Comprehensive molecular characterization of new anaplastic thyroid carcinoma cell lines reveals RhoB as a molecular target for therapy

MAYO CLINIC Cancer Center

#165

A Comprehensive Cancer Center Designated by the National Cancer Institute — NCI CCC

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ABSTRACT

Anaplastic thyroid carcinoma (ATC) is a highly aggressive carcinoma with a mortality rate near 100% and is in need of new therapeutic options. One critical component of drug discovery is the availability of well-characterized cell lines derived from patient tumors for identification of molecular mechanisms related to tumor biology and drug responsiveness. A recent report indicates that up to 42% of thyroid cancer cell lines, including ATC, are redundant or not of correct tissue origin. We report four new ATC cell lines and the methodologies used to characterize the originating tumor tissue and derivative cell lines. To validate the integrity of our newly derived cell lines, we confirmed histologically the original diagnosis of the ATC tumor tissues and demonstrated identical matching of the short tandem DNA repeats (STR) and mutational status to the tumor-derived cell lines. This is the first time that molecular descriptions of thyroid cell lines are matched to the originating tumor tissues. In addition, we fully characterized the cell lines for proliferative growth, mRNA expression of seven thyroid markers, four oncogenes and array CGH to identify novel deletions and amplifications. Previously in other cell lines, we showed that RhoB is a key signaling node for the growth inhibition of ATC. Using our new cell lines, we have identified five classes of compounds (FTI-277, GGTI-286, lovastatin, romidepsin, UCN-01) that upregulate RhoB and inhibit cell proliferation in a dose-responsive fashion. In conclusion, we have developed four molecularly characterized cell lines that further implicate RhoB as a molecular target for therapy in genuine ATC cell lines.

INTRODUCTION

Anaplastic thyroid carcinoma (ATC) represents only 1-2 % of all malignant thyroid diseases while accounting for over half of thyroid carcinoma related deaths in the United States. ATC is characterized as poorly differentiated and highly aggressive with mortality rates near 100% and median survival of 3 to 7 months after diagnosis (Sherman 2003). The attempt to manage ATC has been very unsuccessful. As a result, molecularly targeted therapies are being investigated to improve management of persistent and recurrent ATCs by interfering with signal transduction pathways to inhibit tumor growth and angiogenesis (Baudin and Schlumberger 2007).

In order to thoroughly investigate signaling in ATC for the discovery of novel therapies, cell lines derived from human tumor tissue must be utilized to serve as preclinical models. However, the integrity of cultured cell lines are rarely verified despite that up to 42% of thyroid cancer cell lines, including ATC, are redundant or not of correct tissue origin. Even when obtained from the source, many cell lines are actually already cross-contaminated with older established cell lines (Schweppe *et al* 2008). Due to this problem, we comprehensively characterized four new ATC cell lines to verify their integrity and identity.

Using genuine ATC cell lines, we identified RhoB as a key signaling node for the growth inhibition of ATC in response to therapeutics. RhoB is a member of the Ras superfamily of isoprenylated small GTPases that normally regulates actin stress fibers and vesicle transport. Although RhoB is not mutated in cancer, it has altered expression and activity which modulates proliferation, survival, invasion and angiogenesis. RhoB's activity appears crucial for potentially regulating cancer progression and therapeutic responses (Prendergast 2001).

RESULTS

Confirmation of thyroid origin

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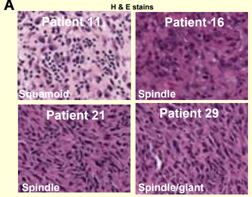

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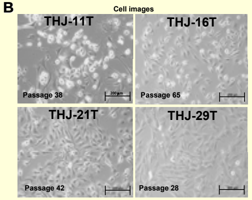

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Short Tandem DNA Repeat (STR) sequences

Patient Cell line	D7S484	D13S158	D10S197	D14S70	mycL	D21S1252	D8S262	D17250	D15S1002	D16S520	D2S2368	D6S441
Patient 11 THJ-11T p.10	106,115 106,115	122,128 122,128	173,175 173,175	110,110 110,110	165,169 165,169	148,164 148,164	117, 123 117, 123	151, 167 151, 167	111, 121 111, 121	157, 157 157, 157	102, 108 102, 108	182, 182 182, 182
Patient 16 THJ-16T p.10	109,113 109,113	124, 130 124, 130	171, 175 171, 175	105, 108 105, 108	184, 203 184, 203	148, 152 148, 152	121, 126 121, 126	159, 161 159, 161	121, 121 121, 121	155, 157 155, 157	90 ,108 90 ,108	176, 184 176, 184
Patient 21 THJ-21T p.10	109, 115 109, 115	124, 126 124, 126	171, 175 171, 175	108, 110 108, 110	181, 207 181, 207	158, **161** 158, **158**	123, 123 123, 123	155, 157 155, 157	109, 121 109, 121	153, **157** 153, **153**	90, 110 90, 110	172, 186 172, 186
Patient 29 THJ-29T p.3	102, 109 102, 109	128, **132** 128, **128**	171, 173 171, 173	98, 105 98, 105	177, 182 177, 182	144, 157 144, 157	**121**, 123 **123**, 123	155, 159 155, 159	113, 117 113, 117	159, 163 159, 163	104, 110 104, 110	170, 178 170, 178

