Newly characterized follicular thyroid carcinoma cell lines demonstrate antitumor synergy in response to combined MEK and Akt inhibitors

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ABSTRACT

Follicular thyroid carcinoma (FTC) is a well-differentiated cancer that encompasses 15-20% of all thyroid cancers and invasion is frequently seen in the vascular structures within the thyroid gland and neck. FTC is associated with up to 40% of all thyroid cancer related deaths with no curative treatments beyond surgery and ^{131}Iodine. We report three new authenticated FTC cell lines (SDAR1, SDAR2, SDAR3) from a patient diagnosed with metastatic FTC to a lymph node and neck mass. A fourth FTC cell line (THJ-306T) was derived from another FTC patient, all of which had features of radioiodide insensitive tumors. Short tandem DNA repeat (STR) analysis validated that the cell lines were derived from the patient tumor tissues. Interestingly, the SDAR cell lines were not mutated for retinoblastoma (Rb), KRas, HRas, PI3K, BRAFV600E or PAX8/PPARgamma. However, the primary tumor cell line, SDAR1, was mutant for p53. Expression of Pax8 and TSHR mRNA were present while PDS, DIO2, TPO and NIS were absent. For the 4th FTC cell line, THJ-306T, contained Rb and NRas codon 61 mutations and expressed TTF1 and Pax8 mRNA. With respect to oncogenic signaling pathways, pERK and pAkt were elevated in all four cell lines. Based upon these findings we tested and demonstrated antitumor proliferative synergy using a MEK inhibitor (GSK-1120212) combined with an Akt inhibitor (MK-2206). Using IC50 concentrations and fixed ratio drug concentrations, CI values for combinatorial therapy were 0.071, 0.29, 0.24 and 0.29 respectively for SDAR1, SDAR2, SDAR3 and THJ-306T. Propidium iodide measurement of cell death revealed synergy of the combinatorial therapy. Thus, inhibiting these two oncogenic pathways in four new FTC cell lines demonstrated strong antitumor synergy. We are currently examining the role of RhoB which may play a role in the observed synergy. Moreover, with the recent discovery that inhibition of the Akt pathway leads to NIS re-expression and radioiodide uptake, we are exploring whether these cells when exposed to combinatorial therapy respond to iodine uptake and retention.

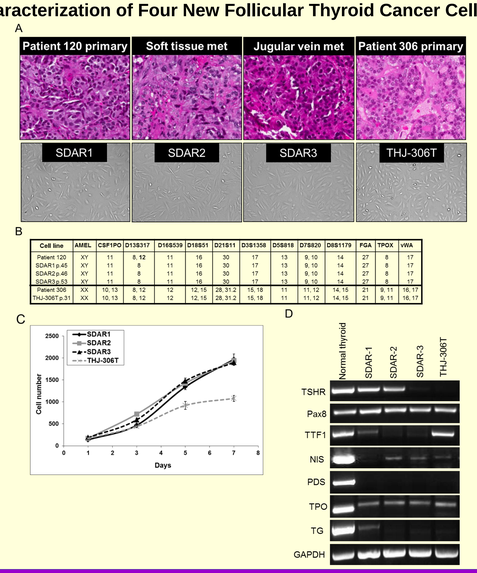
INTRODUCTION

Follicular thyroid carcinoma (FTC) accounts for ~15-20% of thyroid cancers and ~40% of the thyroid cancer associated deaths. FTC consists mainly of differentiated thyroid carcinoma (DTC) and most patients have an excellent prognosis after standard treatment including surgery, adjuvant radioiodine, and L-thyroxine suppression therapy, when detected early. However, aggressive, recurrent and metastatic disease occurs in 10-15% patients, wherein half of these patients became nonresponsive to radioiodine therapy and tumors can display a poorly differentiated phenotype. Although distant metastases are rare at the time of diagnosis of DTC, FTC metastasizes via the vascular system to distant organs and often has a poor prognosis with a high recurrence rate. Currently, no effective therapy is available for this subgroup of patients; thus, their survival rate is poor, preempting a need for new therapeutics and treatment modalities for these advanced carcinomas.

