Development of detection technique for minimal residual diseases through detection of circulating tumor DNA in oral cancer patients

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Development of detection technique for minimal residual diseases through detection of circulating tumor DNA in oral cancer patients

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2020년 12월

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ABSTRACT

Development of detection technique for minimal residual diseases through detection of circulating tumor DNA in oral cancer patients

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Surgical pathology is the standard for tumor diagnosis while clinical examination and radiology can also be used in the diagnosis of oral cancer. However, those classical methods have various limitations in evaluating therapeutic outcomes and recurrence during treatment process of oral cancer patients. Minimal Residual Disease (MRD) refers to a microscopic tumor that remains during or after treatment and can be diagnosed by genetic analysis. Hence this study attempted to develop a method to detect circulating tumor DNA in oral cancer patients from before surgery to 6 months after surgery, using tissue, saliva, and plasma. 11 patients were enrolled in this study and tumor specific mutations were selected by paired analysis of whole exome sequencing between tumor tissues and whole

blood of each patient. Frequently mutated genes were selected from the head and neck cancer data of TCGA and COSMIC to design the panel for targeted deep sequencing. Both plasma and saliva were collected from all patients before surgery, 1 month, 3 months and 6 months after surgery, cell free DNA was isolated for analysis through the panel. As a result, MRD was successfully detected in 5 of the 6 patients with this novel method.

Key words: Liquid biopsy, Circulating tumor DNA, Minimal residual disease, Cell free DNA, Surveillance

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I. INTRODUCTION

The current clinical methods for cancer diagnosis includes clinical examinations, surgical biopsy and imaging such as computed tomography (CT), magnetic resonance images (MRI), positron emission tomography (PET). Among them, surgical biopsy and subsequent pathologic diagnosis are the gold standard of oral cancer, but it has some limitations. Biopsy is an invasive, time consuming procedures and difficult to repeat (Bellairs, et al., 2017; Lauritano, et al., 2019; van Ginkel, et al., 2017). Also it does not reflect the spatiotemporal heterogeneity of a solid tumor because it consists of only a single

site of the tumor in a single point of time (Gerlinger, et al., 2012). Moreover, as there is a paucity of disease specific biomarker in oral cancer, clinicians tend to rely on conventional diagnostic tools (Binahmed, et al., 2007; Paczkowska, et al., 2017).

Although radiologic examinations are useful in oral cancer diagnosis, they are occasionally hampered by some restrictions. Those radiologic modalities have difficulty in observing orofacial lesions due to not only metal artifacts from prosthesis, dental implants, fixation plates, screws, but also post operation fibrosis, inflammation through all periods of treatment (Zhao and Rao, 2017). In this regard, early surveillance by PET-CT may not be accurate until 12 months after initial treatment (Ho, et al., 2013).

Perioperative surveillance is closely related to tumor recurrence and overall survival. Several clinical factors, such as nodal status, invasion depth or surgical margin are already announced in prognosis of oral cancer (Binahmed, et al., 2007; Chandu, et al., 2005; D'Cruz, et al., 2015). As early recurrence tends to significantly decrease overall survival in oral cancer (Kernohan, et al., 2010; Mucke, et al., 2009), early detection of tumor recurrence is important in improving the survival rate.

Recently minimal residual disease (MRD) has emerged importantly in tumor surveillance. MRD refers to a microscopic tumor that remains during or after treatment and cannot be diagnosed by conventional clinical or imaging methods (Pantel and Alix-Panabieres, 2019; Pantel, et al., 2009). In the case of hematologic cancer, MRD detection and monitoring are established and widely used (Ivey, et al., 2016; van Dongen, et al., 1999), but their application in solid tumor is still challenging owing to difficulty in sampling the low concentrations of circulating cells (CTCs) or other factors shed from the cancer cells into the bloodstream (Pantel and Alix-Panabieres, 2019).

In this regard, there are unmet needs in oral cancer diagnosis and surveillance with serial quantitative methods. Recent liquid biopsy technique can be an alternative choice of diagnostic modality in oral cancer (Galot, et al., 2020; Mes, et al., 2020; Wang, et al., 2015). However, there have been no prospective studies reporting quantitative serial detection of tumor recurrence based on complex mutation subsets in oral cancer patients with liquid biopsy before and after treatment. Hence, this study aimed to detect minimal residual disease in oral cancer patients with serial liquid biopsy.

