SCD1 is a novel target in Anaplastic Thyroid Carcinoma

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ABSTRACT

Introduction:

Anaplastic Thyroid Carcinomas (ATC) are aggressive, undifferentiated tumors for which there currently is no cure. Although ATC accounts for a mere 1.7 percent of all thyroid cancers in the United States, it is disproportionately responsible for a large fraction of thyroid cancer related deaths. Patients diagnosed with ATC have grim prognoses, with a median survival rate of 5 months. ATC not only is resistant to traditional radio and chemotherapeutics, but targeted applications as well. Furthermore, due to its highly invasive nature, surgical resection is often not an option. Clearly, new therapies for ATC are sorely needed. Stearoyl-CoA Desaturase 1 (SCD1) is a fatty acid metabolism enzyme whose expression has been implicated in pro-tumor survival of several subsets of cancer.

Methods:

SCD1 expression patterns were examined in patient samples of normal thyroid, follicular adenoma (FA), papillary thyroid carcinoma (PTC), high grade follicular thyroid carcinoma (FTC), and ATC via quantitative real-time PCR as well as by immunohistochemistry. Activity of the SCD1 inhibitor A939572 was tested in representative patient derived cell lines.

Results:

SCD1 demonstrates high levels of expression in papillary thyroid carcinoma (PTC), high grade follicular thyroid carcinoma (FTC), and ATC. Application of an SCD1 inhibitor (A939572) surprisingly yielded strong anti-tumor proliferation and induction of apoptosis specifically in ATC cell lines, but had little to no activity among the other subtypes of thyroid carcinomas. In addition, A939572 induced the endoplasmic reticulum (ER) stress pathway, leading to endoplasmic reticulum associated degradation (ERAD) activation- a proteasome mediated survival response to ER stress. Sequential application of a proteasome inhibitor with A939572 led to synergistic anti-tumor activity in ATC cell lines.

Conclusions:

We propose that administration of an SCD1 inhibitor concomitantly with a proteasome inhibitor is a novel course of treatment that may provide a clinical benefit for patients presenting with ATC.

BACKGROUND

ATC:

- The origin of ATC is unknown, but is thought to arise from less aggressive pre-existing thyroid cancers such as the papillary variant
- ATC progresses rapidly, and requires immediate medical intervention once diagnosed
- Mean survival rate of 3 to 9 months post diagnosis, with less than 10-15% of patients surviving 2+ years
- 20-50% of patients present with distant metastasis at time of diagnosis, and 25% develop new metastasis during disease progression
- ATC is classified as stage IV regardless of tumor size or presence of lymph node or distant metastasis (American Joint Committee on Cancer)
- Radical tumor resection of ATC confers a substantial survival benefit, with complete resection identified as a prognostic factor for survival
- Common mutations reported in ATC include p53 (55%), RAS (22%), BRAF (26%), β-catenin (38%), PIK3CA (17%), Axin (82%), APC (9%), PTEN (12%)

SCD1:

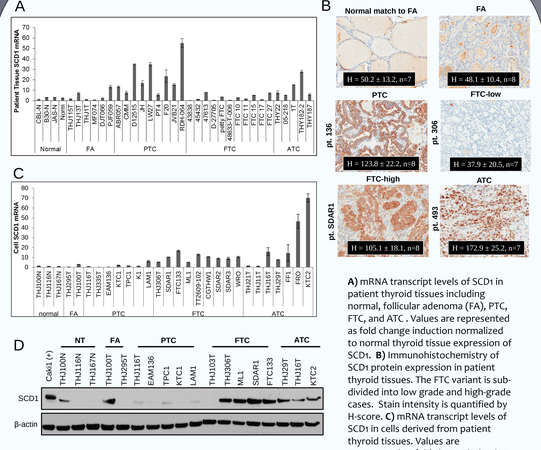
- SCD1 is a fatty acid metabolism enzyme belonging to a family of fatty acyl desaturases, whose role is to catalyze the biosynthesis of Δ9 monounsaturated fatty acids (MUFA), oleic and palmitoleic acid, from the saturated fatty acids (SFA) stearic and palmitic acid
- SCD1 has been proposed as a therapeutic target in other aggressive cancers including renal, breast , prostate, lung, and ovarian carcinomas
- Inhibition of SCD1 activity has been shown to induce the ER stress response in cancer cells

