Molecular features of Thymoma

SUNIL BADVE

INDIANA UNIVERSITY

USA

Issues

Rare tumors

No standard format of treatment

Poor followup data

Histology

- Lymphocytes

Comprehensive Genomic Analysis Reveals Clinically Relevant Molecular Distinctions between Thymic Carcinomas and Thymomas

Nicolas Girard,[1] Ronglai Shen,[2] Tianhua Guo,[3] Maureen F. Zakowski,[3] Adriana Heguy,[4] Gregory J. Riely,[5] James Huang,[6] Christopher Lau,[1] Alex E. Lash,[7] Marc Ladanyi,[1,3] Agnes Viale,[8] Cristina R. Antonescu,[3] William D. Travis,[3] Valerie W. Rusch,[6] Mark G. Kris,[5,9] and William Pao[1,5,9]

Abstract

Purpose: Thymomas and thymic carcinomas are rare intrathoracic malignancies that can be invasive and refractory to conventional treatment. Because these tumors both originate from the thymus, they are often grouped together clinically. However, whether the underlying biology of these tumors warrants such clustering is unclear, and the optimum treatment of either entity is unknown.

Experimental Design: All thymic tumors were profiled for mutations in genes encoding components of the EGFR and KIT signaling pathways, assessed for EGFR and KIT expression by immunohistochemistry, and analyzed by array-based comparative genomic hybridization. Previously untreated tumors were subjected to global gene expression arrays.

Results: We analyzed 45 thymic tumors [thymoma, $n = 38$ (type A, $n = 8$; type B2, $n = 22$; type B3, $n = 8$); thymic carcinoma, $n = 7$]. One thymoma and one thymic carcinoma harbored *KRAS* mutations (G12A and G12V, respectively), and one thymoma had a G13V *HRAS* mutation. Three tumors displayed strong KIT staining. Two thymic carcinomas harbored somatic *KIT* mutations (V560del and H697Y). In cell viability assays, the V560del mutant was associated with similar sensitivities to imatinib and sunitinib, whereas the H697Y mutant displayed greater sensitivity to sunitinib. Genomic profiling revealed distinct differences between type A to B2 thymomas versus type B3 and thymic carcinomas. Moreover, array-based comparative genomic hybridization could readily distinguish squamous cell carcinomas of the thymus versus the lung, which can often present a diagnostic challenge.

Conclusions: Comprehensive genomic analysis suggests that thymic carcinomas are molecularly distinct from thymomas. These data have clinical, pathologic, and therapeutic implications for the treatment of thymic malignancies. (Clin Cancer Res 2009;15(22):6790–9)

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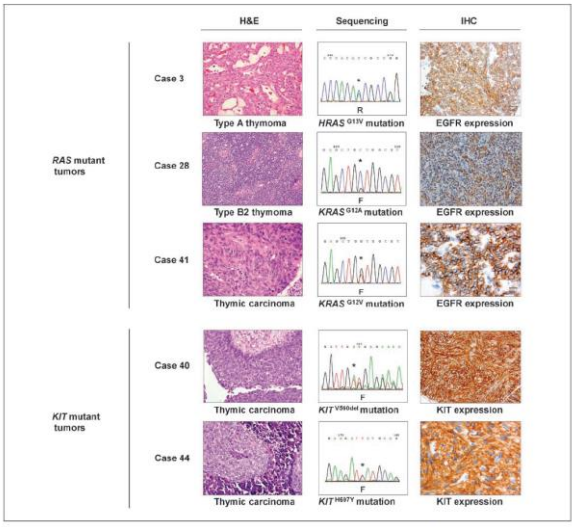

Fig. 1. *RAS* and *KIT* mutant thymic tumors. Left, hematoxylin and eosin (H&E) stainings. Original magnification, ×40. Middle, sequencing chromatograms. Right, immunohistochemical studies with anti-EGFR or anti-KIT antibodies. Asterisks, mutations.

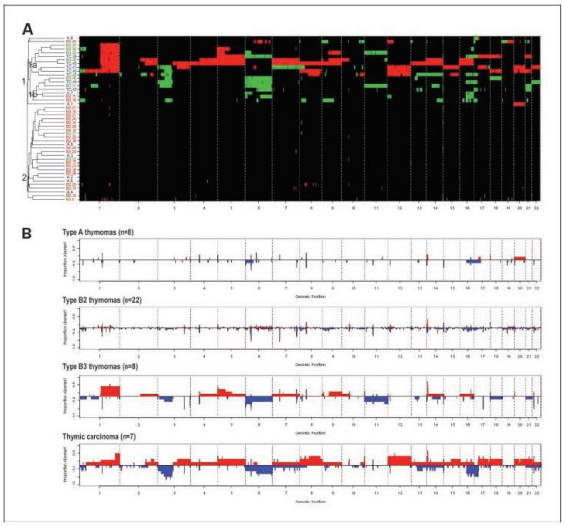

Fig. 4. Genomic profiles of 45 thymic tumors. *A*, unsupervised clustering analysis. Red, gains; green, losses (by genomic position along the 22 chromosomes). *B*, genomic profiles and recurrent copy number alterations in type A and B thymomas and in thymic carcinomas. Red, gains; blue, losses.