II. MATERIALS AND METHODS

1. Samples

We studied tumor tissues and liquid samples from 11 oral cancer patients. The study was approved by the institutional review board of Yonsei University Dental Hospital (Approval No. 2018-0061) and from all subjects signed informed consent was obtained. Fresh tumor tissues were collected during operation following macro dissection to ensure that neoplastic cellularity was over 20% and snap-frozen at liquid nitrogen. In case of fresh tumor tissue was insufficient (LB-005, 007), formalin fixed paraffin embedded (FFPE) tissues were collected through laser captured microdissection (Leica LMD 6500, Leica LMD membrane slide) with achieving tumor cellularity over 80%. Genomic DNA isolation from tumor tissue was done with QIAamp DNA micro kit (Qiagen, Hilden, Germany) following manufacturer's instruction. 3mL of paired whole blood was used for isolation of genomic DNA of each patient with Intron G-Dex^{TM}IIb (Intron, Korea). Saliva was collected using sterilized 50mL tubes (Eppendorf, Germany) after oral rinsing with sterilized saline. In each collection, 5~15mL saliva were collected and snap frozen for store. Chewing paraffin (Ivoclar Vivadent, USA) were used in patients who underwent radiotherapy (RT) or concurrent chemoradiotherapy (CCRT) if they complained xerostomia. Saliva was centrifuged at 3000 rpm for 10 minutes at 4 ℃, then transferred the clear supernatant to a new 50mL tube, and added centrifugation at 3000 rpm for 5 minutes at 4 ℃ for the study. 10mL of whole blood was collected into Cell-Free DNA BCT (Streck, USA) for circulating

tumor DNA (ctDNA) harvest. Blood samples were centrifuged at 1,500g for 15 min. Plasma was then separated by centrifugation at 16,000g for 10 min to remove cell debris, after which 1 mL aliquots were placed in Eppendorf tubes and stored at −80˚C before extraction. Cell free DNA (cfDNA) was isolated from both saliva and whole blood with MagMax (Thermo Fisher Scientific, USA) following manufacturer's instruction. Mean 4mL of plasma was used for cfDNA isolation. All DNA samples were stored at -80℃ and quantified with Qubit fluorometer (Invitrogen, USA) and evaluated the size distribution with 2200 Tapestation (Agilent Technologies, USA). The schematic workflow of sample collection, DNA isolation and sequencing were depicted in Figure 1. Both saliva and plasma for isolation of cfDNA were longitudinally collected at 4 time point. The first one (T1) was immediate before surgery and the second (T2), third (T3), fourth (T4) were 1, 3, 6 months after surgery respectively.

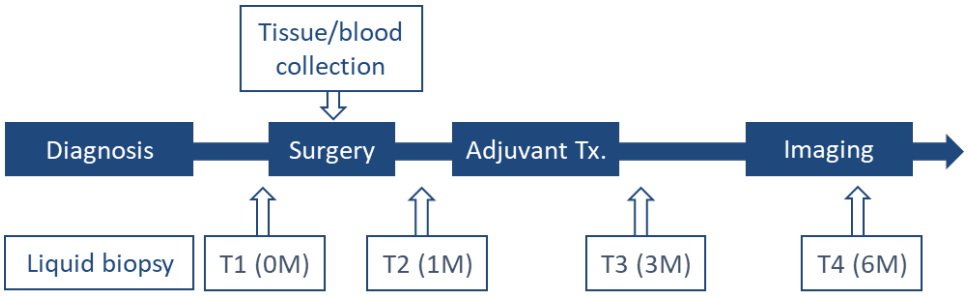


Figure 1. Schematic diagram of longitudinal study design. Liquid biopsy was designed to collect both plasma and saliva for evaluating each therapeutic modality. For example, sampling on T2 estimated therapeutic effect of surgery based on T1 sampling. In this regard, T3 sampling evaluate the efficiency of adjuvant therapies compared to T2.

2. Next generation sequencing

Whole Exome Sequencing (WES) for tumor tissue and paired whole blood samples were done for searching tumor specific somatic mutations. Targeted deep sequencing for cell free DNA from liquid samples were done to detect ctDNA. Briefly, genomic DNA from each samples were fragmented by acoustic shearing on a Covaris S2 instrument and cfDNAs were skipped this step. Fragments of 150–300 bp were ligated to Illumina's adapters and PCR-amplified. The samples were concentrated and hybridized with RNA probes, SureSelect XT Human All Exon V5 Capture library for WES or oral cancer specific customized gene panel for targeted deep sequencing. To make the panel, tumor specific high impact somatic mutations were chosen compared to germline mutations of paired whole blood. In addition to patient driven mutations, frequent mutations of head and neck cancer were chosen based on both The Cancer Genome Atlas (TCGA) from GDC data portal (National Cancer Institute) and Cosmic Mutant Census v87 over than 10 contig.