D

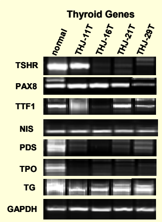
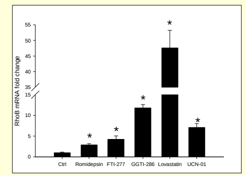


Figure 1. **A**. H & E of patient anaplastic thyroid tissue with predominant subtype. **B**. Phase contrast images of live human anaplastic thyroid carcinoma cell lines derived from patient tissues. **C**. STR sequences of both patient and cell lines confirm identities of derived cultures. Passage number of cell lines are as indicated. **D**. RT-PCR analysis confirms de-differentiation in anaplastic cell lines with transcription of some thyroid genes implicating thyroid origin.

RhoB is a molecular target

A



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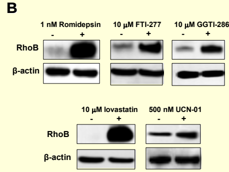

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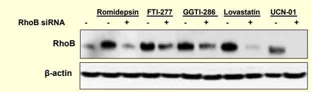

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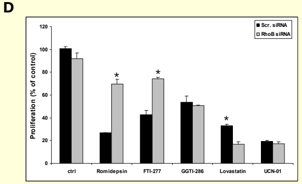

Figure 4. **A**. Real time PCR of RhoB mRNA upregulation in response to drug treatment after 24 hours. **B**. Western analysis confirms RhoB expression upregulation. **C**. Western of cells transfected with scrambled and RhoB siRNA illustrates that RhoB induction can be blocked by siRNA. **D**. Proliferation assay to examine RhoB dependent effects. Cells were transfected with scrambled and RhoB siRNA for 24 hours followed by 3 day drug exposure.

Molecular characterization

A

Mutations

Cell line	p53	RB	BRAF	KRAS	RET / PTC	PAX8 / PPARγ	Amplified genes	Deleted genes
THJ-11T	WT	WT	WT	mut	neg	neg	Myst4	GSTT1
THJ-16T	mut	mut	WT	WT	neg	neg	TNFAIP8L2	PARP12, GSTT1
THJ-21T	mut	mut	mut	WT	neg	neg	JAK2, SYN3, MMP20, MMP27, MMP10, MMP13	GLIS3
THJ-29T	WT	mut	WT	WT	neg	neg	PDGFRA, cKIT, RAD18, IRAK2	TIAM1

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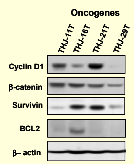

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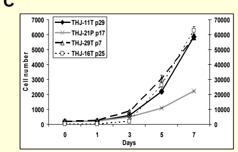

Figure 2. **A**. Summary table of mutations, translocations, and uniquely altered genes. Neither of the ATC cell lines harbor the RET/PTC nor PAX8/PPARγ fusion as routinely seen in other thyroid carcinoma subtypes. **B**. Western analysis of frequently over-expressed oncogenic proteins in ATC that can lead to cell survival. **C**. Cellular proliferation curve over seven days demonstrates proliferative potential of the cell lines. Passage numbers are as indicated.

Drug responsiveness

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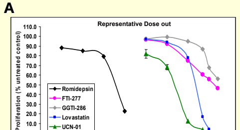

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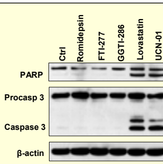

Figure 3. **A**. Representative graph of 4 day treatment with 5 different classes of drugs demonstrating dose dependent inhibition. **B**. Western analysis of PARP and cleaved caspase 3 to check for apoptotic responsiveness after 48 hrs.

CONCLUSIONS AND FUTURE DIRECTIONS

• STR analysis of both patient tissues and established cell lines is essential for verifying identity to avoid redundancy and misidentification.

• Four new ATC cell lines have been comprehensively characterized exhibiting unique profiles and phenotypes to be used for preclinical models.

• All ATC cell lines exhibit a dose-dependent growth inhibition and RhoB induction in response to HDAC inhibitor (Romidepsin), prenylation inhibitors (FTI-277, GGTI-286), HMG co-A reductase inhibitor (lovastatin) and Chk1 inhibitor (UCN-01).

• Lovastatin and UCN-01 induce apoptosis in ATC in vitro

• As a signaling node, RhoB was silenced to demonstrate growth inhibition dependence in at least Romidepsin and FTI-277

• Future studies include verification of GGTI-286, lovastatin and UCN-01 's RhoB independence