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Molecular Analysis of Thymoma

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Abstract

Histologic classification of thymomas has significant limitations with respect to both subtype definitions and consistency. In order to better understand the biology of the disease processes, we performed whole genome gene expression analysis. RNA was extracted from fresh frozen tumors from 34 patients with thymomas and followup data was available. Using the Illumina BeadStudio® platform and Human Ref-8 Beadchip, gene expression data was analyzed with Partek Genomics Suite®, and Ingenuity Pathways Analysis (IPA). Unsupervised clustering of gene expression data, representing one of the largest series in literature, resulted in identification of four molecular clusters of tumors (C1–C4), which correlated with histology ($P = 0.002$). However, neither histology nor clusters correlated with clinical outcomes. Correlation of gene expression data with clinical data showed that a number of genes were associated with either advanced stage at diagnosis or development of recurrence or metastases. The top pathways associated with metastases were amino acid metabolisms, biosynthesis of steroids and glycosphingolipids, cell cycle checkpoint proteins and Notch signaling. The differential expression of some of the top genes related to both metastases and stage was confirmed by RT-PCR in all cases of metastases and matched nonmetastatic cases. A number of potential candidates for therapeutics were also identified.

RNA-profiling

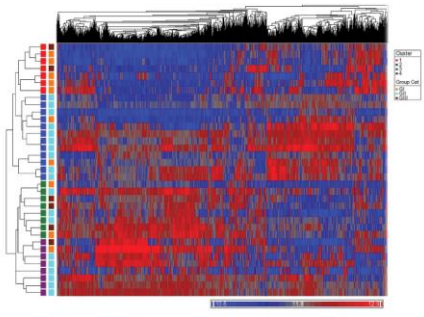


Figure 1. Unsupervised hierarchical clustering of the 34 fresh-frozen thymomas showing four distinct clusters (C1-C4). One sample is included as the duplicate.
doi:10.1371/journal.pone.0042669.g001

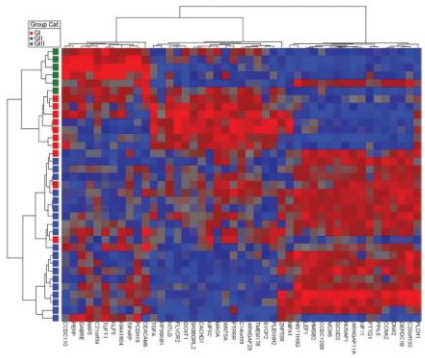
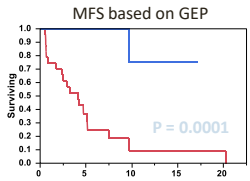


Figure 2. Supervised hierarchical clustering of the fresh-frozen thymomas based on the histologic groups. The figure comprises of 34 tumors (GI = group I (type AB), GII = group II (types B1-B2), and GIII = group III (type B3) and one duplicate.
doi:10.1371/journal.pone.0042669.g002

Metastasis Free survival: 50 patient sample set



Top graph – MFS based on PTA GEP prediction; Class 1 (blue) ; Class 2 (red)
Bottom graph – MFS based on stage; I/II (blue) ; III/IV (red)

GOKMEN-POLAR ET AL LOEHRER/BADVE PLOS ONE

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A Gene Signature to Determine Metastatic Behavior in Thymomas

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Abstract

Purpose: Thymoma represents one of the rarest of all malignancies. Stage and completeness of resection have been used to ascertain postoperative therapeutic strategies albeit with limited prognostic accuracy. A molecular classifier would be useful to improve the assessment of metastatic behaviour and optimize patient management.

Methods: qRT-PCR assay for 23 genes (19 test and four reference genes) was performed on multi-institutional archival primary thymomas (n = 36). Gene expression levels were used to compute a signature, classifying tumors into classes 1 and 2, corresponding to low or high likelihood for metastases. The signature was validated in an independent multi-institutional cohort of patients (n = 75).

Results: A nine-gene signature that can predict metastatic behavior of thymomas was developed and validated. Using radial basis machine modelling in the training set, 5-year and 10-year metastasis-free survival rates were 77% and 26% for predicted low (class 1) and high (class 2) risk of metastasis ($P = 0.0047$, log-rank), respectively. For the validation set, 5-year metastasis-free survival rates were 97% and 33% for predicted low- and high-risk patients ($P = 0.0004$, log-rank), respectively. The 5-year metastasis-free survival rates for the validation set were 49% and 41% for Masaoka stages I/II and III/IV ($P = 0.0537$, log-rank), respectively. In univariate and multivariate Cox models evaluating common prognostic factors for thymoma metastasis, the nine-gene signature was the only independent indicator of metastases ($P = 0.016$).