Few bonafide FTC cell lines and preclinical models exist for FTC for the purpose of better understanding the biology of FTC (disease onset, progression and recurrence) and the development of efficacious therapies. We have now developed four new FTC cell lines from two patients. We provide detailed genetic and molecular characterization of these cell lines compared to the parental FTC tumor tissues as well as to currently available FTC cell lines (WRO, FTC-133, TT2609, ML-1 and CGHTHW). We have also evaluated the most common oncogenic signaling pathways known to be impacted in thyroid cancer within these cell lines and demonstrate a couple of them to be potential molecular targets for therapy.

RESULTS

Characterization of Four New Follicular Thyroid Cancer Cell Lines



Cell line	AMEL	CSF1PO	D13S317	D16S539	D18S51	D21S11	D3S1358	D5S818	D7S820	D8S1179	FGA	TPOX	vWA
Patient 120	XY	11	8, 12	11	16	30	17	13	9, 10	14	27	8	17
SDAR1.p.45	XY	11	8	11	16	30	17	13	9, 10	14	27	8	17
SDAR2.p.46	XY	11	8	11	16	30	17	13	9, 10	14	27	8	17
SDAR3.p.53	XY	11	8	11	16	30	17	13	9, 10	14	27	8	17
Patient 306	XX	10, 13	8, 12	12	12, 15	28, 31.2	15, 18	11	11, 12	14, 15	21	9, 11	16, 17
THJ-306T.p.31	XX	10, 13	8, 12	12	12, 15	28, 31.2	15, 18	11	11, 12	14, 15	21	9, 11	16, 17

Oncogenic Signaling Identified in FTC Cell Lines

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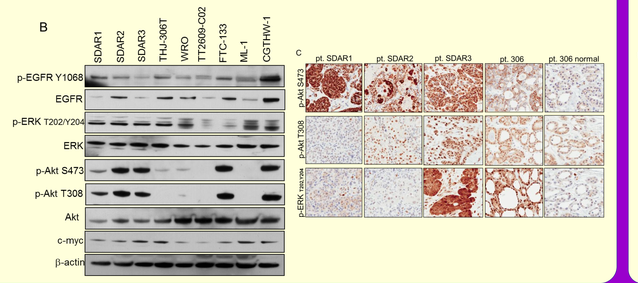
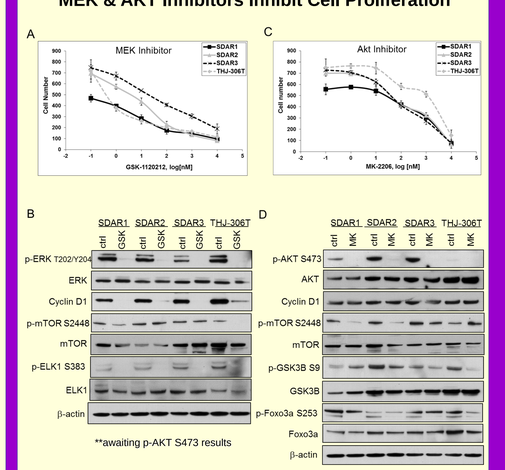
Cell line	Sex	Lesion	Origin Reference	Tumorigenic	TP53	RB	PTEN	KRAS / HRAS exon 12, 13, 61	NRAS exon 12, 13, 61	BRAF V600E	PI3KCA exon 9, 20	PAX8/PPARγ fusion
SDAR1	M	FTC	N/A	No	mut[a]	wt[a]	wt[a]	wt[a]	wt[a]	wt[a]	wt	neg[a, e, g]
SDAR2	M	FTC met	N/A	No	wt	wt[a]	wt[a]	wt[a]	wt[a]	wt[a]	wt	neg[a, e, g]
SDAR3	M	FTC met	N/A	No	wt	wt[a]	wt[a]	wt[a]	wt[a]	wt[a]	wt	neg[a, e, g]
THJ-306T	F	FTC	N/A	No	wt	mut[a]	mut[a]	wt[a]	codon 61[a] (CAA→CGA)	wt[a]	wt	neg[a, g]
WRO	F	FTC	Estour *et al* 1989	Yes	mut[b]	mut[b]	mut[b]	wt[a]	wt[a]	mut[a,g]	wt	neg[c]
TT2609-C02	M	FTC	Geldof *et al* 2001	Yes	mut[b]	wt[c]	mut[c]	wt[a]	codon 61[a] (CAA→CGA)	wt[a]	wt	neg[c]
FTC-133	M	FTC	Goretzki *et al* 1990	Yes	mut[c]	wt[c]	wt[c]	wt[a]	wt[a]	wt[c]	wt	neg[c]
ML-1	F	FTC, rec	Schonberger *et al* 2000	Yes	wt[c]	wt[c]	mut[c]	wt[a]	wt[a]	wt[c]	wt	neg[c]
CGTHW-1	F	FTC met	Lin *et al* 1996	Yes	mut[c]	mut[c]	mut[c]	wt[a]	wt[a]	wt[a]	wt	neg[c]

