Preliminary data of NGS analysis of Thymic carcinoma

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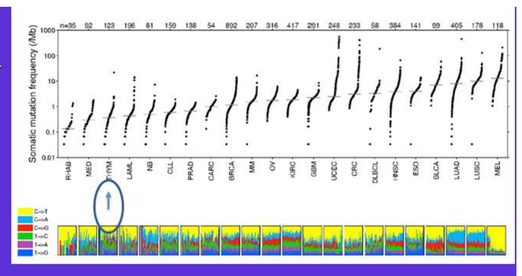
Background – Among rare tumors, in the last years the National Cancer Institute of US included the Thymoma and Thymic carcinoma (TC) (collectively indicated as TET, Thymic Epithelial Tumors) among cancers to be investigated by the Cancer Genome Atlas (TCGA) Project. Our center contributed cases of tissue and matched blood from our Biobank and participated actively to the data analysis (1).
However, the complex genomic events leading to TC development are still largely unknown. At the Pathology Dept. of the IRCCS Regina Elena Natl. Cancer Institute NGS facilities (S5, Thermofisher) are available together with a highly motivated team of molecular biologists.

The results of the TCGA-THYM study showed a low mutational burden in Thymic epithelial tumors (TET) in the series investigated



Tumor mutational burden in TET compared to other 21 different cancer cell types profiled by the TCGA-projects

Radovich et al., 2018, Cancer Cell 33, 244–258
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Therefore a NGS mutational analysis of other cases could allow the identification of further genomic alterations

STUDY DESIGN

- **AIM - We performed a Next Generation Analysys (NGS) to investigate the pathogenesis of TC and identify novel targets for therapy. This would provide biological data with relevant prognostic/predictive value.**
- **MATERIALS Ten TC cases were examined, derived from biopsies/surgical specimens and one matched peritumoral thymic sample. In one case a lung metastatic nodule was available. Six epidermoid carcinoma, 3 undifferentiated carcinoma and one lymphoepithelioma-like carcinoma were investigated. The percentage of neoplastic cells was not < 70-80% of total cells.**

METHODS - The DNA was extracted using the QIAcube and QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA) from microdissected 5 µm formalin-fixed, paraffin-embedded (FFPE) tissue sections
Libraries from Ion AmpliSeq Cancer Hotspot Panel v2 were prepared and sequenced by Ion Chef and S5 system. Data analysis was conducted by using the dedicated Ion Reporter Software.

NGS was performed on Ion Torrent S5 platform by Ion AmpliSeq Cancer Hotspot Panel v2 targeting 50 genes.

- **the Ion AmpliSeq™Cancer Hotspot Panel v2 was used; this panel is designed to amplify 207 amplicons covering over 2,800 COSMIC mutations from 50 oncogenes and tumor suppressor genes.**

Study Design

Ion AmpliSeq Cancer Hotspot Panel v2 targets 50 genes

ABL1	EGFR	GNAS	KRAS	PTPN11
AKT1	ERBB2	GNAQ	MET	RB1
ALK	ERBB4	HNF1A	MLH1	RET
APC	EZH2	HRAS	MPL	SMAD4
ATM	FBXW7	IDH1	NOTCH1	SMARCB1
BRAF	FGFR1	JAK2	NPM1	SMO
CDH1	FGFR2	JAK3	NRAS	SRC
CDKN2A	FGFR3	IDH2	PDGFRA	STK11
CSF1R	FLT3	KDR	PIK3CA	TP53
CTNNB1	GNA11	KIT	PTEN	VHL

More than 2800 COSMIC HotSpots

The most used cancer panel for solid tumors to identify mutations for sensitivity and resistance to targeted therapies

Results

TP53 Gene Variants	Effect
c.826G>A; p.A276T (ex. 8)	Affecting the DNA Binding domain
c.322G>A; p. G108S (ex. 4)	
c.824G>T; p.C275F (ex. 8)	
c.846_847insG; p.R283fs (ex. 8)	The reported p53 mutations are described in the international database of Cancer genomics study Cbioportal as causing a protein loss of function in different cancer types
KIT Gene Variants	**Effect**
c.2515G>A; p.E839K (ex. 18)	This variant affects the Kinase domain: this mutation causes resistence to Imatinib; however, preclinical studies indicate that these mutations are sensitive to PKC412 .
c.1900C>T; p.R634W (ex. 13)	This KIT identified variant R634W, exon 13, could be considered relevant for target therapy with the Sorafenib multikinase inhibitor

Results

- The Ion AmpliSeq™Cancer Hotspot Panel applied contained part of the common mutations found by the TCGA study in TC.

Variants described in Haematopoietic and lymphoid tissue	Variants described in Thymomas and thymic carcinoma	Variants described in other tissues
20	2	14

GENE	TP53	KDR	KIT	APC	GNAQ	STK11	AKT	EGFR	HRAS	KRAS	JAK3	MET	PIK3CA	PDGFRA	SMAD4	CDKN2A	ATM
No.Variants	10 (3 with silent polymorphism)	8	3	2	2	2	1	1	1	1	1	1	1	1	1	1	1

- **The sequencing of 50 genes detected nonsynonymous mutations in all the 10 Thymic Carcinomas, affecting the genes: TP53, KDR, KIT, APC, GNAQ, STK11, ATM, EGFR, HRAS, KRAS, JAK3, MET, PIK3CA, PDGFRA, SMAD4, CDKN2A**

Results

AKT Gene Variant	Effect
c.49G>A; p.E17K (ex. 3) .	this variant E17K, previously reported as a deleterious variant in various cancer types, is possibly associated to aberrant AKT activation. Therapeutic targeting by AZD5363, an oral pan-AKT inhibitor, was described Hyman et al, J Clin Oncol 35, 2251, 2017
KRAS Gene Variant	**Effect**
c.35G>T; p.G12V (ex. 2)	Described in Thymic Carcinoma N. Girard et al, Clin Cancer Res 2009;15(22) November 15, 2009)
KDR (VEGFR2) Gene Variant	**Effect**
c.1416A>T; p.Q472H (ex. 11) rs1870377	This KDR variant Q472H represents a described Single Nucleotide Polymorphism (SNP) which was reported to be correlated with longer OS in patients with thymic tumor Berardi et al, Impact of VEGF, VEGFR, PDGFR, HIF and ERCC1 gene polymorphisms on thymic malignancies outcome after thymectomy Oncotarget 2015 Aug 7;6(22):19305-15.

Conclusions – in the limited series so far analyzed we found in TC non recurrent variants. In particular we observed p53 variants affecting the DNA binding domain and KIT variants known to affect the kinase domain, as well as a deleterious (E17K) variant in AKT and a KRAS variant already described in TC, inhibiting the use of EGFR targeting therapies.

These preliminary data could have clinical, pathologic, and therapeutic implications for the treatment of thymic malignancies. A wider series of TC will be analyzed. A Transcriptome sequencing would be advisable as next molecular analysis.

The Authors declare no conflict of interests

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