After hybridization, the captured targets were pulled down by biotinylated probe/target hybrids using streptavidin-coated magnetic beads (Dynabeads My One Streptavidin T1; Life Technologies Ltd.) and buffers. The selected regions were then PCR-amplified using Illumina PCR primers. Libraries were quantified using the Agilent 2100 Bioanalyzer (Agilent Technologies) and KAPA Library Quantification Kit (KK4824, Kapa Biosystems). The resulting purified libraries were applied to an Illumina flow cell for cluster generation and sequenced using 100 bp paired-end reads on an Illumina NovaSeq6000 sequencer by following the manufacturer's protocols.

3. Sequencing quality control

The quality of the reads was checked using fastQC (v.0.10.1), helping the understanding for the basic quality for sequence quality score, GC content, N content, length distribution and duplication level. After checking the read quality, the low-quality base below Q20 were trimmed using Cutadapt (v.1.8.1) (Martin, 2011)

4. Sequencing alignment, variant call and annotation

High quality reads were then aligned to the human reference genome hg19 using Burrows Wheeler Aligner (BWA) (v.0.7.12) (Li and Durbin, 2009) with minimum seed length of 45. After the alignment of the reads to reference genome, the duplicated reads were further removed using MarkDuplicates.jar in PicardTools (v.1.98). For the read alignment, the suspicious intervals were examined for the more accurate realignment using RealignerTargetCreator tool in GenomeAnalysisTK (v.2.3.9) (McKenna, et al., 2010) and Mills-and-1000G-gold.standard-INDELs.hg19 was referred as the known indel set. Then, Base quality score recalibration (BQSR) process was done to adjust the quality score using BaseRecalibrator Tool in GATK. For the realigned and recalibrated reads, variants were called using UnifiedGenotyper tool in GATK. All variants were then annotated using SnpEff (v.4.1) (Cingolani, et al., 2012) in context of the prediction of damaging or clinical effect of the variants using dbNSFP (Liu, et al., 2016), Cosmic (Forbes, et al., 2008) and ClinVar (Landrum, et al., 2014). Allele frequencies were referred from 1000 Genomes

(Genomes Project, et al., 2015), ESP6500 and ExAC database (Karczewski, et al., 2017). Mutation allele frequency over than 0.1% was regarded for existence of ctDNA in liquid samples. In contrast, high frequency mutations over than 7.0% collected from open source data were regarded as noise. Besides mutations collected from TCGA, COSMIC were regarded as true mutation when they were detected on pre-treatment liquid samples.

III. RESULTS

1. Clinical features and mutations in primary tumors

Total 11 patients were enrolled in prospective, longitudinal study. All patients were diagnosed as squamous cell carcinoma before surgery by experienced oral and maxillofacial pathologist. Their average age was 64 and the majority (73%) were female. All samples were from oral cavity. Except individual tongue and cheek sample, 9 samples were from gingiva (5 from maxilla, 4 from mandible). Five patients (45%) had early (stage II) disease, and the remaining six patients (55%) had advanced (stage III or IV) disease. Among them, three patients had history of recurrent tumor. Especially, LB-002 patient had five times of tumor recurrence only in oral cavity with three times of surgery, two times of RT history. Also, LB-011 patient had history of definitive RT on right tongue on 10 years before. Only one patient (LB-007) recurred at same site within 8 months since initial surgical treatment before this study (Table 1).

No.	Age	Sex	Primary site	TNM Stage
LB-001	40	F	Tongue, left	3
LB-002*	67	F	Mx. gingiva, left	2
LB-003	52	M	Mn. gingiva, left	2
LB-004	73	F	Mx. gingiva, anterior	2
LB-005	77	F	RMT, right	4a
LB-006	63	M	Mx. gingiva, right	4a
LB-007*	57	F	Cheek, right	4b
LB-008	75	M	Mx. gingiva, right	4a
LB-009	84	F	Mx. gingiva, right	2
LB-010	53	F	Mn. gingiva, right	3
LB-011*	62	F	Mn. gingiva, left	2

Table 1. Summarized clinical information of patients enrolled in the study. Asterisk (*) means recurrent tumors at the beginning of the study. TNM stage were decided based on AJCC 8th edition.

To begin this study, we attempted to identify tumor only somatic mutations in every patient through WES compared to germline mutations from whole blood. TP53 mutation was a driver event in six patients (55%), while CASP8, AJUBA, CDKN2A and NOTCH1 mutation were relatively frequent (Figure 2). PIC3CA was not observed in primary tumor of out cohort.