Treatment:

- ***Currently there is no defined standard of care therapy for patients with ATC***
- ATC does not respond to radioiodine therapy or TSH suppression with thyroxine
- Treatment includes combination of radiotherapy with chemotherapy (cisplatin, doxorubicin, paclitaxil, carboplatin) and targeted therapy
- Targeted therapies under investigation in ATC: Anti-angiogenic therapy (Pazopanib), vascular disrupting agents (Combretastatin A4 phosphate), Tyrosine kinase inhibitors (Imatinib, Sorafenib), Histone deacetylase inhibitors (valproic acid), and PPARY agonists (Efatutazone)

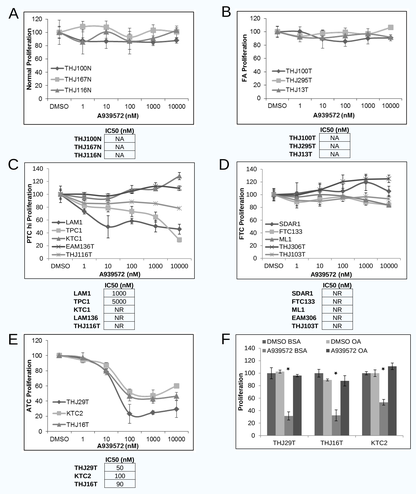
RESULTS

Figure 1: Expression Analysis of SCD1 in Thyroid Cancer



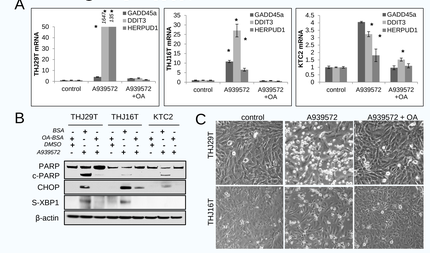
A) mRNA transcript levels of SCD1 in patient thyroid tissues including normal, follicular adenoma (FA), PTC, FTC, and ATC . Values are represented as fold change induction normalized to normal thyroid tissue expression of SCD1. **B)** Immunohistochemistry of SCD1 protein expression in patient thyroid tissues. The FTC variant is sub-divided into low grade and high-grade cases. Stain intensity is quantified by H-score. **C)** mRNA transcript levels of SCDn in cells derived from patient thyroid tissues. Values are represented as fold change induction normalized to normal thyroid cell expression of SCD1. **D)** Western blot of SCD1 expression in cells derived from patient thyroid tissues.

Figure 2: A939572 Inhibits ATC Cell Proliferation



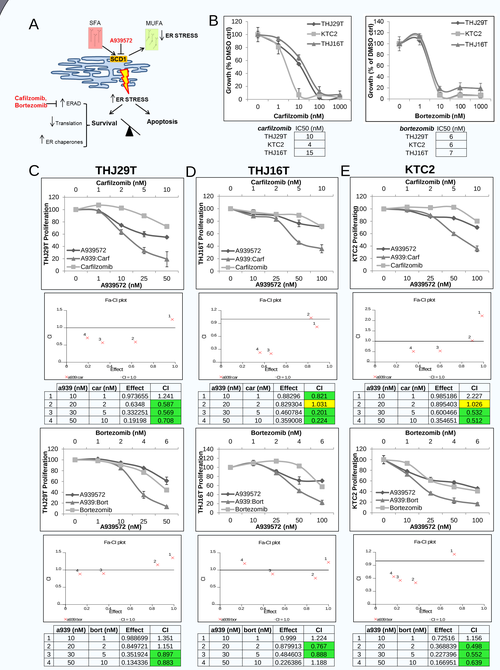
Drug dose response of thyroid cells including **(A)** normal, **(B)** follicular adenoma (FA), **(C)** PTC, **(D)** FTC, and **(E)** ATC cells to A939572, a small molecule selective inhibitor of SCD1 activity. **(F)** Target specificity was confirmed by rescue of cell proliferation in A939572 treated (IC50 dose) ATC cells with oleic acid (OA), the primary product of SCD1 .

