecules. This suggests a potential higher need for them in dynamic or rapidly dividing cells such as cancer cells.

RESULTS

SCD1 is overexpressed in ccRCC

Figure 1. QPCR of total mRNA extracted from patient stage I through IV and metastatic ccRCC tumor tissues vs. matched distant site normal tissue analyzed for gene expression of *SCD1* shows overexpression in all tumor samples (Fig 1A). IHC staining for SCD1 protein expression in patient matched tumor and normal tissue confirms elevated expression in tumor samples across all stages (Fig 1B). Increased SCD1 expression in metastatic samples is confirmed by western blot (Fig 1B). To validate cell models, QPCR and western blot analysis of six primary NRE and six ccRCC cell lines for SCD1 expression (Fig 1C,D) was performed and yielded expression patterns analogous to those observed in tissue.

SCD1 KD induces tumor specific cell death

Figure 2. Two lentiviral constructs that target SCD1 (shSCD780, shSCD1200) demonstrate over 80% mRNA knockdown in two NRE (K359N, K360N) and two ccRCC cell lines (Caki1, A498) (Fig 2A). Growth analysis resulted in over an 80% decrease in proliferation among tumor samples at day 5 post-infection (Fig 2B) but not in NRE samples (Fig 2C). Western blot analysis for poly-ADP-ribose polymerase (PARP) cleavage, a marker for apoptosis, confirmed PARP cleavage in both Caki1 and A498 cells infected with each shSCD lentiviral construct compared to NT controls (Fig 2D), and indicates that loss of proliferation is due in part by induction of programmed cell death. Specificity of shSCD780 and shSCD1200 for SCD1 was confirmed by western blot (Fig 2D).