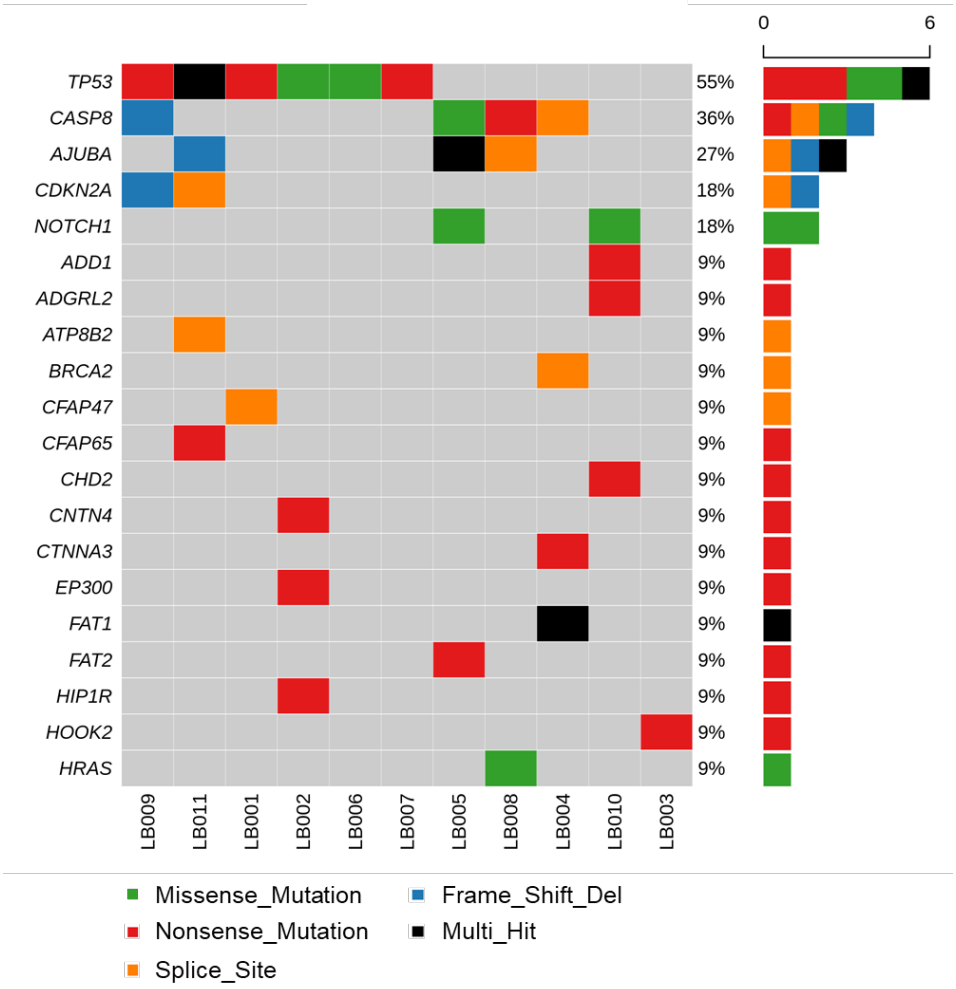


Figure 2. Oncoplot of Top 20 genes analyzed by whole exome sequencing from index tumor of 11 patients. Sequencing depth was 200x on index tumor, 100x on whole blood.

2. Variants selection for panel design

In addition to tumor specific mutations from tissue samples, frequent somatic mutations were searched upon TCGA and COSMIC to make panel being optimized to head and neck squamous cell carcinoma (HNSCC) involving oral cancer (Supplement Table 1,2).

308 mutations were selected from total 508 TCGA HNSCC samples. TP53 was the most frequently mutated gene in overall with various mutations (531/834, 63.7%). The most frequent independent mutation in TCGA was PIK3CA c.1633G>A (p.Glu545Lys) with overall 5% frequency. TP53 c.524G>A (p.Arg175H) mutation was the most frequent mutation among several TP53 mutations with overall 2% frequency. Among the 185 mutations summarized from COSMIC, TP53 showed the most frequent mutation count (588/3613, 16.3%) and CDKN2A, PIK3CA followed the next. Intriguingly, although TP53 was the most mutated gene in both TCGA and COSMIC, their frequency was differed in each set.

Also, previous reported driver events were added in designing the panel involving FAT1, AJUBA, NOTCH1, HRAS, NRAS, FBXW7, KMT2D and NSD1 (Stransky, et al., 2011; Wang, et al., 2015). All those given mutations were summarized to make panel with the most frequent genes (Supplement Table 3).

3. Longitudinal liquid biopsy for oral cancer surveillance

Longitudinal liquid biopsy with saliva as well as plasma were done on 11 patients. As 9 samples (5 plasma, 4 saliva) were missed and 2 saliva samples were not able to pass the DNA quality control, total 77 liquid samples underwent deep sequencing. Targeted deep sequencing was performed to detect the ctDNA based on previously designed panel.

