Original Article

Mutational differences between primary cancer tissue and circulating tumor cells in early-stage non-small cell lung cancer

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Background: Early-stage non-small cell lung cancer (NSCLC) has a high recurrence rate despite proper management, including curative surgery. Circulating tumor cells (CTCs) are believed to play a key role in the distant metastasis of lung cancer. Immunofluorescence imaging studies of CTCs have revealed that they are associated with the prognosis of NSCLC. However, the mutational profiling of CTCs from early-stage NSCLC has not been extensively explored. We hypothesized that CTCs could be detected by mutational analysis using panel sequencing and would have distinct mutations associated with distant metastasis compared to those of primary cancer tissue in early-stage NSCLC. Thus, this study examined the DNA from CTCs using targeted panel sequencing to identify mutations and compared them with mutations found in primary cancer tissue in patients with resectable early-stage NSCLC.

Methods: Overall, 45 patients with resectable NSCLC were prospectively enrolled from September to December 2023. Matched whole blood samples and primary cancer tissues were collected during curative surgery. Then, 405-gene targeted panel sequencing was performed on DNA from primary cancer tissues and CTCs.

Results: In this study, 37 patients (82%) had adenocarcinoma, and 30 (67%) were classified as having pathologic stage 1 disease. Mutated genes were detected in all (100%) and 31 patients (69%) for primary cancer tissue and CTCs from panel sequencing, respectively. The partial concordance rate of mutations between primary cancer tissue and CTCs was 17.8%, with the top 10 mutated genes differing significantly. Among primary cancer tissue samples, mutated genes differed by stage and histologic type; these findings were not observed in CTCs. CTCs predominantly displayed mutations in tumor suppressor genes, whereas primary cancer tissues exhibited mutations in both oncogenes and tumor suppressor genes.

Conclusions: CTCs exhibited unique mutations, showing low concordance with mutations found in primary cancer tissue. CTCs may possess specific mutations independent from those of primary cancer tissue in early-stage NSCLC.

Keywords: Circulating tumor cells (CTCs); next-generation sequencing; non-small cell lung cancer (NSCLC)

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Introduction

Non-small cell lung cancer (NSCLC) is recognized as a malignant disease with a high recurrence rate, even with appropriate management (1). Circulating tumor cells (CTCs), as a kind of liquid biopsies, offer advantages such as reduced invasiveness and the ease of repeated sampling. These benefits position CTCs as a breakthrough in various areas, including prognosis prediction, treatment monitoring, and the detection of drug resistance, especially for advanced-stage disease (2,3). However, limited data are available regarding the potential diagnostic, predictive, and staging value of CTCs in early-stage NSCLC.

Distant recurrence of early-stage NSCLC frequently occurs despite curative surgery (4,5). CTCs are believed to directly relate to metastasis through the process of epithelial-mesenchymal transition (EMT) (6). Several studies have suggested that the presence of CTCs, as detected by immunofluorescence imaging, is associated with poor prognosis or recurrence in patients with NSCLC (7-10).

We thought that CTCs harbor unique gene mutations that promote the EMT of primary cancer tissue. Examining the mutational profiles of CTCs could reveal additional characteristics associated with distant recurrence in early-stage NSCLC. However, most studies have been limited to the sequencing analysis of cell-free DNA, immunofluorescence imaging of CTCs, or single-cell analysis of CTC (7,11-14). The rarity of CTCs has led to a paucity of research on their mutational profiles or on direct comparisons using targeted panel sequencing between CTCs and primary cancer tissue, particularly in the context of early-stage NSCLC.

Thus, this study was conducted to analyze DNA from CTCs and compare the findings with those of primary cancer tissue using 405-gene targeted panel sequencing in patients with resectable early-stage NSCLC. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-709/rc).

Highlight box

Key findings

- The mutational profiles of primary cancer tissue and circulating tumor cells in resectable early-stage non-small cell lung cancer (NSCLC) were significantly different. The concordance rate between the two samples was 17.8% using 405-gene targeted panel sequencing.

What is known and what is new?

- Circulating tumor cells are known to exhibit distinct mutations from those of primary cancer tissue based on whole exome sequencing or single cell analysis.
- Our study performed targeted panel sequencing for 405 cancer-associated genes in matched samples of primary cancer tissue and circulating tumor cells from 45 patients with early-stage NSCLC. The mutational roles of circulating tumor cells were different from those of primary cancer tissue. The number of mutations in circulating tumor cells did not show statistically significant associations with stage or histology.

What is the implication, and what should change now?

- Mutations in circulating tumor cells occurred independently from those in primary cancer tissue; therefore, some mutations in circulating tumor cells might be associated with the epithelial-mesenchymal transition. Further research is needed on the relationship between circulating tumor cells and distant recurrence.

Methods

Ethical statement

The study protocol was approved by the Institutional Review Board of Seoul National University Bundang Hospital (approval No. B-2211-791-304) and conformed to the principles of the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent before enrollment.

Patients

A total of 45 patients were enrolled who were postoperatively diagnosed with NSCLC at Seoul National University Bundang Hospital between September and December 2023. The following exclusion criteria were applied: (I) patients with a histologic type other than adenocarcinoma or squamous cell carcinoma; (II) patients diagnosed with another malignancy within the past 5 years; (III) patients suspected of having any distant metastasis; and (IV) patients who refused to participate in this study.