Inhibition of SCD1 in ccRCC cells induces cell death through ER stress activation
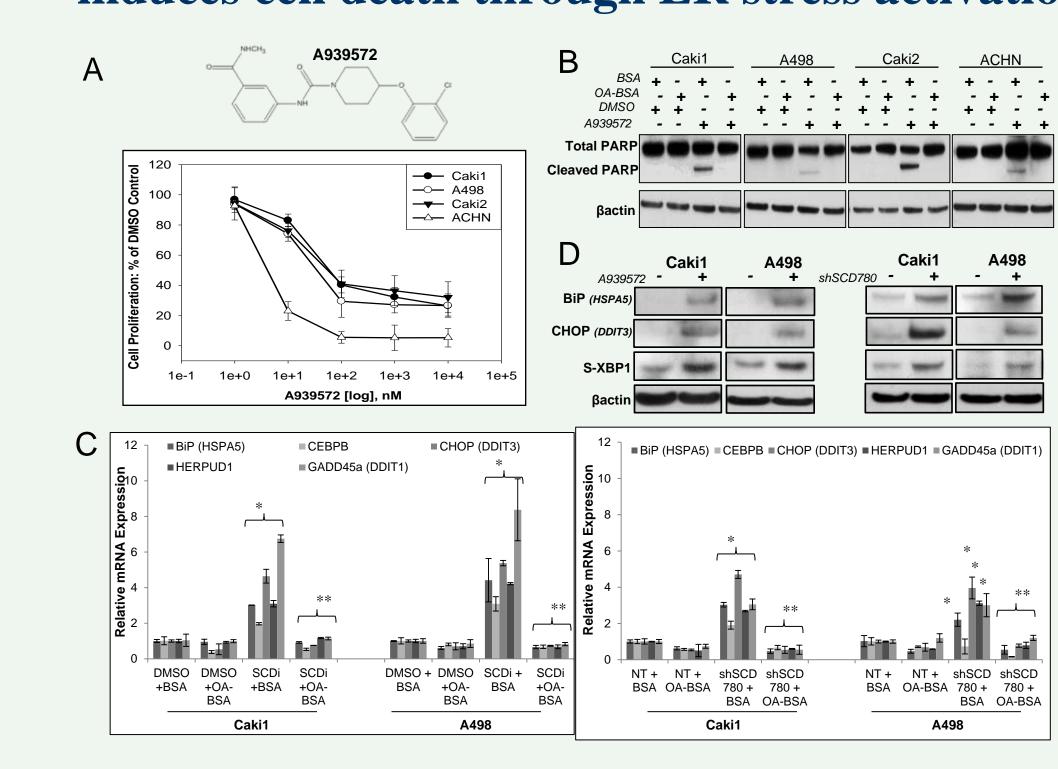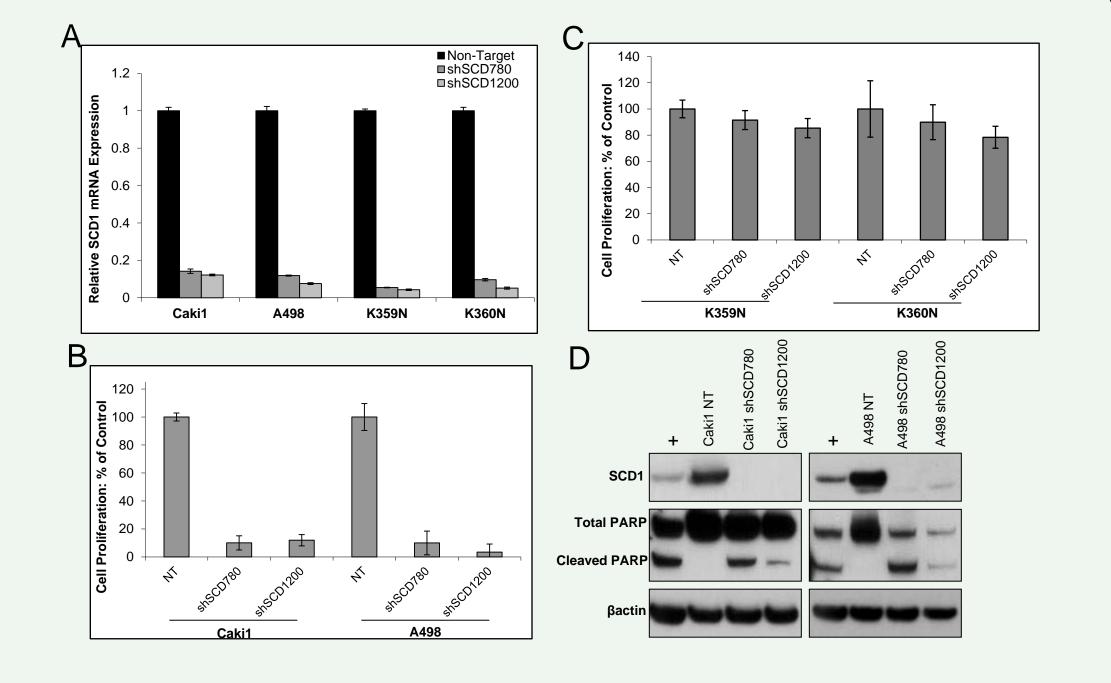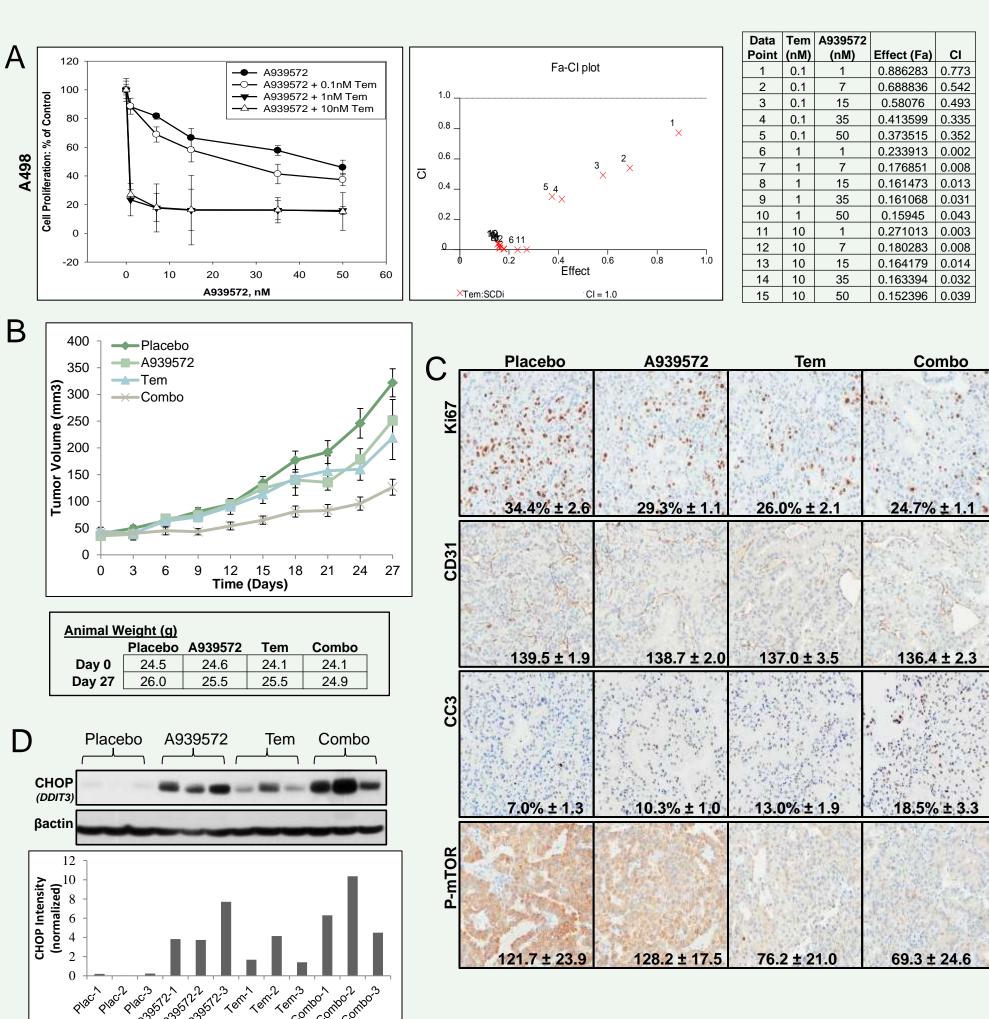

