Clin. Lab. 2020;66:1-4

ORIGINAL ARTICLE

Next-Generation Sequencing of Plasma Cell-Free DNA for Treatment Monitoring in Advanced Head and Neck Cancer Patients

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SUMMARY

Background: **Liquid biopsy, especially circulating tumor DNA (ctDNA), has been reported to provide information on tumor genetic features and possible disseminated disease in many kinds of solid tumors. In this pilot study, we investigated the utilization of ctDNA for monitoring treatment response and progression of the disease in patients with head and neck cancers (HNC) by next-generation sequencing (NGS).**
Methods: We sequenced ctDNA from 24 plasma samples collected at different time points from 19 HNC patients undergoing surgery or chemotherapy using a targeted gene panel composed of 61 actionable genes recurrently mutated in human malignancies in Tongren Hospital from November 2016 to February 2018. Also, the presence or absence of the residual ctDNA after chemotherapy in 9 patients was associated with the treatment efficacy evaluated by computed tomography (CT).
Results: **We found that *TP53* (2/6) and *PIK3CA* (2/6) are the most commonly recurring mutated genes. Out of 7 patients with matched tissue and pre-treatment blood samples, we confirmed concordance of ctDNA mutationin 5 patients. In 4 post-surgery patients with undetectable ctDNA mutation, ctDNA did not reoccur during the follow-up period of over 20 months.**
Conclusions: **These results suggested that NGS detection of ctDNA may contribute to minimal residual disease (MRD) detection and chemotherapy efficacy prediction in HNC.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.191150)

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Manuscript accepted December 26, 2019

Supplementary Table

Table S1. Clinical characteristics of 19 patients with head and neck cancer.

Tumor site	TNM stage	Tumor differen-tiation	Smok-ing	Alcohol	Tissue sampling time	Tissue acquisition method	Tissue gDNA QC	Blood sampling time			cfDNA concentration (ng/ml plasma)		
								Pre-treatment	Postoper-ative	During chemotherapy	Pre-treatment	Postoper-ative	During chemotherapy
Hypopharynx	III	High	N	N	2017/4/24	Laryngoscope	Pass	2017/3/3	/	2017/3/30	8.88	/	14.15
Hypopharynx	IV	Medium	Y	Y	2017/6/5	Laryngoscope	Pass	2017/3/8	/	2017/4/1	2.56 (Fail)	/	7.11
Laryngeal	III	Medium	Y	Y	2017/4/27	Surgery	Pass	2017/4/20	2017/5/8	/	13.55	27.20	/
Hypopharynx	IV	Medium	Y	Y	2017/7/3	Laryngoscope	Pass	2017/5/9	/	/	5.20	/	/
Laryngeal	IV	Medium	Y	Y	/	/	/	/	/	2018/1/24	/	/	22.00
Nasopharyngeal	IV	Low	N	N	/	/	/	/	/	2018/1/24	/	/	14.96
Hypopharynx	IV	Low	Y	Y	2017/7/14	Laryngoscope	Pass	2017/5/9	/	/	6.32	/	/
Laryngeal	III	High	N	N	2017/4/26	Surgery	Pass	/	2017/5/3	/	/	426.92	/
Hypopharynx	IV	High	N	N	/	/	/	2017/6/3	/	/	6.53	/	/
Laryngeal	IV	High	Y	Y	2016/11/15	Surgery	Pass	2016/11/10	2016/11/17	/	21.51	9.05	/
Hypopharynx	IV	Low	Y	Y	2017/5/16	Laryngoscope	Pass	2017/3/8	/	2017/4/3	3.93	/	9.24
Hypopharynx	IV	Low	N	Y	2017/9/12	Laryngoscope	Fail	2017/3/10	/	2017/3/30	11.50	/	7.58
Laryngeal	IV	High	Y	Y	2017/6/19	Laryngoscope	Fail	2017/3/20	/	/	5.28	/	/
Hypopharynx	IV	Medium	Y	Y	/	/	/	2017/3/23	/	/	7.81	/	/
Laryngeal	III	Medium	Y	Y	2017/3/24	Surgery	Pass	/	2017/3/27	/	/	15.38	/
Laryngeal	IV	NA	N	N	/	/	/	2018/1/24	/	/	21.86	/	/
Nasopharyngeal	IV	NA	Y	Y	/	/	/	/	/	2018/1/31	/	/	13.73
Hypopharynx	IV	Medium	Y	Y	/	/	/	/	/	2018/1/31	/	/	15.85
Tonsil	IV	Medium	Y	Y	/	/	/	/	/	2018/2/1	/	/	9.77

Table S2. List of Mutations Detectable by Accu-Act Panel.