Conclusion: A nine-gene signature was established and validated which predicts the likelihood of metastasis more accurately than traditional staging. This further underscores the biologic determinants of the clinical course of thymoma and may improve patient management.

Citation: Gökmen-Polar Y, Cook RW, Goswami CP, Wilkinson J, Maetzold D, et al. (2013) A Gene Signature to Determine Metastatic Behavior in Thymomas. PLoS ONE 8(7): e69047. doi:10.1371/journal.pone.0069047

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Competing Interests: YG-P: Other Remuneration – Indiana University. RWC: Manager, Scientific Relations – Castle Biosciences; Stock Ownership – Castle Biosciences; Research Funding – Castle Biosciences. CPG: Other Remuneration – Indiana University. DM: President and CEO – Castle Biosciences; Stock Ownership – Castle Biosciences. KMO: Director of Operations – Castle Biosciences; Stock Ownership – Castle Biosciences. FULL: Other Remuneration – Indiana University. SB: Other Remuneration – Indiana University. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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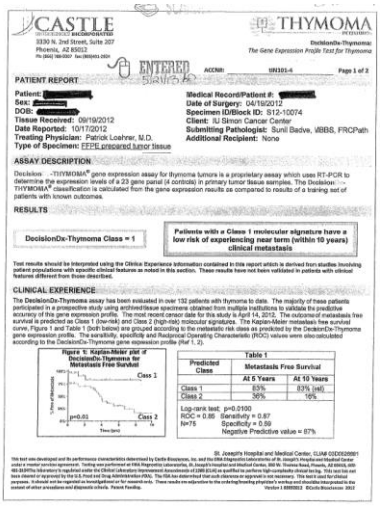
¶ These authors also contributed equally to this work.

Introduction

Thymomas and thymic carcinomas are rare epithelial tumors arising from the thymus gland in the anterior mediastinum. The exact incidence of thymomas is not well documented, but estimated at 0.15/100,000 person-years [1]. The rarity and morphological heterogeneity of these tumors have significantly contributed to difficulties in predicting the behaviour of these tumors.

The World Health Organization (WHO) unifying schema recognizes "organotypic" thymoma-like type A, AB, B1, B2, and B3 thymomas and "nonorganotypic" thymic carcinomas. The type A, AB, and B1 were believed to be benign with rare recurrences/progression of disease [2,3], while B3 tumors were aggressive. However, the WHO schema is difficult to apply uniformly; in addition, a significant number of tumors show mixed morphological features [4]. The Masaoka system is most commonly used to stage thymomas. Briefly, stages I and II



CASTLE BIOSCIENCES, INC.
3330 N. 2nd Street, Suite 207
Phoenix, AZ 85012

DecisionDx-Thymoma
The Gene Expression Profile Test for Thymoma

ENTERED

ACCN#: UN101-4 Page 1 of 2

PATIENT REPORT

Patient: [redacted]
Sex: [redacted]
DOB: [redacted]
Tissue Received: 09/19/2012
Date Reported: 10/17/2012
Treating Physician: Patrick Loehrer, M.D.
Type of Specimen: FFPE prepared tumor tissue

Medical Record/Patient #: [redacted]
Date of Surgery: 04/19/2012
Specimen ID/Block ID: S12-10074
Client: IU Simon Cancer Center
Submitting Pathologist: Sunil Badve, MBBS, FRCPath
Additional Recipient: None

ASSAY DESCRIPTION

Decision- -THYMOMA® gene expression assay for thymoma tumors is a proprietary assay which uses RT-PCR to determine the expression levels of a 23 gene panel (4 controls) in primary tumor tissue samples. The Decision- -THYMOMA® classification is calculated from the gene expression results as compared to results of a training set of patients with known outcomes.

RESULTS

DecisionDx-Thymoma Class = 1

Patients with a Class 1 molecular signature have a low risk of experiencing near term (within 10 years) clinical metastasis

Test results should be interpreted using the Clinical Experience information contained in this report which is derived from studies involving patient populations with specific clinical features as noted in this section. These results have not been validated in patients with clinical features different from those described.

CLINICAL EXPERIENCE

The DecisionDx-Thymoma assay has been evaluated in over 132 patients with thymoma to date. The majority of these patients participated in a prospective study using archived tissue specimens obtained from multiple institutions to validate the predictive accuracy of this gene expression profile. The most recent censor date for this study is April 14, 2012. The outcome of metastasis free survival is predicted as Class 1 (low-risk) and Class 2 (high-risk) molecular signatures. The Kaplan-Meier metastasis free survival curve, Figure 1 and Table 1 (both below) are grouped according to the metastatic risk class as predicted by the DecisionDx-Thymoma gene expression profile. The sensitivity, specificity and Reciprocal Operating Characteristic (ROC) values were also calculated according to the DecisionDx-Thymoma gene expression profile (Ref 1, 2).

