#1458

Characterization of novel thyroid PDX models and their response to combination therapies

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Abstract

Patient derived xenografts (PDX) mouse models for many types of cancer have demonstrated therapeutic responses similar to those seen in patients. Thus, resected patient tumor tissue directly implanted into immune compromised mice followed by therapy and tumor growth analysis is thought to be the closest preclinical model to predict patient therapeutic responses. To date, few models are available for the different histotypes of thyroid cancer derived from follicular thyrocytes; these include papillary, follicular, Hurthle cell, squamous and anaplastic thyroid carcinoma. We have developed eight PDX models in athymic nude mice representing many of these subtypes. The models have been extensively characterized for mutational status (i.e. BRAF, RAS) as well as validation by short tandem repeat (STR) analysis to match that of the originating patient tumor tissue. Squamous and anaplastic thyroid cancers are rare tumor types with no FDA approved therapies. Each model demonstrated their own unique responses to radiation, cytotoxic therapies such as doxorubicin, cisplatin, paclitaxel or molecular targeted therapies such as carfilzomib (proteasome inhibitor), sorafenib, sunitinib, and pazopanib (tyrosine kinase inhibitors). We expect that these models may provide useful *in vivo* models for thyroid cancer research as well as models for therapeutic guidance based upon histotype, mutational status and response to therapies.

Background

Thyroid cancer is the most common endocrine cancer and is categorized into 4 main subtypes: papillary, follicular, medullary and anaplastic . Papillary thyroid (PTC) and Follicular thyroid (FTC) are well-differentiated accounting for 80-90% of all thyroid cancers. Variants include tall cell, insular, columnar and Hurthle cell. Medullary thyroid (MTC) arises from neuroendocrine cells and accounts for 3-4%. Generally, PTC, FTC, and MTC are managed successfully with the current standard-of-care if detected early; however, up to 30% will have recurrence even decades later. Anaplastic thyroid (ATC) is rare (accounts for 1-2%) and is undifferentiated leading to its very aggressive nature and poor prognosis. Squamous cell (SCTC) is also very rare (<1%) with no known origin. SCTC is also highly aggressive with poor prognosis. For recurring or more aggressive thyroid cancers that are refractory to radioactive iodine, improved therapeutics need to be established.

The use of patient-derived xenografts (PDX) mouse models for therapeutic studies is thought to closely resemble therapeutic responses seen in the patients. Thus, the number the PDX models available have readily increased. Creating PDX models has its challenges and various success rates depending upon the tissue type.

Thyroid PDX success rate = 16.1% (as of 4/6/2015)

Figure 1: Histology of thyroid PDX models

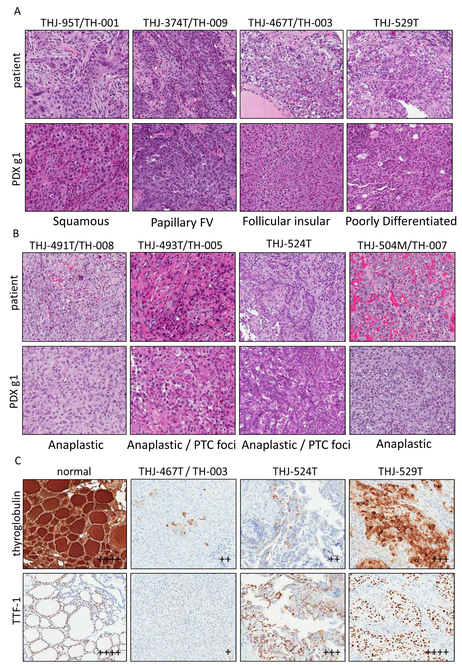

Table 1. DNA STR profile of validated PDX models

	AMEL	D5S818	D13S317	D7S820	vWA	TH01	TPOX	CSF1PO	D18S51	D3S1358	D8S1179	FGA
patient 95	XY	12,13	9	10,11	16,17	6,9	8,11	12	15	14,17	13	22,23
THJ-95T / TH-001	XY	12,13	9	10,11	16,17	6,9	8,11	12	15	14,17	13	22,23
patient 374	XX	11,13	8,12	10,12	14,18	6	8,11	10,12	11,14	15,17	12,15	21,24
THJ-374T /TH-009	XX	11,13	8,12	10,12	14,18	6	8,11	10,12	11,14	15,17	12,15	21,24
patient 467	XY	13	11	10	15,17	9,9.3	8,9	10,11	12,15	14,15	14	19,20
THJ-467T / TH-003	XY	13	11	10	15,17	9,9.3	8, 9	10,11	12,15	14,15	14	19,20
patient 491	XX	11,13	9,11	9,11	17,18	9.3	8,11	11	11,16	16	12,13	21,24
THJ-491T / TH-008	XX	11,13	9,11	9,11	17,18	9.3	8,11	11	11,16	16	12,13	21,24
patient 493	XY	-----	11,12	12	14,15	7	8,11	10, 13	12, 19	16,17	11, 14	19,24
THJ-493T / TH-005	XY	-----	11,12	12	14,15	7	8,11	10,13	12,19	16,17	11,14	19,24
patient 504	XY	11	11,12	10,11	15,17	9.3	10,12	10	12,15	15,17	10,13	19,21
THJ-504M / TH-007	XY	11	11,12	10,11	15,17	9.3	10,12	10	12,15	15,17	10,13	19,21
patient 524	XY	11,13	9,10	9,10	14,16	6,7	11	10,12	12,15	15,18	13	20,24.2
THJ-524T PDX	XY	11,13	9,10	9,10	14,16	6,7	11	10,12	12,15	15,18	13	20,24.2
patient 529	XY	10,12	12	10,12	16,18	6,9	8	10,11	14,18	15,19	13,14	24,24.2
THJ-529T PDX	XY	10,12	12	10,12	16,18	6,9	8	10,11	14,18	15,19	13,14	24,24.2

