Genomic alterations of anaplastic thyroid carcinoma detected by targeted, massive parallel sequencing in a BRAF^{V600E} mutation-prevalent area

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Introduction

▪ **Anaplastic thyroid carcinoma (ATC)** is the most aggressive type of thyroid cancer without effective therapy. Even though the prevalence of ATC is less than 1T of all thyroid cancer, it is important to understand genetic alterations and find effective molecular targets of ATC because of the dismal prognosis of ATC.

▪ Genetic studies of ATC presented **frequent somatic mutations in *BRAF* and *RAS*** which are also commonly mutated in papillary thyroid cancer (PTC) or follicular thyroid cancer (FTC) **and additional *PI3KCA, PTEN, TP53, CTNNB1, ALK* and *TERT* promoter mutations** are also commonly observed in ATCs. However, the precise molecular mechanisms of dedifferentiation and progression of cancer are largely unknown.

▪ In this study, we performed **targeted next-generation sequencing to investigate the mutational profile of ATC** using **massively parallel sequencing approach**.

Methods

1. Tissue samples and DNA extraction
   ▪ We enrolled **11 patients with ATC** who underwent thyroid surgery between 2009 and 2013 in Asan Medical Center, Seoul, Korea.
   ▪ **Both ATC tissues and matched normal tissues** were selected for DNA isolation.
   ▪ Genomic DNAs from formalin-fixed paraffin-embedded (FFPE) tissues was extracted using NEXprep FFPE Tissue Kit (Geneslabs, Korea).
2. **Targeted next generation sequencing (NGS)**
   ▪ Targeted NGS was performed using the MiSeq platform (Illumina) with OncoPanel version 2 (OP_v2) for capturing exons of **505 cancer related genes** plus **partial introns from 15 genes often rearranged** in cancer.
   ▪ Germ-line variants were filtered out with panel of normal.
   ▪ We validated genetic alterations detected by targeted NGS with **mass spectrometric genotyping** using Sequenom's MassArray technology platform (Sequenom, San Diego, CA).

Results

1.Clinical characteristics of 11 ATC patients

- Mean age : 74.4 years (median 75 years)
- Female/Male : 8 (73%)/3 (27%)
- Mean maximal size of tumor : 4.5 cm
- All patients were died (Median survival time : 4.3 months)

2.Targeted NGS

- **48 genetic variations were detected and 47 were confirmed** after validation process.