Of the 11 patients involved in the study, 6 patients were confirmed clinical recurrence until 18 months follow up after initial treatment. Four of them were advanced stage (stage III, IV) and most of them had positive surgical margin (5/6, 83.3%) while the rest one had close margin (lesser the 5mm, 1/6, 16.7%). None of them had free of margin in pathologic report. In contrary, non recurrent five patients had free of margin in four patients (4/5, 80%) and the rest one had close margin (1/5, 20%). Four of recurred six patients (4/6, 66.7%) received RT (one patient) or CCRT (three patients) for adjuvant therapy while only one patient underwent adjuvant RT in non recurrent group (1/5, 20%) (Table 2).

The presence of ctDNA in liquid biopsy means substantial increased possibility for recurrence. The earlier ctDNA detection implicates the needs for close clinical monitoring. In this study, we successfully detected the presence of ctDNA through combination of liquid samples mainly dependent on saliva from recurred 5 patients (Table 2, Supplement Table 4). Only one recurred patient (LB-003) who clinically recurred at 12 months did not show the presence of ctDNA until 6 months after initial treatment. Notably, the recurred lesion was observed on left temporal space while the primary tumor was found on ipsilateral gingiva of mandible. Mean time difference between first ctDNA detection and

clinical recurrence diagnosis was 4.4 months (mean 2.4 months versus 6.8 months) on recurred 5 patients with longitudinal ctDNA detection. Most of clinical recurrences were found on regular imaging studies (CT, MRI) and clinical examinations such as naked eye inspection or palpation (Table 2).

Among the non recurrent patients, LB-009 patient showed the presence of TP53 c.818G>A single nucleotide variant (SNV) in both plasma and saliva at initial and 1 month after surgery. However, those ctDNA SNV was disappeared on 3 months even though the patient did not receive any adjuvant therapy. Intriguingly, the surgical margin of this patient was positive only in maxilla hard tissue but free in soft tissue. This patient suffered from oroantral fistula and post operation necrosis on surgical site after surgery until 6 months.

No.	ctDNA detection				Clinical recurrence	Recurrence recognition time (month)	Adjuvant therapy	Recurrence detection method
	0	1M	3M	6M				
LB-001	O	na	O	O	Recur	6	RT	Clinic, CT
LB-002	O	O	O	O	Recur	8.5	-	Clinic, pathology
LB-003	O	-	-	-	Recur	12	CCRT	Clinic, MRI
LB-004	O	-	-	-	-	-	-	-
LB-005	O	-	na	-	-	-	-	-
LB-006	O	-	-	O	Recur	6	CCRT	MRI
LB-007	O	O	na	na	Recur	7.5	CCRT	MRI, CT
LB-008	O	O	O	O	Recur	6	-	MRI, CT
LB-009	O	O	-	-	-	-	-	-
LB-010	O	-	-	-	-	-	RT	-
LB-011	O	na	-	-	-	-	-	-

Table 2. Summary of longitudinal ctDNA detection results and clinical recurrence.

Five of six logoregional recurred patients were detected by liquid biopsy earlier than classical methods. (na means not available)

The efficiency of liquid biopsy for oral cancer was 100% at before of initial treatment when combining plasma and saliva while the latter showed much higher efficiency. Though ctDNA detection with saliva missed only one patient (LB-006) who was stage IV maxilla gingiva cancer, the presence of plasma ctDNA of same patient compensated it.

When patients were grouped following the clinical recurrence, ctDNA detection efficiency was relatively low at T2, T3 (1 month after initial treatment) (Table 3). Considering that there was a partial missing in liquid sample collection, ctDNA in saliva was detected more sensitively than plasma immediately after surgery. In particular, sequencing could not be performed on the two T3 saliva samples of the recurred group as they did not meet the DNA quality control criteria. Although the detection rate of ctDNA in saliva decreased in the recurred group at time point T4 when ctDNA in plasma began to be detected, the overall tumor monitoring efficiency was increased when saliva and plasma were combined.