Figure 1: Kaplan-Meier plot of DecisionDx-Thymoma for Metastasis Free Survival

Table 1

Predicted Class	Metastasis Free Survival	
	At 5 Years	At 10 Years
Class 1	83%	83% (est)
Class 2	36%	18%

Log-rank test: p=0.0100
ROC = 0.86 Sensitivity = 0.67
NPT5 Specificity = 0.89
Negative Predictive value = 87%

St. Joseph's Hospital and Medical Center, CLIA# 03D0529991

This test was developed and its performance characteristics determined by Castle Biosciences, Inc. and the DNA Diagnostics Laboratories of St. Joseph's Hospital and Medical Center under a master services agreement. Testing was performed at DNA Diagnostics Laboratories, St. Joseph's Hospital and Medical Center, 350 W. Thomas Road, Phoenix, AZ 85013, 602-406-3048The laboratory is regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing. This test has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary for clinical purposes. It should not be regarded as investigational or for research only. These results are adjunctive to the ordering/treating physician's workup and should be interpreted in the context of other procedures and diagnostic criteria. Patent Pending.

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DecisionDx-Thymoma
The Gene Expression Profile Test for Thymoma

ACCN#: UN101-4 Page 2 of 2

RAW DATA AND CALCULATION OF DECISIONDX-THYMOMA CLASS 1 AND 2

DecisionDx-Thymoma® assay uses RT-PCR to determine the expression levels of a 23 gene panel (4 control) in primary thymoma tumor tissue. The nineteen genes of interest are: PDGFRL, FCGBP, PRKX1, SERPINF1, NGB, SCUBE2, MAB21L1, LEPR, LCA5, CPB2, FSTL3, ETV1, BTG3, SLC7A11, GALT8 ,ZFH1, ANTXR1, DDB1/AK, and GLOM2. The four control genes are: IPO8, TPRC, UBC, and PGK1. The optimized molecular model determines Δ Cq values for each of the nineteen genes of interest. The Δ Cq values are imported into a radial basis machine learning algorithm (RBM), which analyses their combined expression profile. RBM calculates a predicted classification that describes how comparable the gene expression profile of the patient tissue sample is to the gene expression profiles contained in the training set. The predicted classification is reported as Class 1 for low risk of metastasis and Class 2 for high risk of metastasis.

ADDITIONAL BACKGROUND INFORMATION

Comparison of the DecisionDx-Thymoma assay to the Masaoka staging system and other traditional prognostic factors

The validation study for the DecisionDx-Thymoma assay included a direct comparison to the Masaoka staging system and other traditional prognostic factors. The Masaoka staging system is commonly used in the U.S. and assigns stage based upon the degree of tumor invasion and dissemination throughout the pleural cavity and body (Ref 3, 4). Kaplan-Meier at risk metastasis free survival comparisons between the DecisionDx-Thymoma test and Masaoka staging (non-invasive stage I/II vs invasive stage III/IV) at 5 years and 10 years are shown in Table 2.

Table 2. Comparison of DecisionDx-Thymoma to Masaoka Staging System

		Metastasis Free Survival (MFS)		
		At 5 years	At 10 years	p-value
DecisionDx-Thymoma Class	Class 1	83%	83% (est)	p=0.010
	Class 2	36%	18%	
Masaoka Stage	Stage I / II	63%	53% (est)	p=0.0320
	Stage III/V	41%	20%	

Univariate analysis from the validation study confirms that the DecisionDx-Thymoma assay is an independent predictor of metastasis free survival (Table 3; Ref 2). Cox multivariate analysis was not performed due to the fact that the DecisionDx-Thymoma Class was the only statistically significant factor under univariate analyses.

Table 3

	Univariate	
	Hazard Ratio	p-value
DecisionDx-Thymoma Class	3.34	0.008
Masaoka Stage (I/II vs III/V	2.25	0.016
Extent of resection – RC	2.10	0.066
WHO malignant class	1.62	0.093
Autoimmune disease	1.10	0.902
Gender	0.73	0.330
Age > 60 years	0.48	0.005
Cox univariate analysis (Cox Hazard ratio = Exp(b))		

John F. Sloan, PhD FACMG; Laboratory Director

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The integrated genome landscape of thymic epithelial tumors: a report by the TCGA research network

M. RADOVICH, C.R. PICKERING, I. FELAU, G. HA, H. ZHANG, H. JO, K.A. HOADLEY, P. ANUR, J. ZHANG, M. MCLELLAN, R. BOWLBY, T. MATTHEW, L. DANILOVA, A.M. HEGDE, J. KIM, M. LEISERSON, G. SETHI, C. LU, M. RYAN, X. SU, A.D. CHERNIACK, G. ROBERTSON, R. AKBANI, P. SPELLMAN, J.N. WEINSTEIN, D.N. HAYES, B. RAPHAEL, T. LICHTENBERG, K. LERAAS, J.C. ZENKLUSEN, THE CANCER GENOME ATLAS NETWORK, J. FUJIMOTO, C. SCAPULATEMPO-NETO, A.L. MOREIRA, D. HWANG, J. HUANG, M. MARINO, R. KORST, G. GIACCONE, Y. GOKMEN-POLAR, S. BADVE, A. RAJAN, P. STRÖBEL, N. GIRARD, M.S. TSAO, A. MARX, A.S. TSAO, P.J. LOEHRER

