Exploring the genomic heterogeneity of the combined B2-B3 Thymoma and nodal metastasis

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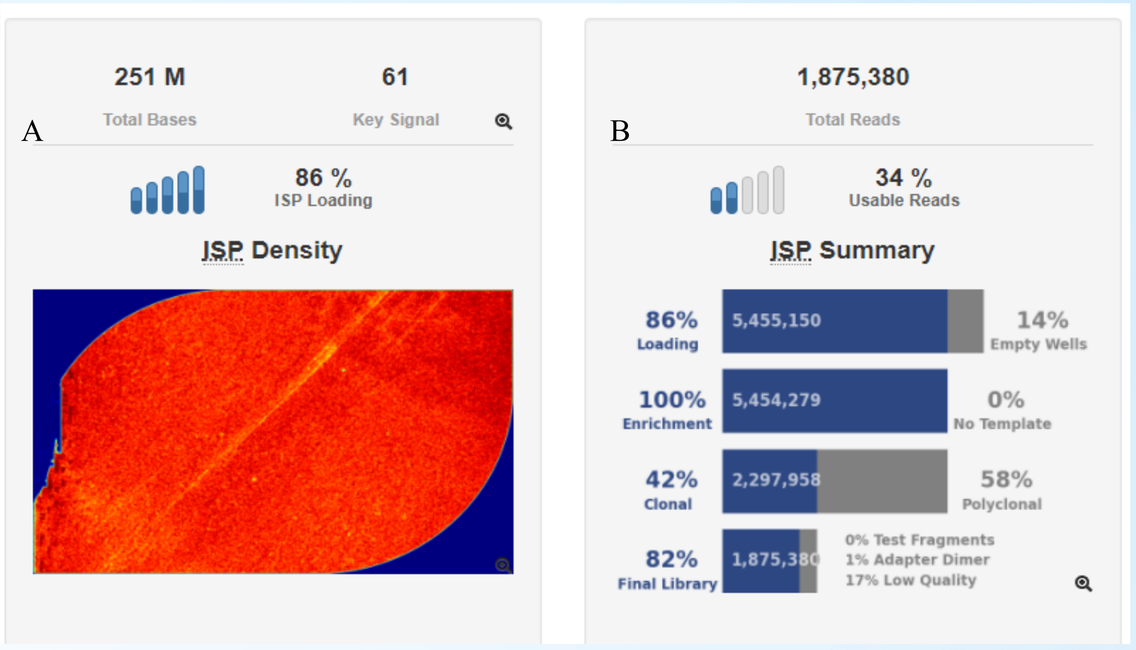
BACKGROUND

The association between thymic epithelial tumors (TETs) and other malignancies, mostly reported as hematologic neoplasm, is widely known, however the uninvolved pathogenic mechanism remains clear. Large scale efforts to shed light on genetic mutations in cancer such as the cancer genome atlas (TCGA) have analysed samples obtained from resection of the primary tumor, however, genomic data in the field of advanced-stage TETs about primary and metastatic tumor are still lacking, except for sporadic reports of *TP53* and *KIT* mutations in thymic carcinoma. Moreover, recent published data, have underlined the low mutational burden and the rarity of common cancer-related mutated genes in thymoma samples. The identified mutational load of an unusual case of B2-B3 thymoma is here explored.

MATERIAL AND METHODS

A 57-yr-old caucasian female with a history of multiple malignancies (cervical squamous carcinoma, clear cell renal carcinoma, chronic myeloid leukemia CML) underwent radical thymectomy with a diagnosis of B2-B3 thymoma. Two years later a latero-cervical lymph node was resected and histologically confirmed as thymoma metastasis. DNA was extracted from clinical tissue samples; microdissection procedure carried out on stained slides to selectively distinguish two primary tumor and one lymph node metastasis sections respectively. DNA for each sample was used for Libraries construction and purification on the Ion Chef (Thermofisher) by the Ion AmpliSeq Cancer Hotspot Panel v2 (Life Technologies) (Figure 1). This panel gives 207 amplicons covering 2800 mutational hotspot regions in 50 genes. Templates were sequenced on PGM (Life Technologies). All detected variants were reviewed with the Integrative Genomics Viewer (IGV V.2.1, Broad Institute, Cambridge, Massachusetts, USA) and only variants with greater than or equal to 5X allele coverage and a quality score greater than or equal to 20, within an amplicon that covered at least 100X alleles, were called, and the frequency of each mutant allele was recorded.

Figure 1. Ion Torrent images



Loading density (A) and performance parameters (B) of an Ion Torrent sequencing run, carried out using a 316 chip, are shown.

RESULTS

In each samples of primary tumor, 9 genes (18%) were mutated, with a total of 15 genes (30%) considering both samples (Table 1). Only 2 genes were mutated in the nodal metastasis sample and the PIK3CA mutated gene has been not found in the primary tumor. The same KIT mutation has been identified in all the samples with a high frequency (Figure 2), other common cancer-related mutated genes such as: IDH1, FBXW7, SMO, NOTCH-1, PTEN, ATM, RB1, SMAD4, EGFR, BRAF, RAS, TP53, SRC, FBW7, were identified.