Specimen processing

Following the induction of anesthesia, a total of 10 mL of fresh blood was drawn from the peripheral arterial blood into an ACD-A tube for CTC analysis. After the surgical specimen was removed, primary tissue samples from both normal and cancerous areas, each measuring 5 mm × 5 mm × 5 mm, were excised. These samples were promptly

transported to BeyondDx Inc. (Gwangmyeong, Republic of Korea) for CTC extraction, which was performed within 6 hours of collection. Subsequently, DNA from the CTCs and primary tissue from the normal and cancerous lung regions were sent to Geninus (Seoul, Republic of Korea) for targeted panel sequencing.

Primary cancer tissue sequencing

DNA was extracted from the normal and cancerous tissues using a QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). The concentration and purity of the DNA were assessed with a NanoDrop One UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit 4.0 Fluorometer (Life Technologies, Grand Island, NY, USA). The degree of DNA degradation was evaluated using a 4200 TapeStation instrument (Agilent Technologies, Santa Clara, CA, USA). Subsequently, the DNA was used to construct a library with the Complete Library Preparation Kit (Twist Bioscience, San Francisco, CA, USA) and CancerSCAN panel probes (Geninus), targeting 405 cancer-associated genes (Box S1). Sequencing was performed with the NovaSeq 6000 platform (Illumina, San Diego, CA, USA). Mutations in the primary cancer tissue were identified using the sequencing results from the normal lung tissue as controls.

CTC staining and sequencing

Peripheral blood mononuclear cells (PBMCs) were isolated from the ACD-A tube, which was stored at room temperature, using Ficoll-Hypaque density gradient centrifugation. For CTC staining analysis, CD45+ PBMCs were depleted using Dynabeads (Invitrogen, Waltham, MA, USA). The remaining cells were stained with anti-epithelial cell adhesion molecule (EpCAM)-fluorescein isothiocyanate (FITC), anti-cytokeratin (CK)-FITC, and anti-CD45-phycoerythrin antibodies and were counterstained with 4',6-diamidino-2-phenylindole (DAPI). MCF7 and Jurkat cells served as staining controls for CTCs and PBMCs, respectively. Images were captured with a Cytation 5 imaging system (Biotek Inc., Winooski, VT, USA) and analyzed using Gen5 software to enumerate the CTCs. CTCs were identified as $DAPI^+/CD45^-$ and either double-positive ($EpCAM^+/CK^+$) or single-positive ($EpCAM^+/CK^-$ or $EpCAM^-/CK^+$). In contrast, PBMCs were identified as $DAPI^+$, $EpCAM^-/CK^-/CD45^+$ (*Figure 1*). Separately, candidate CTCs for sequencing were isolated using EpCAM, EGFR, and HER2 biomarkers for targeted capture. Genomic DNA from CTCs expressing any of these markers—EpCAM, EGFR, or HER2—was extracted and amplified with the REPLI-g Single Cell kit (Qiagen Inc.) according to the manufacturer's protocol. The amplified DNA was used to construct a library, which was then sequenced using the CancerSCAN panel, following the same methodology applied to primary cancer tissue samples.

CTC positivity and mutation classification

On an immunofluorescent image, CTC positivity was defined as the presence of one or more CTCs per 2×10^6 PBMCs. For primary cancer tissue and CTCs, positive mutation status was indicated by the presence of one or more single nucleotide variant or insertion/deletion mutations. If a case exhibited at least one mutation common to primary cancer tissue and CTCs, it was considered to show partial concordance. Mutated genes were categorized based on their function; oncogenes included growth factor receptors, transcription factors, and signal transducers, while tumor suppressors consisted of genes functioning as caretakers and gatekeepers. A subset of genes, termed cancer hallmark genes, was identified using the methodology described by Liang *et al.* (15). Cancer hallmark genes were further classified into 11 categories or pathways: deregulated metabolism, evasion of anti-growth signaling, angiogenesis, immune system evasion, resistance to cell death, replicative immortality, sustained proliferative signaling, tissue invasion and metastasis, tumor-promoting inflammation, the tumor microenvironment, and genome instability. Comparative analysis was conducted using all available primary cancer tissue and CTC samples.

Statistical analysis

The primary objective of this study was to compare mutations between primary cancer tissue and CTCs based on sequencing profiles in early-stage NSCLC. The secondary objective was to examine the classification and roles of mutations between these samples. Categorical variables were reported as numbers and percentages, while continuous variables were expressed as medians with interquartile ranges (IQRs) and compared using the Student's *t*-test. Discrete variables were analyzed using the chi-square method. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