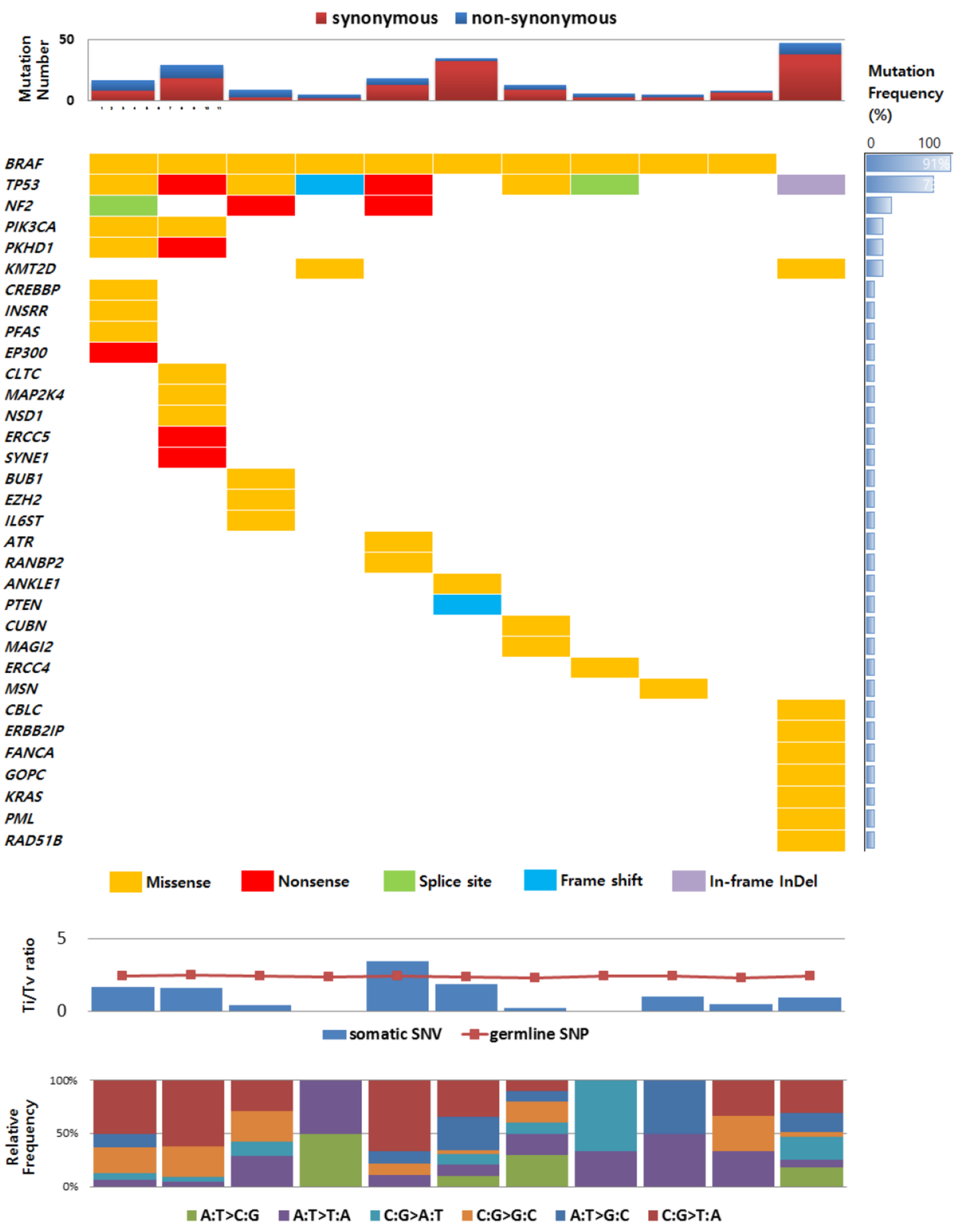

Figure. Somatic mutations in anaplastic thyroid carcinomas detected by targeted massive parallel sequencing

3. Summary of mutations frequently detected among 11 ATCs

Gene	Mutation (amio acid)	Nucleotide change	Mutation type	No. of cases
ATC related genes				
BRAF	V600E	T>A	missense	10
KRAS	G12V	G>T	missense	1
TP53	K132R	A>G	missense	1
	T125K	C>A	missense	1
	R248Q	G>A	missense	1
	Q331*	C>T	non-sense	1
	Q317*	C>T	non-sense	1
	X331	G>T	splicing site	1
	N131d	delCAA	deletion	1
	F328Sfs*17	del T	frame shift	1
PIK3CA	G364R	G>A	missense	1*
	G364E	G>A	missense	1*
	E365K	G>A	missense	1*
	E545K	G>A	missense	1
PTEN	V45Yfs*9	133delG	Frame shift	1
Recurrently mutated novel genes				
NF2	S228*	C>G	non-sense	1
	Q470*	C>T	non-sense	1
	X200	A>G	splicing site	1
KMT2D	V5245G	T>G	missense	1
	R3714K	G>A	missense	1
PKHD1	Q1665*	C>T	non-sense	1
	E4004K	G>A	missense	1

* These 3 different mutations in PIK3CA were detected in one sample.

Summary & Conclusions

1. We found 5 ATC related gene mutations and 28 novel gene mutations in 11 ATCs and confirmed the heterogenus genetic background of ATCs.

2. ***BRAF^{V600E}* mutation** was the most frequently observed mutations and the prevalence was 91%. This high prevalence of BRAF mutation in our ATCs might be related with frequent *BRAF* mutations of PTCs in Korea.

3. Among novel mutations we found, ***NF2*** and ***KMT2D* gene mutations** were repeatedly observed. They were known to **be tumor suppressors** in other cancers. Further studies were needed to find detailed molecular mechanisms how loss of function mutations of *NF2* or *KMT2D* affects the pathogenesis of ATCs.