NIH THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

Introduction

The Cancer Genome Atlas (TCGA) is an NIH initiative to create a comprehensive compendium of genomic alterations across 33 cancer lineages

The Thymic Epithelial Tumors (TET) project was the last project to be initiated by the TCGA as part of its rare tumor initiative

- The TCGA TET Analysis Working Group (AWG) is comprised of clinicians, scientists, and bioinformaticians tasked with the official analysis of the data.
  - 47 members from 24 institutions and 6 countries.

Tissue from **117 patients** were submitted to the TCGA from 18 institutions for comprehensive multi-omics analyses

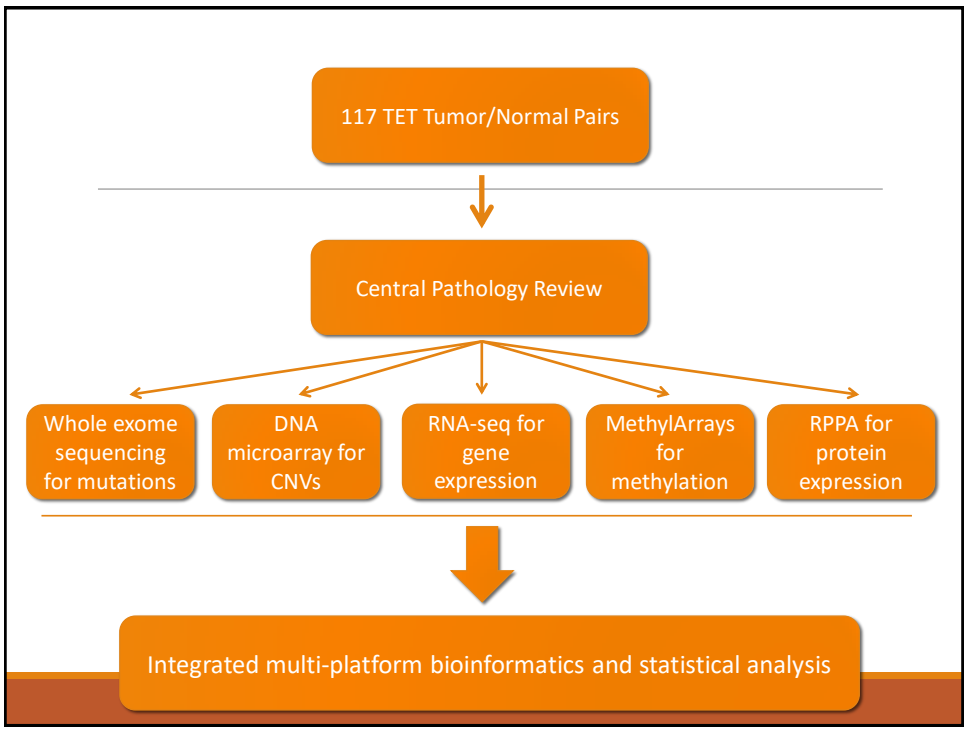
NIH THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

Patient Demographics

Total number	**117**
Median age in years(range)	60 (17 – 84)
Male /Female	61 (52%)/56 (48%)
Race	
Caucasian	97 (83%)
Black	6 (5%)
Asian	12 (10%)
Data missing	2 (2%)
Masaoka stage	
I	36 (31%)
IIA	39 (33%)
IIB	19 (16%)
III	15 (13%)
IVA	1 (1%)
IVB	5 (4%)
Data missing	2 (2%)
Histologic subgroup:	
Type A	10 (9%)
Type AB	48 (41%)
Type B1	12 (10%)
Type B2	25 (21%)
Type B3	10 (9%)
Type TC	10 (9%)
Type MN-T	2(2%)