Figure 3. A939572 is a small molecule that specifically inhibits SCD1 enzymatic activity (1) and demonstrates a dose-dependent decrease in proliferation and induces cell death in Caki1, A498, Caki2, and ACHN (Fig 3A,B). Target specificity was confirmed by rescue of cell death in A939572 treated (IC50 dose) ccRCC cells with oleic acid (OA-BSA), the primary product of SCD1 (Figure 3B). ER stress signaling is induced by SCD1 inhibition in Caki1 and A498 cells treated with either A939572 or shSCD780 as demonstrated by QPCR analysis of five ER stress genes (*BiP, CHOP, HERPUD1, GADD45a,* and *CEBPβ),* and can be rescued with OA-BSA (Figure 3C). Western blot of Caki1 and A498 cells for protein expression of ER stress markers BiP, CHOP, and spliced-XBP1 revealed amplified expression in both drug treated and shSCD780 lentiviral knockdown cells (Figure 3D), confirming induction of ER stress upon loss of SCD1 expression.

Combinatorial Inhibition of SCD1 and mTOR synergistically decreases tumor cell growth *in vivo*

Figure 4. Combinatorial treatment utilizing A939572 in congruence with temsirolimus (Tem), an mTOR inhibitor currently FDA approved for treatment of advanced ccRCC, yielded strong synergy in four ccRCC cell lines (Fig 4A, data not shown) indicated by the combination index (CI) determined using CalcuSyn® (2). Athymic nude (nu/nu) mice bearing A498 ccRCC xenografts were treated with A939572 and Tem individually or in combination, and tumor volume (mm³) was recorded (Fig 4B). IHC analysis of tumors were examined for proliferation (KI67), cell death (cleaved caspase 3, CC3), microvessel density (CD31) and mTOR phosphorylation (Fig 4C). ER stress was examined via western blot of total protein extractions prepared from randomly selected tumor tissue samples representing each treatment group, and increased expression of CHOP was confirmed in all samples treated with A939572 (A939572 and Combo) (Fig 4D) confirming that inhibition of SCD1 in ccRCC contributes to ER stress *in vivo*.
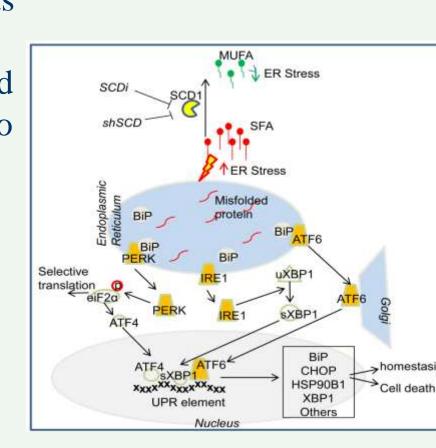
CONCLUSIONS

- SCD1 is frequently overexpressed in ccRCC, and may serve as a biomarker to identify patients who are appropriate candidates for anti-SCD1 therapy

- Inhibition of SCD1 both genetically and pharmacologically abrogates tumor cell growth, induces apoptosis, and promotes ER stress both *in vitro* and *in vivo*

- Anti-tumor activity is mediated by the ER stress response, and therefore ER stress factors may serve as biomarkers for response to anti-SCD1 therapy

- The SCD1 inhibitor A939572 when combined with the mTOR inhibitor temsirolimus yields strong anti-tumor synergy *in vitro* and *in vivo*, providing a novel multi-targeting strategy which should be investigated for ccRCC patients presenting with advanced or metastatic disease

FUTURE DIRECTIONS

Current Literature:

SCD1 converts SFA into MUFA and therefore inhibition of SCD1 likely leads to an increase in SFA content in cells. Current literature suggests that increased exposure of cells to SFAs corresponds to an accumulation of SFA content in membrane structures, altering the morphology and decreasing membrane fluidity (3). This may compromise the integrity as well as the functionality of the membranes, including those of the ER, leading to a stress response (3-5). Desaturation of fatty acids is thought to counter these effects, and is protective against SFA mediated stress (3, 6-7).

Future Directions:

- Identify molecular mechanisms regulating SCD1 expression in ccRCC in order to ascertain the role of SCD1 in ccRCC initiation, development, and progression

- Identify how loss of SCD1 mediates the ER stress response (direct vs. indirect) in order to understand the mechanism by which SCD1 promotes ccRCC tumorigenicity

- The preclinical data shown here strongly supports the investigation of anti-SCD1 therapy alone or in combination with an mTOR inhibitor in a phase 1 clinical trial for patients with advanced or metastatic ccRCC.

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