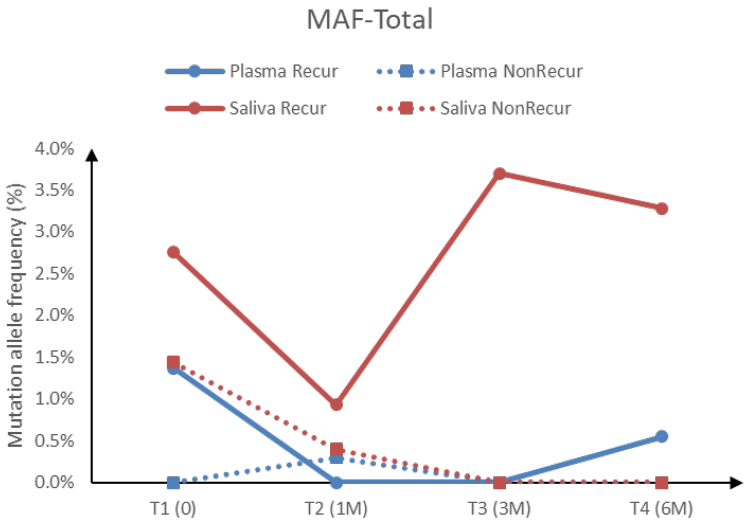
Intriguingly mean allele frequency (MAF) and ctDNA count through targeted deep sequencing provided quantitative information in plasma and saliva. Saliva constantly showed much higher MAF and sum of ctDNA count than plasma (Figure 3). Saliva also exhibited that the local residual tumor was not present in the group that did not recur as time goes by. Although plasma had a similar expression pattern with saliva, saliva had much higher quantitative values in both MAF and ctDNA count.

Time point	Recur	Plasma(%)	Saliva(%)	Saliva or Plasma (%)
0	-	3/11 (27)	10/11 (91)	11/11 (100)
1M	Non Recur	1/4 (25)	1/4 (25)	1/4 (25)
	Recur	0/5 (0)	3/6 (50)	3/6 (50)
3M	Non Recur	0/4 (0)	0/4 (0)	0/4 (0)
	Recur	0/5 (0)	3/4 (75)	3/6 (50)
6M	Non Recur	0/5 (0)	0/5 (0)	0/5 (0)
	Recur	2/5 (40)	2/5 (40)	4/5 (80)

Table 3. Summary of tumor recurrence surveillance by longitudinal liquid biopsy.

Saliva had better efficiency than plasma in oral cancer surveillance. Some of recurred patients exhibited a little amount of ctDNA in saliva at one and three months after surgery.

A.



B.

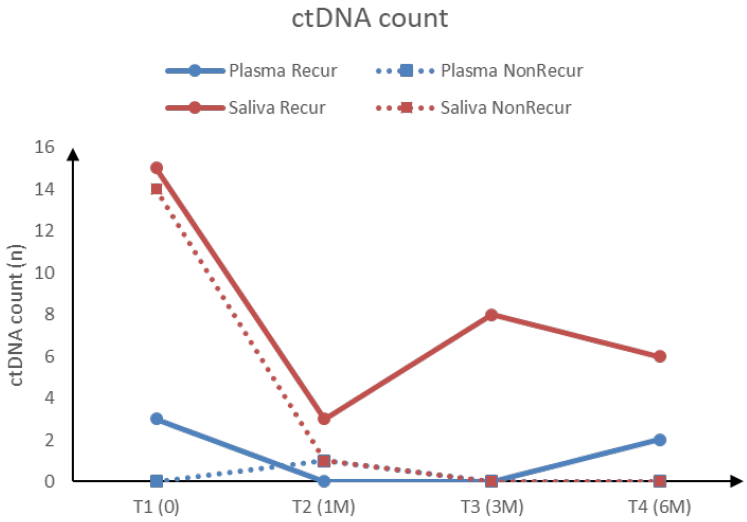
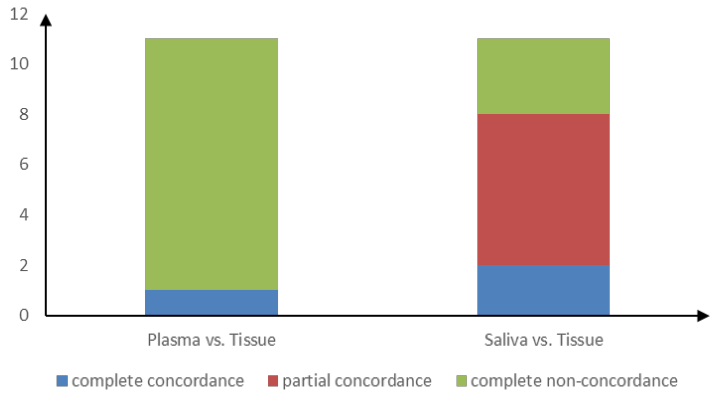


Figure 3. Mean allele frequency and ctDNA count difference between plasma and saliva based on liquid biopsy follow up. A. MAF of both plasma and saliva, B. Sum of ctDNA count in both plasma and saliva.