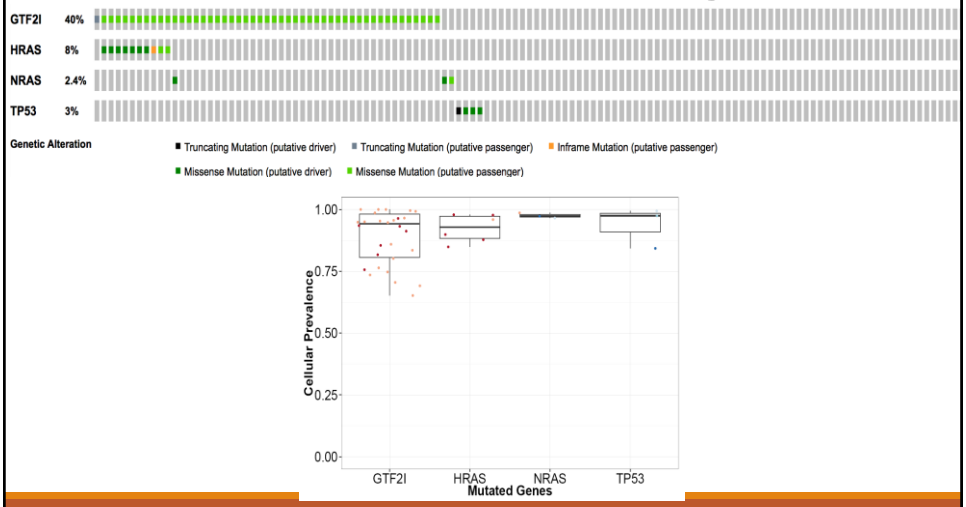
Clinical Characteristics

Adjuvant radiation therapy	39 (33%)
Adjuvant systemic therapy (total 14)	14 (12%)
Platinum- and/or anthracycline-containing combination	6
Other systemic therapy*	4
Targeted therapy*	2
Data missing	2
Autoimmune disease (total 39)*	39 (33%)
Myasthenia gravis only	32
Non-myasthenia gravis autoimmune disease only	7
Data missing**	6
Onset of myasthenia gravis (total 32)	32
Myasthenia gravis diagnosed prior to thymoma	20
Myasthenia gravis and thymoma diagnosed simultaneously	7
Myasthenia gravis diagnosed after thymoma	4
Data not available	1
Secondary malignancy (total 22)	
Diagnosed after thymic tumor	10
Diagnosed prior to thymic tumor	9
Diagnosed synchronously	3



Common mutated oncogenes

N=117 TETs

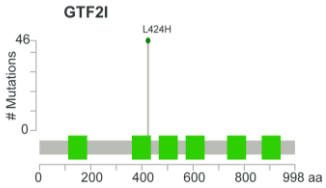


The majority of these mutations are clonal = 100% of tumor cells

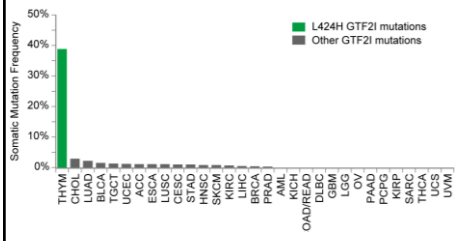
GTF2I: a thymoma specific oncogene

GTF2I = General Transcription Factor II-I

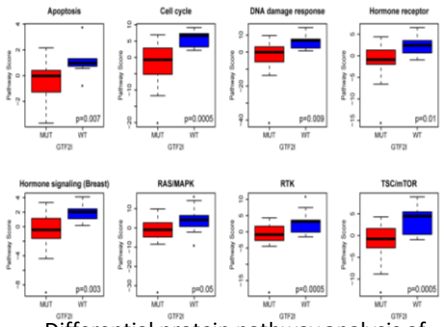
- Mutated in 40% of TETs (exclusively in A & AB thymomas, first observed by Petrini et al. 2014)
- GTF2I protein was first discovered in 1997 as a phosphorylation target of Bruton's Tyrosine Kinase (BTK)
- Phosphoprotein with roles in transcription and signal transduction
- Germline deletion is responsible for mental retardation observed in Williams-Beuren syndrome