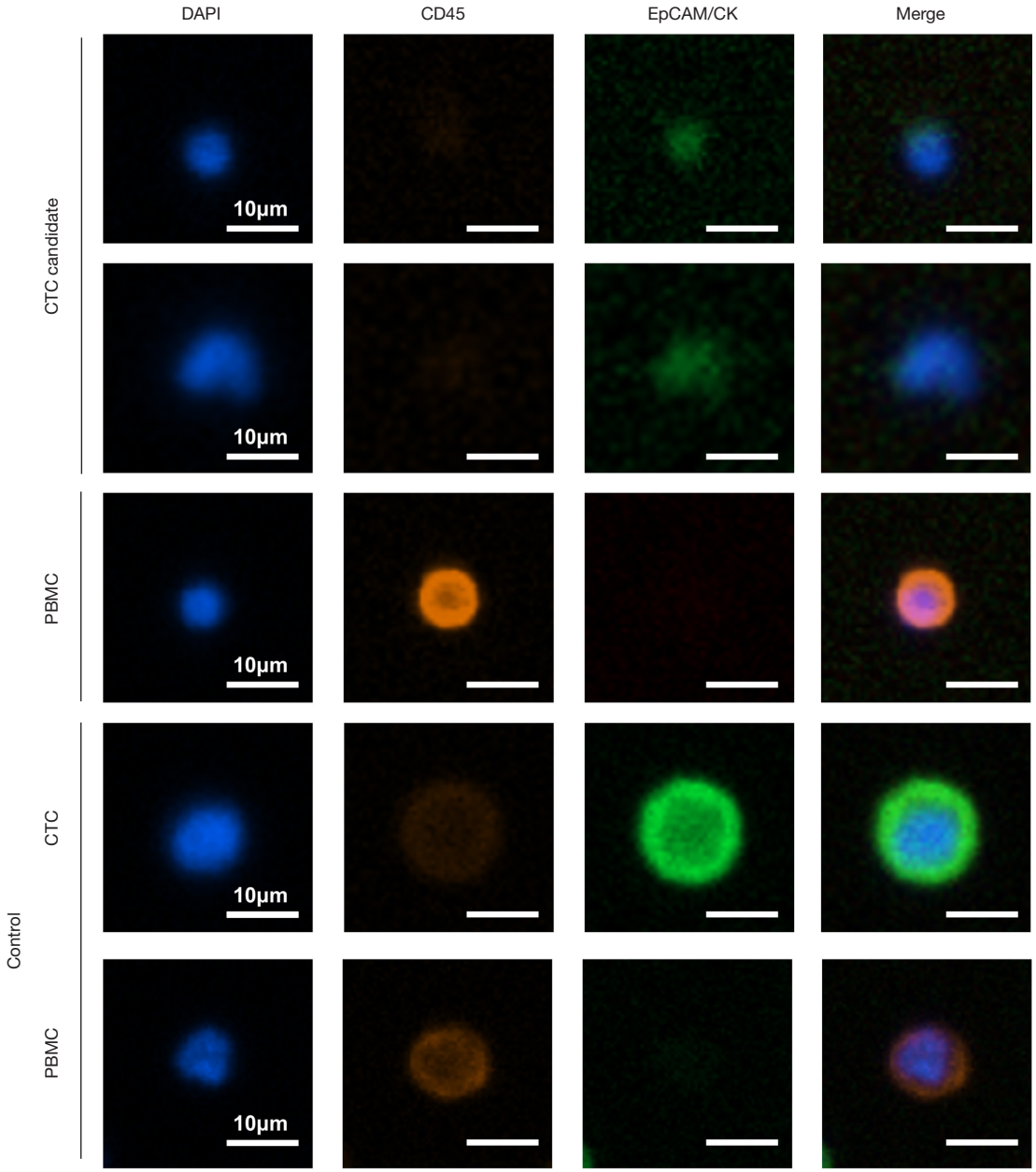

Figure 1 Immunofluorescence staining image of a CTC. CTCs were identified as DAPI-positive and CD45-negative ($DAPI^+/CD45^-$) and were either doubly or singly positive for anti-EpCAM and CK, resulting in a profile of $EpCAM^+/CK^+$, $EpCAM^+/CK^-$, or $EpCAM^-/CK^+$. PBMCs were characterized as $DAPI^+$, $EpCAM^-/CK^-/CD45^-$. The staining image from an enrolled patient's sample is depicted from the first to the third line of the image, while the fourth and fifth lines of the figure display control images for CTCs and PBMCs, respectively. Scale bar in each figure represents 10 μm. CTC, circulating tumor cell; DAPI, 4',6-diamidino-2-phenylindole; EpCAM, epithelial cell adhesion molecule; CK, cytokeratin; PBMCs, peripheral blood mononuclear cells.

Table 1 Pathologic status of patients

Characteristics	Values (n=45), n (%)
Histology	
Adenocarcinoma	37 (82.2)
Squamous cell carcinoma	8 (17.8)
Pathologic stage	
I	30 (66.7)
II	6 (13.3)
III	9 (20.0)
Lymph node metastasis	9 (20.0)
Visceral pleural invasion	15 (33.3)
Parietal pleural invasion	3 (6.7)
Vascular invasion	5 (11.1)
Lymphatic invasion	11 (24.4)
Perineural invasion	0
Tumor spread through air spaces	25 (55.6)
Necrosis	18 (40.0)

Results

Baseline and pathologic characteristics

The mean age of the 45 patients was 68.7±8.4 years, and 26 (57.8%) were male. Additional baseline and preoperative characteristics are detailed in Table S1. Of the patients, 37 (82.2%) were diagnosed with adenocarcinoma and 8 (17.8%) with squamous cell carcinoma. According to the American Joint Committee on Cancer, eighth edition, the pathologic stages were as follows: stage I in 30 patients (66.7%), stage II in 6 patients (13.3%), and stage III in nine patients (20.0%), as shown in *Table 1*.