[a]Fagin *et al* 1993 [b]Geldof *et al* 2001 [c]Gromyko *et al* 2010 [d]Pohl *et al* 2010 [e]Schweppe *et al* 2008
[f]protein accumulation [g]confirmed by Reddi lab [h]confirmed by Grebe lab [i]confirmed in patient tissue

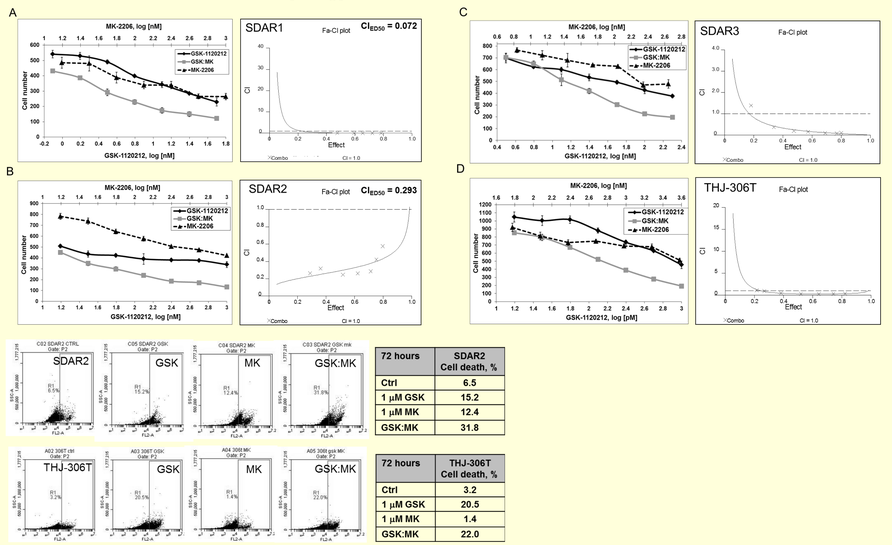


MEK & AKT Inhibitors Inhibit Cell Proliferation



**awaiting p-AKT S473 results

Antitumor Synergy of Combinatorial MEK & AKT Inhibitors



72 hours	SDAR2 Cell death, %
Ctrl	6.5
1 μM GSK	15.2
1 μM MK	12.4
GSK:MK	31.8

72 hours	THJ-306T Cell death, %
Ctrl	3.2
1 μM GSK	20.5
1 μM MK	1.4
GSK:MK	22.0

Results & Summary

1. **We have developed and characterized four new human FTC cell lines, matching genetic and molecular characteristics to that of the parental tumor tissues.**
2. **We observe over-expression of specific oncogenic pathways (pAKT and pERK) and have demonstrated that targeting these molecular pathways results in antitumor synergy.**

Impact and Future Directions

1. **Four new cell lines have been added to the small pool of authenticated FTC-derived cell lines thereby expanding the resources currently available for FTC research.**
2. **These cell lines are sufficiently characterized allowing one to track genetic drift over time and to always be certain of its origin.**
3. **Screening for oncogenic signaling pathways such as pAKT and pERK in patient tissue microarrays (TMA) will determine the incidence of oncogenic overexpression and may provide predictive value for combinatorial molecular targeted therapy.**
4. **The ability to derive cell lines from patient tumors, identify the oncogenic pathway involved, and evaluate available therapies, allows for a personalized treatment regimen in the near future, particularly for those cases with recurrent/metastatic disease unresponsive to routinely used therapies.**