All mutations occur at the same locus

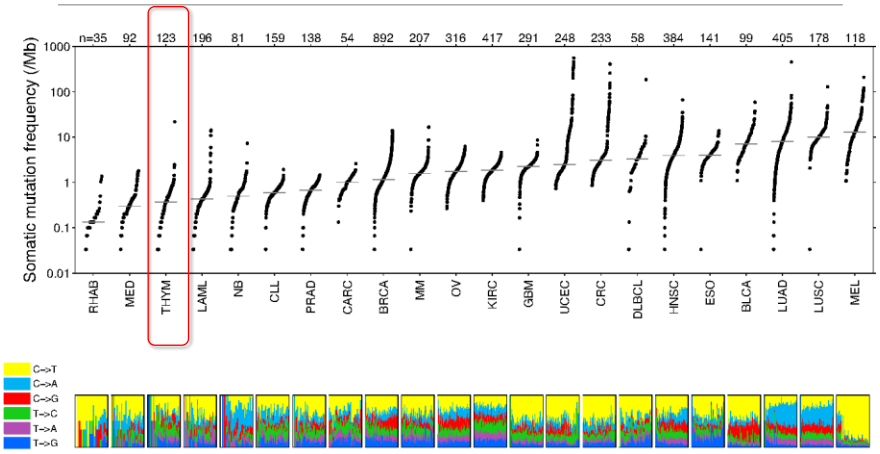


L424H mutations occur only in thymoma

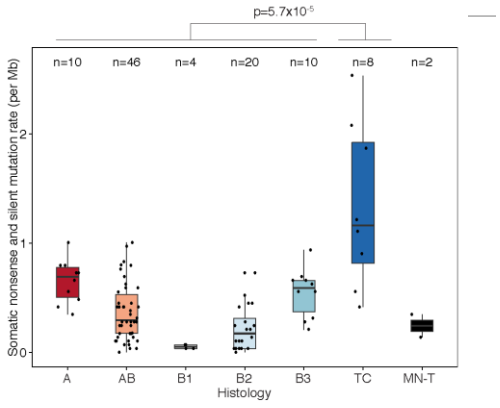


Differential protein pathway analysis of GTF2I mutant vs. WT in A & AB tumors

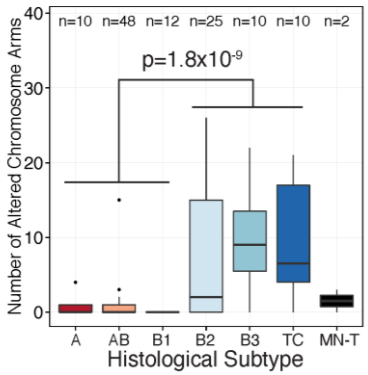
TETs have the lowest mutational burden among adult cancers