Panel sequencing data

Positive CTCs were identified by immunofluorescence imaging in 11 patients (24.4%), with a median count of 4.0 CTCs (IQR, 1.5–7.5). No clinical or pathologic factors were found to be associated with CTC positivity. Mutations were detected in all 45 primary cancer tissue samples (100%) and in 31 CTC samples (68.9%) (*Figure 2*). Regarding these mutations, a partial concordance rate of 17.8% was found between matched primary cancer tissue and CTCs.

Figure 3 presents the top 10 mutated genes in primary cancer tissues and CTCs. The most frequently mutated gene in primary cancer tissue was *EGFR* (n=17, 37.8%), followed by *TP53* (n=12, 26.7%). In contrast, *MSH6* mutations were found in all CTCs (n=31, 100%), with *FANCE* mutations being the second most prevalent among these cells (n=28, 90.3%). The mutation profiles of these top 10 genes in CTCs differed significantly from those in primary cancer tissues, with higher percentages for the mutations within CTCs.

Table 2 presents the top 10 genes for primary cancer tissue and CTCs, categorized by pathologic stage and histologic type. In stage 1 disease, the most frequently mutated genes in primary cancer tissue were *EGFR* (n=14, 46.7%) and *TP53* (n=6, 20.0%). In contrast, in stage 2 or higher, *TP53* (n=6, 40.0%) and *KRAS* (n=4, 26.7%) were the most commonly mutated genes identified through panel sequencing. When primary cancer tissue was divided by histologic type, *EGFR* (n=17, 45.9%) and *TP53* (n=8, 21.6%) were the predominant mutated genes in adenocarcinoma, while *TP53* (n=4, 50.0%) and *NF1* (n=3, 37.5%) were the most frequent in squamous cell carcinoma. For CTCs, *MSH6* was the most commonly mutated gene, followed by *FANCE*, irrespective of stage or histologic type. The number of mutations in primary cancer tissue was higher in patients with a pathologic stage of 2 or above relative to stage 1 (P=0.008). However, the number of CTCs detected on immunofluorescent imaging and the mutation count in CTCs did not exhibit statistically significant differences (P=0.84 and P=0.56, respectively, *Table 3*). Additionally, no significant difference in the number of mutations was found in either sample across histologic types (*Table 4*).

Roles of mutated genes

Figure 4 presents the confirmed mutational profiles of genes in primary cancer tissue and CTCs, reflecting the roles of each gene. Primary cancer tissue exhibited mutations in both oncogenes, like growth factor receptors, and tumor suppressor genes, such as gatekeepers. In contrast, CTCs predominantly displayed mutations in tumor suppressor genes, including caretakers and gatekeepers, rather than oncogenes.

The hallmark pathways of cancer for each sample are depicted in *Figure 5A,5B*, while the proportional differences between the two samples are illustrated in *Figure 5C*. The hallmark pathways of the primary cancer tissue were characterized by sustained proliferation, resistance to cell

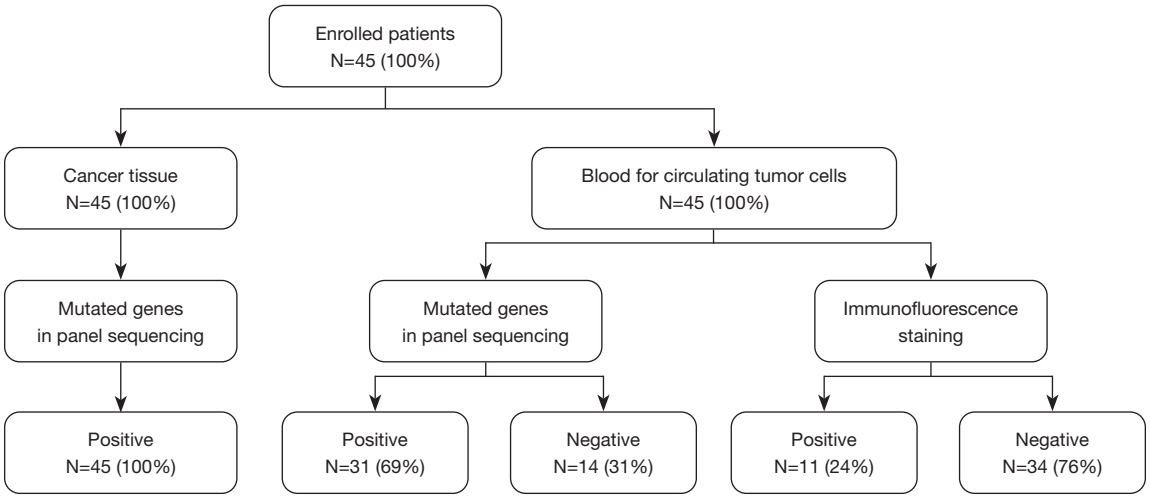

Figure 2 The number of enrolled patients and the positivity rates for targeted panel sequencing of mutated genes in samples and immunofluorescent imaging of circulating tumor cells from each procedure. Mutations in cancer tissue genes were detected in all enrolled patients, whereas mutations in circulating tumor cells were identified in 31 patients through panel sequencing. Immunofluorescent imaging confirmed the presence of circulating tumor cells in 11 patients.