AR	***CDK6***	***EZH2***	***GNAS***	***KRAS***	***PDGFRA***	***SMAD4***
ABL1	***CDKN2A***	***FBXW7***	***HNF1A***	***MAP2K1***	***PIK3CA***	***SMACRB1***
AKT1	***CSF1R***	***FGFR1***	***HRAS***	***MET***	***PTCH1***	***SMO***
ALK	***CTNNB1***	***FGFR2***	***IDH1***	***MLH1***	***PTEN***	***SRC***
APC	***DDR2***	***FGFR3***	***IDH2***	***MPL***	***PTPN11***	***TERT***
ATM	***DNMT3A***	***FLT3***	***JAK2***	***MSH6***	***RB1***	***TP53***
BRAF	***EGFR***	***FOXL2***	***JAK3***	***NOTCH1***	***RET***	***TSC1***
CDH1	***ERBB2***	***GNA11***	***KDR***	***NPM1***	***ROS1***	
CDK4	***ERBB4***	***GNAQ***	***KIT***	***NRAS***	***STK11***	

Underline indicates the gene includes all exons, white background indicates the detection of gene rearrangement.

Table S3. Tumor tissue DNA driver mutations in 9 patients with head and neck cancer.

Patient ID	Gene	Mutation type	Nucleotide change	Mutation	Allelic frequency
TRHN001	ERBB2	Intron	C>T/G>A	*ERBB2*g.37863431C>T	14.52%
	TP53	Nonsense	G>T/C>A	*TP53*p.E180*, c.1015G>T	15.27%
TRHN002	ND *	/	/	/	/
TRHN003	KRAS	Missense	G>T/C>A	*KRAS*p.G13V, c.38G>T	36.55%
TRHN004	CDK4	Missense	C>A/G>T	*CDK4*p.T102K, c.305C>A	23.15%
	TP53	Missense	T>A/A>T	*TP53*p.S215R, c.645T>A	34.51%
	TP53	Missense	G>A/C>T	*TP53*p.S215N, c.644G>A	34.51%
	PIK3CA	Missense	G>T/C>A	*PIK3CA*p.D1029Y, c.3085G>T	39.62%
TRHN008	TERT	Intron	G>A/C>T	*TERT*g.1295250G>A	19.76%
	TP53	Missense	C>T/G>A	*TP53*p.R114C, c.817C>T	18.78%
TRHN010	ND *	/	/	/	/
TRHN012	TP53	Missense	A>G/T>C	*TP53*p.Y163C, c.488A>G	19.03%
	CDKN2A	Nonsense	G>A/C>T	*CDKN2A*p.W110*, c.330G>A	28.43%
	ROS1	Intron	G>A/C>T	*ROS1*c.5777+585G>A	17.97%
TRHN018	ND *	/	/	/	/
TRHN026	RB1	Nonsense	C>G/G>C	*RB1*p.Y529*, c.1587C>G	27.42%
	PIK3CA	Missense	G>A/C>T	*PIK3CA*p.E545K, c.1633G>A	17.17%
	TERT	Missense	G>A/C>T	*TERT*p.V554I, c.1660G>A	12.84%
	CDKN2A	Missense	A>T/T>A	*CDKN2A*p.D108V, c.323A>T	72.49%

* **ND - not detected.**

Table S4. Plasma cfDNA mutations from 11 pre-treatment patients with head and neck cancer.

Patient ID	Gene	Mutation type	Nucleotide change	Mutation	Allelic frequency
TRHN001	PIK3CA	Missense	G>A/C>T	*PIK3CA*p.E707K, c.2119G>A	0.24%
TRHN002	ND *	/	/	/	/
TRHN003	KRAS	Missense	G>T/C>A	*KRAS*p.G13V, c.38G>T	3.45%
TRHN005	ND *	/	/	/	/
TRHN006	ND *	/	/	/	/
TRHN007	TP53	Missense	G>A/C>T	*TP53*p.M237, c.711G>A	8.36%
TRHN010	PDGFRA	Synonymous	T>C/A>G	*PDGFRA*p.L271=, c.811T>C	0.32%
TRHN017	FBXW7	Missense	C>T/G>A	*FBXW7*p.R465C, c.1393C>T	5.57%
TRHN017	FGFR3	Missense	G>T/C>A	*FGFR3*p.G375V, c.1124G>T	8.06%
TRHN017	ROS1	Intron	A>G/T>C	*ROS1*g.117647952A>G	9.21%
TRHN017	FGFR3	Missense	C>G/G>C	*FGFR3*p.S249C, c.746C>G	11.45%
TRHN017	TP53	Nonsense	G>T/C>A	*TP53*p.E298*, c.892G>T	13.81%
TRHN018	ND *	/	/	/	/
TRHN021	ND *	/	/	/	/
TRHN026	TERT	Missense	G>A/C>T	*TERT*p.V554I, c.1660G>A	14.34%
TRHN026	PIK3CA	Missense	G>A/C>T	*PIK3CA*p.E545K, c.1633G>A	20.92%
TRHN026	RB1	Nonsense	C>G/G>C	*RB1*p.Y529*, c.1587C>G	37.74%

*** ND - not detected.**