4. Concordance of variants between liquid biopsy and index tumor

Concordance of targeted deep sequencing results was estimated in samples from 11 patients with cfDNA and genomic DNA from primary tumor tissues at before initial treatment. The concordance rate between salivary cfDNA and tumor tissue DNA was 72.7% (8/11)) among all patients while plasma cfDNA was 9.1% (1/11). In salivary cfDNA, two patients showed complete concordance and six patients showed partial concordance (Figure 4A). The concordance for each gene were depicted on Figure 4B. Though two patients (LB-001,009) did not exhibit concordance between tumor tissue and liquid samples, TP53 mutations which were not found on WES of index tumor were revealed by deep sequencing of liquid samples.

A.



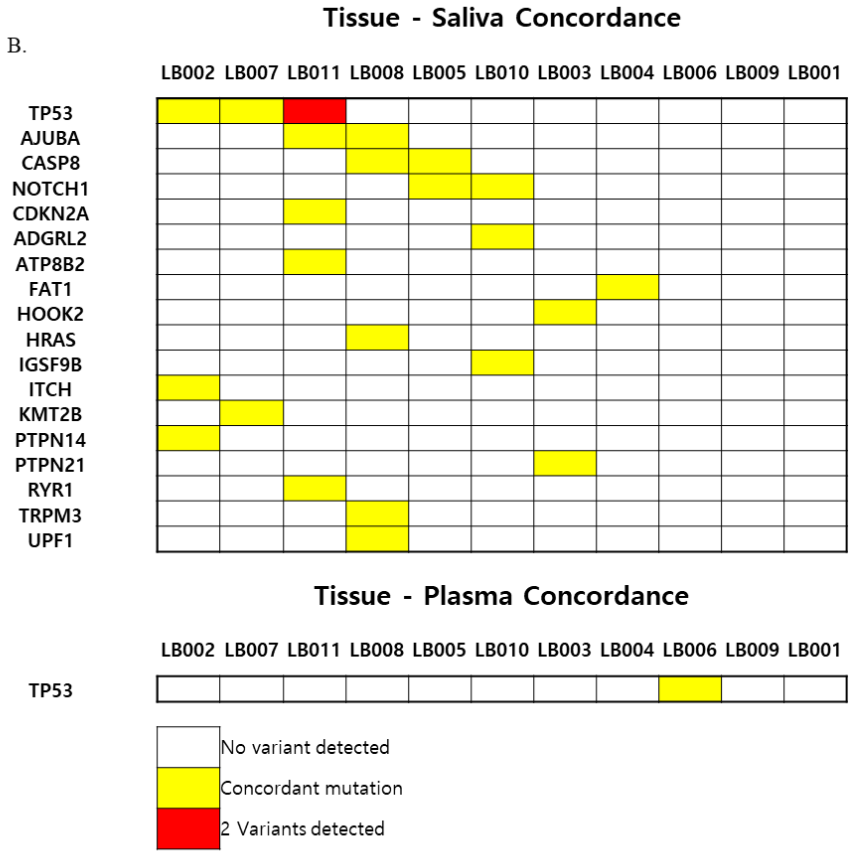


Figure 4. Concordance between cfDNA and index tumor. A. Summarized concordance of plasma, saliva each other, B. Summarized gene based concordance.

IV. DISCUSSION

This study was designed to develop a workflow for detection of SNVs in cfDNA from plasma, saliva of oral cancer patients using the identical sequencing panel, and to evaluate its feasibility in liquid samples of 11 oral cancer patients longitudinally. This method was based on targeted deep sequencing of a panel composed with frequently mutated genes in HNSCC. This panel based multiplex NGS approach provides several advantages in liquid biopsy. High resolution data can be acquired through multiplex analysis by the HNSCC specialized panel. It can also help to overcome the weakness in selection of unique mutation from index tumor which cannot fully cover the evolution tracing, dynamic changes in mutational spectrum as well as intra-tumor genetic heterogeneity (de Roest, et al., 2019; Gerlinger, et al., 2012; Luskin, et al., 2018; Pantel and Alix-Panabieres, 2019).

Overall, we explored the relevance of liquid biopsy to supervise the clinical recurrence using a designed panel at deep coverage. As a result, we successfully detected the presence of ctDNA in both plasma and saliva in oral cancer patients while the latter exhibited much higher resolution. The presence of primary tumor was 100% confirmed at before of initial treatment by liquid biopsy as a previous report (Wang, et al., 2015). Quantitatively, increased SNVs of ctDNA allele frequency was observed around 3 months after surgery mainly dependent on saliva than plasma which suggested possibility of locoregional recurrences. The emergence of SNVs of ctDNA in liquid samples was about 4 months earlier than clinical recurrence by conventional regular clinical, radiological examinations

which were sometimes blinded until 6 months after surgery and continuing post operation adjuvant therapy.