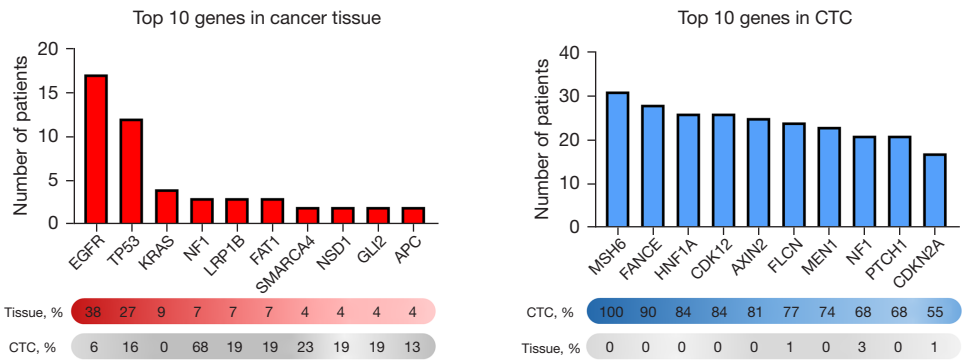

Figure 3 The top 10 mutated genes in cancer tissue and circulating tumor cells, with a comparison of the number of samples in each category derived from targeted panel sequencing. CTC, circulating tumor cell.

death, and altered metabolism. In comparison, CTCs exhibited additional hallmark pathways, such as evasion of anti-growth signaling, replicative immortality, and invasion/metastasis.

Discussion

In this study, the concordance rate of gene mutation profiles between CTCs and the corresponding primary cancer tissue was relatively low in patients with early-stage NSCLC. The classifications and roles of the mutated genes were markedly different between the two sample types. These findings suggest that the mutational forces acting on CTC genomes may differ from those affecting primary cancer tissues. Given the scarcity of studies directly comparing primary cancer tissue and CTCs over 50 matched cases using panel sequencing, our study is meaningful. It represents an analysis of mutations in CTCs and primary cancer tissues, with panel sequencing performed for 45 matched cases of early-stage NSCLC.

Our findings align with those of prior research. Ni *et al.* conducted exome sequencing on single-cell CTCs from patients with lung cancer (14). Their findings revealed that single CTC harbored a greater number of mutations than primary cancer tissue, and the mutations in CTCs more closely resembled those in metastatic tumors than those in

Table 2 Differences in mutation profiles by stage and histology

Rank	Stage				Histology			
	Stage 1 (n=30)		Stage >1 (n=15)		ADC (n=37)		SqCC (n=8)	
	Genes	Number	Genes	Number	Genes	Number	Genes	Number
Primary cancer tissue								
1	*EGFR*	14	*TP53*	6	*EGFR*	17	*TP53*	4
2	*TP53*	6	*KRAS*	4	*TP53*	8	*NF1*	3
3	*NCOR1*	2	*LRP1B*	3	*KRAS*	4	*NSD1*	2
4	*NF1*	2	*EGFR*	3	*LRP1B*	2	*NOTCH3*	2
5	*BRAF*	2	*RB1*	2	*RB1*	2	*RB1*	1
6	*KMT2D*	1	*PTPRD*	2	*PTPRD*	2	*PIK3CA*	1
7	*NSD1*	1	*NOTCH2*	2	*FAT1*	2	*LRP1B*	1
8	*RB1*	1	*SMARCA4*	2	*GLI2*	2	*KMT2D*	1
9	*PIK3CA*	1	*FAT1*	2	*SMARCA4*	2	*CHEK2*	1
10	*NOTCH3*	1	*PIK3CA*	1	*APC*	2	*FBXW7*	1
11	*NTRK3*	1	*NF1*	1	*CREBBP*	2	*PAK5*	1
Circulating tumor cells								
1	*MSH6*	18	*MSH6*	13	*MSH6*	26	*MSH6*	5
2	*FANCE*	16	*FANCE*	12	*FANCE*	23	*FANCE*	5
3	*CDK12*	16	*HNF1A*	12	*CDK12*	22	*HNF1A*	5
4	*AXIN2*	15	*FLCN*	11	*HNF1A*	21	*FLCN*	5
5	*HNF1A*	14	*CDK12*	10	*AXIN2*	21	*CDK12*	4
6	*FLCN*	13	*AXIN2*	10	*FLCN*	19	*AXIN2*	4
7	*MEN1*	13	*MEN1*	10	*MEN1*	19	*MEN1*	4
8	*NF1*	13	*NF1*	8	*NF1*	19	*CDKN2A*	3
9	*PTCH1*	13	*PTCH1*	8	*PTCH1*	19	*NF1*	2
10	*CDKN2A*	11	*CDKN2A*	6	*CDKN2A*	14	*PTCH1*	2
11	*TERT*	6	*TERT*	5	*TERT*	9	*TERT*	2

ADC, adenocarcinoma; SqCC, squamous cell carcinoma.