Table 2. Mutation summary of thyroid PDX models

Cell line	Sex	Lesion	g0 incubation	Mouse strain	BRAF V600E	HRAS codon 12, 13, 61	KRAS codon 12, 13, 61	NRAS codon 12, 13, 61
THJ-95T / TH-001	M	SQ	~5 months	Athymic nude	wt®	wt®	codon 12® GGT→ GTT	wt®*
THJ-374T / TH-009	F	PTC FV	~3.5 months	Athymic nude	wt®	wt®	wt®	wt®
THJ-467T / TH-003	M	FI	~2.5 months	Athymic nude	wt®	wt®	wt®	wt®*
THJ-491T / TH-008	F	ATC	~7 months	Athymic nude	wt®	wt®	wt®	wt®*
THJ-493T / TH-005	M	ATC/PTC	~1.5 months	Athymic nude	wt®	codon 61® CCA→CCG	wt®	wt®*
THJ-504M / TH-007	M	ATC	~2.5 months	Athymic nude	mut®	wt®	wt®	wt®*
THJ-524T	M	ATC/PTC	~3 months	Athymic nude				
THJ-529T	M	PDTC	~4.5 months	NOD SCID Athymic nude				

®confirmed in patient tissue
*PDX model had possible mutation at codon 62 (GAA→GAG), which may be due to artifact
SQ – squamous cell carcinoma
FI – poorly differentiated insular thyroid carcinoma with Hurthle cell features
ATC – anaplastic thyroid carcinoma
PTC – papillary thyroid carcinoma
FV – follicular variant features
PDTC – poorly differentiated thyroid carcinoma

Table 2. Summary of thyroid PDX models.
-g0 incubation indicates the number of months that the tumor was grown in the mouse host until collection.
-BRAF V600E and RAS mutations were analyzed by PCR and confirmed by sequencing.

Figure 2. Anaplastic carcinoma PDX therapy

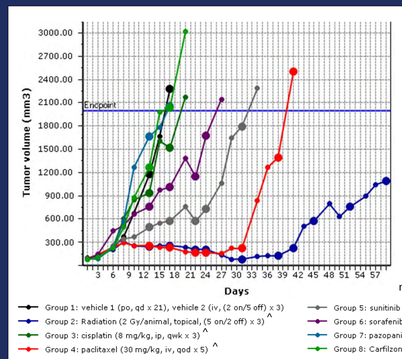

Figure 2. THJ-493T / TH-005 was most responsive to radiation (TTE=59+ days) therapy followed by paclitaxel (TTE=39.2 days) therapy as compared to placebo (TTE=16 days). No response was seen with cisplatin, pazopanib and carfilzomib. ^Significant body weight loss was observed in groups 2, 3, 4.

TTE = time-to-endpoint
^ considered significant with >5.0% loss

Figure 3. Squamous cell carcinoma PDX therapy

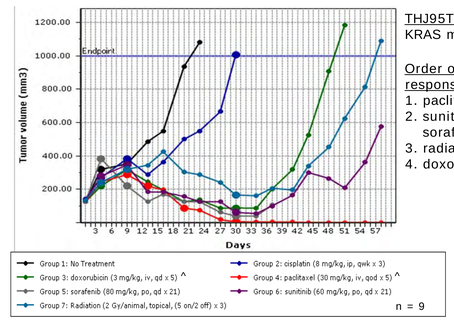

Figure 3. THJ-95T/TH-001 was very responsive to paclitaxel therapy (TTE=58+ days) and most resistant to cisplatin (TTE=29.4 days) therapy as compared to placebo (TTE=22.2 days). ^Significant weight loss observed in groups 3 and 4.

Figure 4. Follicular Insular carcinoma PDX therapy

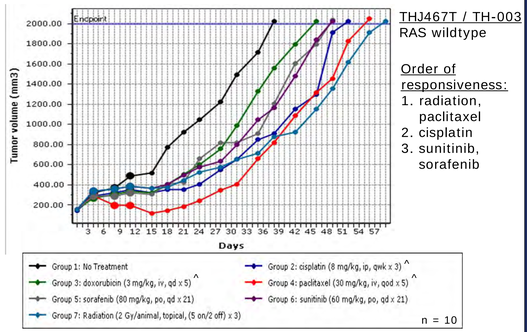

Figure 4. THJ-467T/TH-003 was slightly responsive to radiation (TTE=56 days) and paclitaxel therapy (TTE=54 days) and most resistant to doxorubicin (TTE=42.8 days) therapy as compared to placebo (TTE=37.5 days). ^Significant body weight loss observed in groups 2, 3 and 4.

Summary

We have developed rare thyroid cancer PDX models, some of which have no standard of care. These PDX models may be used to test new therapeutics predicting patient response to new therapies.