More aggressive WHO subtypes have a higher rate of somatic alterations

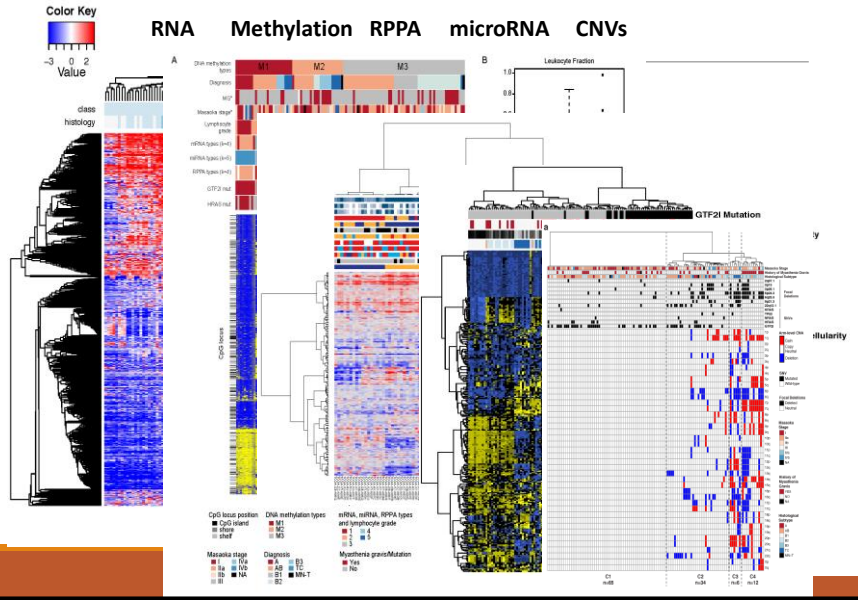


Thymic carcinomas have higher burden of point mutations and indels than thymoma

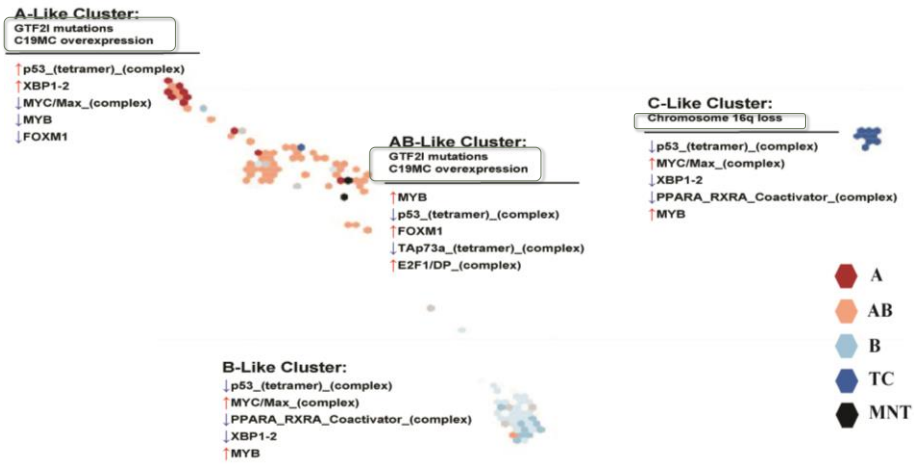


B2, B3, and TC tumors have higher number of altered chromosomal arms

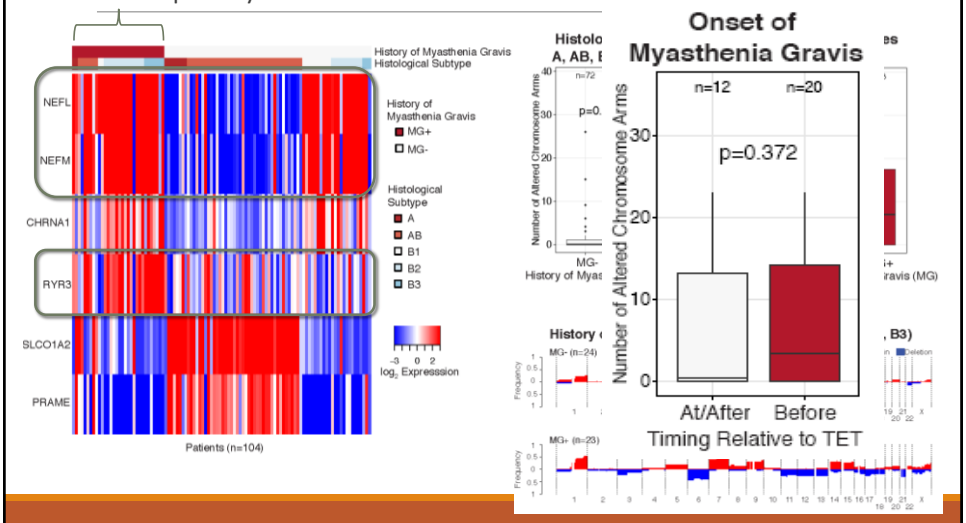
Data, Data, and more Data…



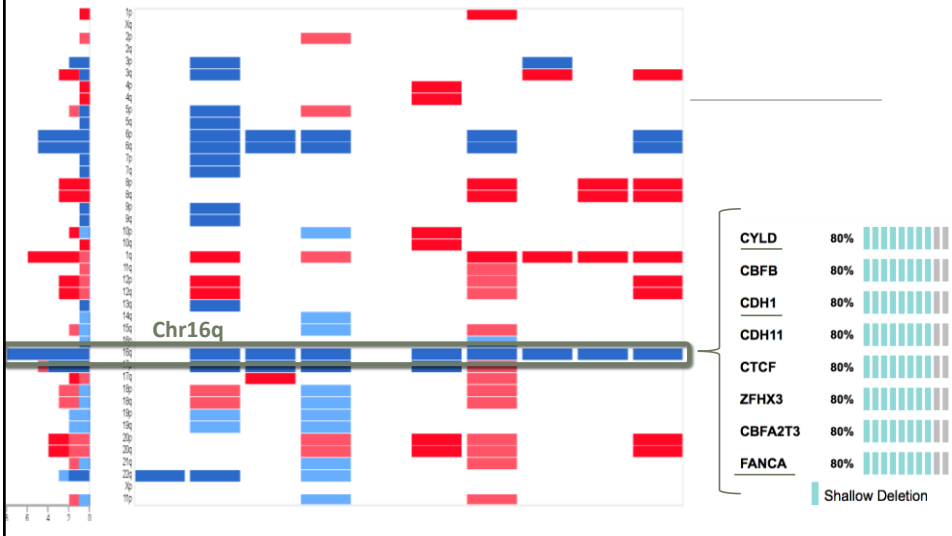
Multi-omics analyses identifies four molecular subtypes



Myasthenia gravis is associated with high tumoral overexpression of muscle auto-antigens and aneuploidy

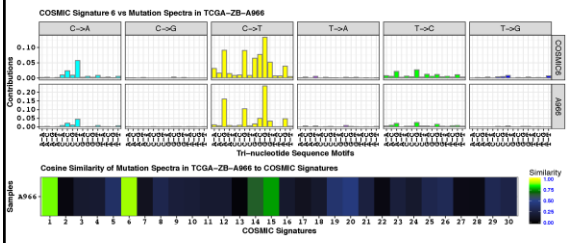


Genomics of thymic carcinoma: Chromosome 16q loss



Tumor suppressors derived from the Sanger Cancer Gene Census

A novel case of an Microsatellite Instability (MSI)-high thymic carcinoma



- 78 year old female diagnosed with Stage IVb thymic carcinoma
- Overall survival: 12 months
- MSI-High, Chr 16q loss +
- Harbored a loss-of-function MLH1 E37* mutation with a concurrent 2.6-fold down-regulation in RNA expression

TCGA Summary

TETs have the lowest mutational burden among adult cancers, however, an enrichment of GTF2I, HRAS, NRAS, TP53, and loss of Chr16q (Type TC) is observed.

We identify four robust molecular subtypes of TETs with associated genomic hallmarks

Myasthenia gravis was linked to the over-expression of muscle auto-antigens and increased aneuploidy

We did not observe the presence of viruses (including polyoma) in TET tissues

We describe a novel case of microsatellite unstable thymic carcinoma - consideration of immune checkpoint therapy in this rare subset

Conclusions

Molecular analysis has some utility

Mutational profile identified
- GTF2i in A/AB
- No specific targets

RNA-based prognostic signature
- Validated in TCGA
- Limited commercial viability (rare tumors)

Acknowledgements
A true group effort! The TCGA TET Analysis Working Group