Table 3 Numbers of CTCs on immunofluorescent imaging and mutated genes in each sample, categorized by stage

Sample	Stage 1 (n=30)	Stage >1 (n=15)	P value
Immunofluorescent imaging of CTCs[†]	5.0 [1.0–8.5]	4.0 [3.0–4.0]	0.84
Mutations of primary cancer tissue	6.0 [4.0–8.0]	11.0 [7.0–14.0]	0.008
Mutations of CTCs	27.0 [20.0–42.0]	28.0 [22.0–43.5]	0.56

Values are presented as median [interquartile range]. [†], the count of CTCs was calculated only for patients (n=11) in whom these cells were detected by immunofluorescent imaging. CTCs, circulating tumor cells.

Table 4 Numbers of CTCs on immunofluorescent imaging and mutated genes in each sample, categorized by histology

Sample	ADC (n=37)	SqCC (n=8)	P value
Immunofluorescent imaging of CTCs[†]	4.0 [1.0–8.0]	0.0 [0.0–0.0]	–
Mutations of primary cancer tissue	7.0 [5.0–8.0]	11.0 [8.5–15.0]	0.17
Mutations of CTCs	28.5 [21.5–45.0]	23.0 [20.5–36.5]	0.45

Values are presented as median [interquartile range]. [†], the count of CTCs was calculated only for patients (n=11) in whom these cells were detected by immunofluorescent imaging. CTCs, circulating tumor cells; ADC, adenocarcinoma; SqCC, squamous cell carcinoma.

primary cancer tissue. Yoo *et al.* performed targeted deep sequencing on amplified DNA from CTCs in patients with lung cancer (16), reporting an absence of shared mutations between CTCs and primary cancer tissue. Furthermore, Kim *et al.* undertook whole exome sequencing in 20 patients with primary bladder cancers (17). Their research indicated no correlation in mutation abundance between primary cancer tissue and CTCs, and the concordance level of mutations was low.

Although the present results also indicated a low concordance rate between samples, our study methods require examination to confirm their validity, as the findings may impact future research on resectable NSCLC. First, we desired to ensure that the CTCs originated from the primary cancer. In our study, CTCs were detected in 24% of patients using immunofluorescence imaging, while 69% of patients exhibited mutated genes in CTCs. The mutational positivity of CTCs was relatively high for early-stage NSCLC. This high positivity rate could have resulted from the amplification and sequencing processes. However, our amplification method, REPLI-g, has demonstrated less bias and a higher yield compared to other methods (18). Furthermore, in another study, the positivity rate of CTCs in resectable early-stage NSCLC was reported to be around 60% using imaging methods (10), similar to our research. We believe that amplifying the DNA of CTCs allows for more sensitive detection than immunofluorescence imaging, especially considering the difficulty of detecting small quantities of CTCs in 10 cc of peripheral blood.

Second, we aimed to address some of the limitations associated with mutational studies of CTCs. Wang *et al.* suggested potential reasons for low concordance rates, such as the selection of subcolonies by the CTC isolation method and the reliance on EpCAM-dependent selection techniques (19). To encompass intra-tumor and CTC heterogeneity, we included gene mutations from various CTC colonies rather than from a single CTC. The EpCAM-dependent method tends to isolate only a limited number of CTCs (20), and EpCAM-positive CTCs have been found to be less frequently detected in NSCLC than in other epithelial tumors (21). Consequently, we employed a strategy that utilized multiple cell surface proteins, including EpCAM, EGFR, and HER2, which have been suggested to improve CTC isolation in NSCLC (21). We believe that this approach enables more specific detection of various CTCs, even in early-stage NSCLC, than other techniques. As an alternative, establishing CTC cultures could facilitate the expansion of CTCs to enough numbers for genotyping and phenotyping (17). However, this method may also be flawed if the cultured CTCs do not retain their original characteristics. Based on these considerations, we believe that our approach is reliable for the analysis of CTC mutations.

Multiple genes identified in CTCs were categorized to tumor suppressor genes, including *MSH6*, *FANCE*, *CDK12*, *HNF1A*, and *AXIN2*. Most of the genes are not directly associated with established mutational profiles of NSCLC (22). In contrast, all primary cancer tissue samples exhibited multiple mutations across both oncogenes (*EGFR*, *NOTCH*, *ROS1*, and *KRAS*) and tumor suppressor genes (*TP53*, *FAT1*, *LRP1B*, and *PKHD1*), which are well-known for their association with NSCLC. We applied Liang's concept of "cancer hallmarks" to identify mutations specifically linked to carcinogenesis based on gene function (15). The CTCs showed a higher prevalence of genes involved in categories such as evasion of anti-growth signaling, replicative immortality, and invasion/metastasis. This indicates that primary cancer tissue likely arises from errors in proliferation or the cell cycle, while CTCs are more prone toward metastasis through invasion and replication, even in cases of early-stage NSCLC.