As genetic variations in cancer underwent dynamic changes from emergence to evolution overcoming therapies, intra-tumor, inter-tumor heterogeneity tended to increase (Campbell, et al., 2008; Gerlinger, et al., 2012). This implies that a few driver mutations from index tumor cannot cover the serial tracing of tumor recurrence. In this regard, frequent mutation selection from both TCGA and COSMIC in addition to index tumor sequencing can be an alternative choice for serial liquid biopsy.

Previous reports used the presence of HPV-DNA in plasma to increase the detection efficiency in HPV positive oropharyngeal cancer (Wang, et al., 2015). However, this method is difficult to apply in oral cancer as most of oral cavity cancer, for example gum, palate, tongue, cheek are HPV negative (Cancer Genome Atlas, 2015). In this regard ctDNA or copy number aberrations (CNA) in local or systemic fluid can be a choice for liquid biopsy in oral cancer patients. Recent report exhibited advantages of low-coverage whole genome sequencing in HNSCC patients while ctDNA detection method is still obscure to apply in patient cohort (Mes, et al., 2020). Although ctDNA in plasma of locoregional recurrent and/or metastatic HNSCC were successfully detected while the latter showed higher probability of detection, this study had a limitation that liquid biopsy were done on incurable end stage cohort as this technique was originally invented and exhibited maximal clinical benefit in early surveillance since initial treatment (Galot, et al., 2020).

Ideally, driver mutation selection from index tumor and subsequent large panel based serial targeted deep sequencing for ctDNA as well as low coverage whole genome

sequencing for CNAs, digital droplet PCR for validation can be a great research method in tracing tumor recurrence, evolution regardless of time, fund and low quantity of cfDNA. The other unpredicted clinical obstacle in collecting saliva for cfDNA study was dry mouth with severely decreased saliva secretion in patient with RT or CCRT. As saliva showed better efficiency in locoregional recurrence monitoring in this study, overcoming low quantity and quality of salivary cfDNA would be a main hurdle in future studies.

In conclusion, we developed a novel method for analysis of somatic mutations in cfDNA from both plasma and saliva of oral cancer patients. Future research should focus on not only clinical protocol establishment for surveillance of locoregional, metastatic disease but also designing novel clinical trials to give practical benefit in those patients.

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V. CONCLUSION

1. Locoregional recurrence of oral cancer can be successfully predicted by longitudinal detection of ctDNA from both plasma and saliva.

2. Liquid biopsy for early detection is carefully suggested to begin 3 months after initial treatment.

3. As driver mutation of index tumor cannot fully cover the cancer progression, panel based NGS method can be useful to overcome tumor heterogeneity.

4. Combined ctDNA deep sequencing with CNAs analysis, ddPCR validation will increase probability of detection in HPV negative HNSCC.

5. Dry mouth is a real clinical hurdle in saliva collection during serial liquid biopsy.

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ABSTRACT (IN KOREAN)

구강암 환자의 순환 종양 DNA 검출을 통한 미세잔존질환 탐지 기법 개발

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최 영

조직 생검과 현미경을 통한 병리학적 진단은 종양 확진의 기준이며 임상검사와 영상검사 또한 구강암 진단에 유용하게 사용되고 있다. 그러나 고전적인 진단 기법들은 구강암 환자의 치료 과정에서 치료 성과와 재발을 평가하는 데에 있어 다양한 한계점을 지니고 있다. 미세잔존질환이란 치료 중이나 치료 후에 남아 있는 소수의 악성 세포를 의미하며 분자생물학적, 유전학적 분석을 통해 진단된다. 이에 본 연구는 구강암 환자의 조직, 타액과 혈액을 활용하여 수술 전부터 수술 후 6개월까지 연속적으로 구강암 환자의 순환 종양 DNA를 검출하는 방법을 개발하고자 하였다. 11명의 구강암 환자가 연구에 포함 되었으며 각 환자의 종양 조직과 혈액을 각각 whole exome sequencing 한 뒤 paired analysis를 통해 tumor specific mutation을 선정하였다. Targeted deep sequencing을 위한 환자 맞춤 패널 설계 및 범용성을 확보하기 위하여 TCGA와 COSMIC에 등재 된 두경부암 유전체 자료 중 빈번한 돌연변이를 함께 선정하였다. 모든 환자에게서 수술 전, 수술 후 1개월, 3개월, 6개월째에 각각 혈액과 타액을 샘플링 하였으며 cell free DNA를 추출한 뒤 제작한 시퀀싱 패널을 활용하여 분석하였다. 그 결과 임상적으로 재발이 확진된 6명의 환자 중 5명의 미세잔존질환을 성공적으로 조기 탐지할 수 있었다.

핵심어 : 액체생검, 순환종양DNA, 미세잔존질환, 무세포DNA, 종양감시