Figure 3: A939572 Induces ER Stress in ATC



Evaluation of ER stress induction in ATC cells treated with A939572 by **(A)** QPCR analysis of three ER stress genes (*GADD45a* , *DDIT3* (*CHOP*), *HERPUD1*) and **(B)** Western blot for protein expression of CHOP and spliced-XBP1. **(C)** Western blot for PARP cleavage (c-PARP) indicating programmed cell death. **(D)** Representative phase-contrast microscopy of ATC cells treated with A939572 +/- OA rescue demonstrating cell death (decreased cell density and cell detachment) and subsequent rescue with oleic acid.

Figure 4: A939572 / Proteosome Inhibitor Synergy in ATC



THJ29T	a939 (nM)	car (nM)	Effect	CI
1	10	1	0.973655	1.241
2	20	2	0.6348	0.597
3	30	5	0.332251	0.569
4	50	10	0.19198	0.708

THJ16T	a939 (nM)	car (nM)	Effect	CI
1	10	1	0.88296	0.821
2	20	2	0.829304	1.031
3	30	5	0.460784	0.201
4	50	10	0.359009	0.224

KTC2	a939 (nM)	car (nM)	Effect	CI
1	10	1	0.985186	2.227
2	20	2	0.895403	1.076
3	30	5	0.600466	0.532
4	50	10	0.354651	0.512

THJ29T	a939 (nM)	bort (nM)	Effect	CI
1	10	1	0.988699	1.351
2	20	2	0.849721	1.151
3	30	5	0.351924	0.897
4	50	10	0.134336	0.883

THJ16T	a939 (nM)	bort (nM)	Effect	CI
1	10	1	0.999	1.224
2	20	2	0.879913	0.767
3	30	5	0.484603	0.388
4	50	10	0.226386	1.188

KTC2	a939 (nM)	bort (nM)	Effect	CI
1	10	1	0.72516	1.156
2	20	2	0.368839	0.496
3	30	5	0.227396	0.552
4	50	10	0.166951	0.639

(A) Proposed model of SCD1 inhibitor and proteosome inhibitor drug synergy. Drug dose response of ATC cells to proteosome inhibitors **(B)** carfilzomib (targets 20S proteosome subunit) and **(C)** bortezomib (targets 26S proteosome subunit). Mono and combinatorial dose response and synergy curves of **(C)** THJ29T, **(D)** THJ16T, and **(E)** KTC2 ATC cells treated with A939572 and either carfilzomib or bortezomib. Synergy is indicated indicated by the combination index (CI) determined using CalcuSyn® where synergistic values are CI<0.9 (green highlight), additive values are 0.9-1.1 (yellow highlight), and agonistic values are 1.1<CI.

CONCLUSIONS

- SCD1 demonstrates elevated expression patterns in PTC, advanced FTC, and ATC subtypes of thyroid carcinoma when compared to normal and benign thyroid tissue
- Presence of SCD1 protein does not dictate response to anti-SCD1 therapy
- ATC cells uniquely demonstrate decreased proliferation and induction of apoptosis in response to SCD1 inhibition (A939572)
- SCD1 inhibition in ATC induces the ER stress response, leading to up-regulation of transcriptional inhibitors, molecular chaperones, and ERAD
- ATC cells are sensitive to mono-therapeutic inhibition of proteosome activity using the inhibitors carfilzomib and bortezomib
- Complementary targeted inhibition of the proteosome degradation pathway using either carfilzomib or bortezomib in addition to SCD1 inhibition results in the synergistic inhibition of ATC proliferation
- Our hypothesized molecular mechanism of drug synergy between A939572 and a proteosome inhibitor is mediated by blockage of ERAD induction and survival caused by SCD1 inhibition
- SCD1 and proteosome activity present as novel therapeutic targets in ATC

FUTURE DIRECTIONS

- Development of an orthotopic preclinical model of ATC to examine effects of drug treatment on tumor growth, local invasion, and metastasis
- Validation of mono-therapeutic activity of an SCD1 inhibitor and a proteosome inhibitor in a preclinical model of ATC
- Validation of combinatorial therapy using an SCD1 inhibitor together with a proteosome inhibitor in a preclinical model of ATC
- Pharmacokinetic and pharmacodynamics assessment of combinatorial therapy
- Investigation of efficacy of SCD1/proteosome inhibitor combinatorial drug therapy in other aggressive tumor models and in cases of refractory disease
- Initiation of a phase I clinical trial investigating tumor response to combinatorial therapy in patients with ATC

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