The potential reasons for the heterogeneity in mutational status between the two samples may include the influence of the microenvironment. Factors such as EMT, mesenchymal-to-epithelial transition, blood flow, and immune cell activity could impact the mutation of CTCs in

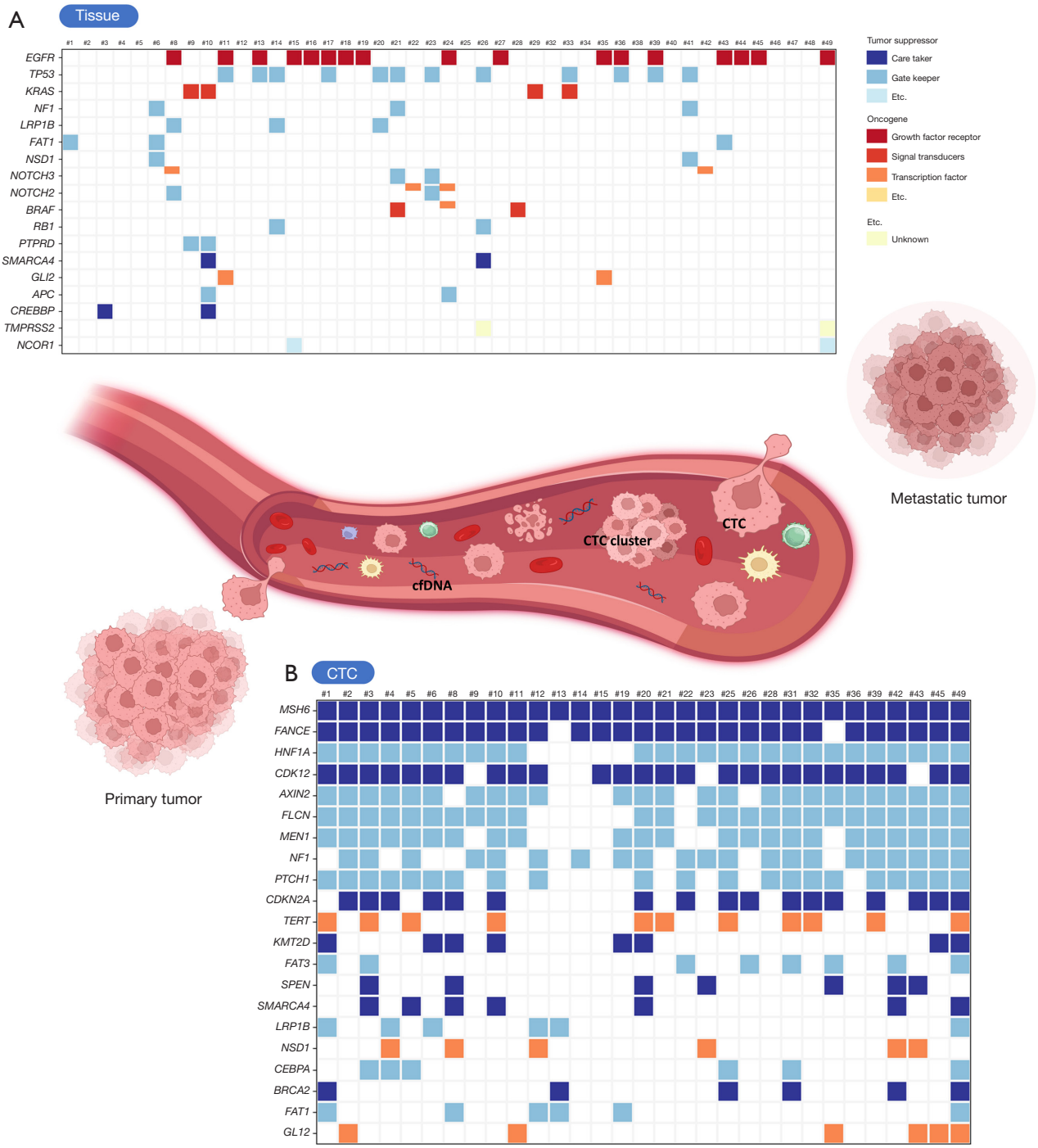

Figure 4 Heatmap image of the genomic profile and functional classification in cancer tissue and circulating tumor cells derived from targeted panel sequencing. CTC, circulating tumor cell.