Figure 2. KIT M541L mutation

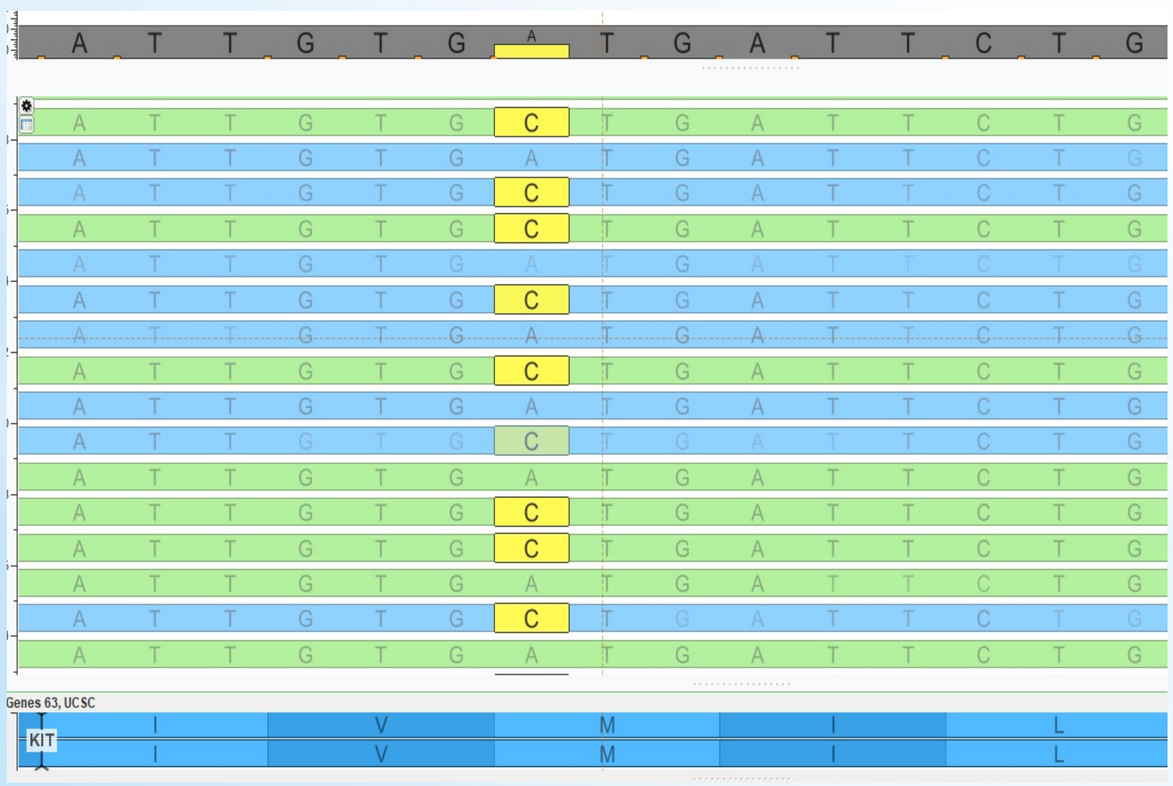


Figure shows KIT M541L mutation. This point mutation was observed with a Genome Brower web app and consists in a single nucleotide substitution at nucleotide position 1621 (A>C).

Table 1. List of mutated cancer-related genes

SAMPLE	GENE	MUTATION	ALLELE FREQUENCY (%)
Primary Tumor (Sample 1)	*IDH1*	p.R132C c.394 C>T	450.00
	KIT	p.M541L c.1621 A>C	59.30
	FBXW7	p.R479Q c.1436 G>A	3.80
	FBXW7	p.R395 c.117 C>T	3.30
	SMO	p.V404M c.1210 G>A	14.70
	NOTCH1	p.V1578 delV c.4732-4734del GTG	2.40
	PTEN	p.R335 c.1003 C>T	4.20
	ATM	p.R3008C c.9022C c.9022 C>T	10.60
	RB1	p.358 c.1072 C>T	11.30
	SMAD4	p.Q245 c.733 C>T	13.30
Primary Tumor (Sample 2)	*ATM*	p.R3008H c.9023 G>A	21.40
	KIT	p.M541L c.1621 A>C	51.40
	KIT	p.V654A c.1961 T>C	2.70
	FBW7	p.R505C c.1513 C>T	3.10
	EGFR	p.R108K c.323 G>A	4.40
	BRAF	p.R444W c.1330 C>T	6.90
	PTEN	p.Q214 c.640 C>T	6.10
	RAS	p.G12D c.35G>A	6.30
	TP53	p.E285K c.853 G>A	6.70
	SRC	p.Q531 c.1591 C>T	7.50
Nodal Metastasis (Sample 3)	*PIK3CA*	p.E545K c. 1633G>A	15.80
	KIT	p.M541L c.1621 A>C	44.70

Table shows genes and hotspot mutations identified in the patient both in primitive tissue and metastasis.

CONCLUSION

According to our knowledge, this is the first case recorded of B2-B3 thymoma with the identification of several mutated genes, already recognized to be involved in tumorigenesis mechanism of other solid tumors. In the light of the medical history of our patient we assume a *KIT* role as driven gene in the tumorigenesis of both thymoma and CML. Up to date the patient is thymoma free and under treatment with Dasatinib for CML control. Deep genomic information are further needed to investigate the development of target therapy.

REFERENCES

- Diagnostic features and subtyping of thymoma lymph node metastases. Sioletic S, Lauriola L, Gallo E, et al., Res Int. 2014
- Consistency and reproducibility of next-generation sequencing and other multigene mutational assays: A worldwide ring trial study on quantitative cytological molecular reference specimens. Malapelle U, Mayo-de-Las-Casas C, Molina-Villa MA et al., Cancer. 2017
- The enlightenments from ITMIG Consensus on WHO histological classification of thymoma and thymic carcinoma: refined definitions, histological criteria, and reporting.Wu J, Fang W, Chen G1. J Thorac Dis. 2016
- Mutations of epigenetics regulatory genes are common in the thymic carcinoma. Yisong Wang et al., Scientific Reports 2014
- Imatinib mesylate in thymic epithelial malignancies. G.Palmieri et al., Cancer Chemoter Phrmacol. 2012