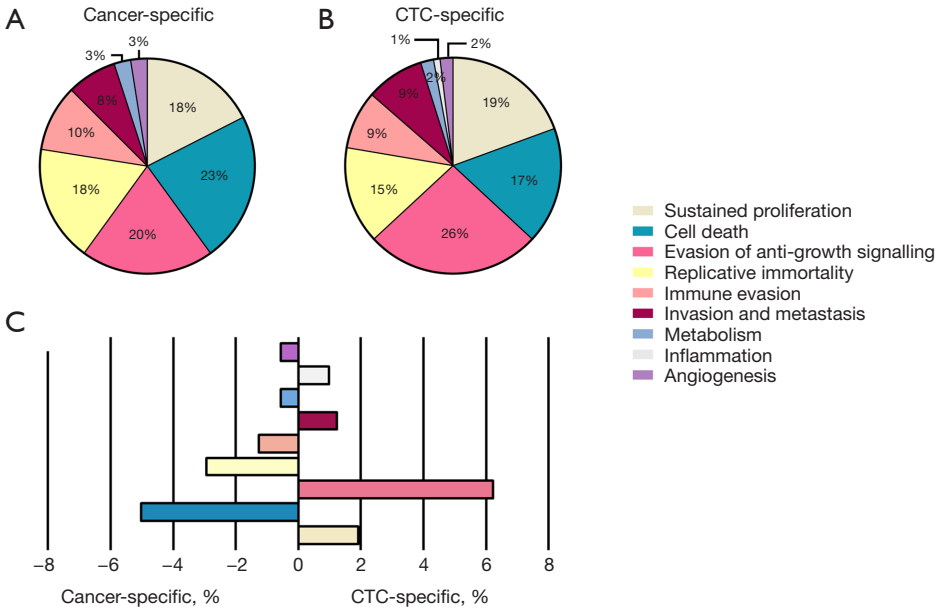

Figure 5 Cancer hallmark pathways based on two samples from targeted panel sequencing. A pie chart depicts the proportion of hallmark pathways specific to cancer tissue (A) and those specific to CTCs (B). Additionally, the proportional difference between the cancer tissue and circulating tumor cells is shown (C). CTC, circulating tumor cell.

a way markedly different from that of the primary tumor. Contrary to expectations, we found no correlation between the numbers or mutations of CTCs and pathologic stage or invasive pathologic factors. In our previous research, the immunophenotypes of captured CTCs exhibited a wide range, even among those at the same stage (23). Similarly, several studies have found that the number of CTCs does not vary significantly by stage in patients with lung cancer (12,24), a phenomenon observed in other types of cancer as well (25). We hypothesize that mutations in CTCs may occur independently of cancer stage or histologic type. These mutations could arise not because of cancer progression, but rather from the progression of the CTCs themselves, as indicated in *Table 2*. This suggests that certain mutations, such as those of *MSH6* and *FANCE*, might be necessary for or associated with the differentiation of cancer cells into CTCs in NSCLC. However, this does not necessarily mean the mutations are critical for actual metastasis, since the CTCs collected using our specific markers did not precisely correspond to EMT. To elucidate the specific relationship between CTC mutations and metastasis, further studies that compare CTCs with actual metastatic tissue are needed.

To investigate which samples were more similar to advanced-stage NSCLC, we compared the mutations found in our study to those reported in advanced-stage NSCLC in other studies. Previous studies have also found *EGFR*, *TP53*, and *KRAS* as common mutations in advanced-stage NSCLC, similar to the mutations in primary cancer tissue in our study (26,27). However, because most prior studies performed panel sequencing that targeted fewer than 50 genes, we thought that it would not be appropriate to directly compare the mutations of advanced-stage NSCLC with those of CTCs in our study. Other studies utilizing panel sequencing with more than 50 genes have reported *CDKN2A*, *PTCH1*, and *FLCN*—which were detected in CTCs in our cohort—as among the top 10 genes (28,29). We hypothesize that advanced-stage NSCLC might have the characteristics of both primary cancer tissue and CTCs in early-stage NSCLC, and expect that it will be possible to detect more gene overlap between CTCs and advanced-stage NSCLC if the same 405-gene panel sequencing is used.

From a clinical perspective, we suggest that conducting a mutational analysis of CTCs can be useful at the time of preoperative evaluation. Patients with NSCLC who test positive for mutations observed in CTCs should be monitored for recurrence more closely during follow-up. If targetable mutations, such as *EGFR*, are detected in CTC samples, we should consider adjuvant systemic therapy after surgical resection. We can then determine

whether to maintain or discontinue adjuvant therapy by conducting follow-up mutational analyses of CTCs. Several studies have suggested that targeted panel sequencing for the initial assessment of advanced-stage NSCLC could be cost-effective (30,31); however, the cost-effectiveness of mutational analysis of CTCs using panel sequencing in patients with early-stage NSCLC is doubtful.

Limitations

This study has several limitations. First, the small number of patients enrolled may not adequately represent the characteristics of primary NSCLC. Second, the follow-up duration for enrolled patients was too brief to establish an association between CTC mutation or positivity and cancer recurrence or survival. We plan to continue monitoring this cohort to understand long-term outcomes. Third, although the mutation profiles differed between the two samples as indicated in previous studies, we worried about differences between the concrete mutation profile of CTCs in this study and prior results. We are confident that the mutation profile of CTCs originated from the actual CTCs in the bloodstream and that their profile differed from that of the primary cancer tissue. However, due to the relatively early stage of the cohort in this study compared to the advanced or metastatic cohorts in previous research, minor mutations were detected more sensitively than the dominant mutations.

Conclusions

The mutational profiles of CTC DNA differed significantly from those of primary cancer tissue in early-stage NSCLC. Mutations in CTCs occurred in specific genes with high frequency and were associated with cancer metastasis, irrespective of stage or histological type. These findings contribute to a deeper understanding of CTCs in patients with early-stage NSCLC, however, further research with more cases is required.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-709/rc

Data Sharing Statement: Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-709/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-709/coif). Jinsu Lee, Soojeong Ji and H.S. are from BeyondDx Inc. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (approval No. B-2211-791-304) and informed consent was obtained from all individual participants.

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