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A retrospective single-centered, comprehensive targeted genetic sequencing analysis of prognostic survival using tissues from Korean patients with metastatic renal cell carcinoma after targeted therapy

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Purpose: To identify candidate gene mutations to significantly predict the risk of survival prognosis after treatment with systemic first-line targeted therapy (TT) in metastatic renal cell carcinoma (mRCC) patients.

Materials and Methods: Between 2005 and 2017, 168 triplet-tissue block samples from 56 mRCC patients were selected for targeted gene sequencing (TGS). Fifty-six patients' medical records including overall survival (OS) and progression-free survival (PFS) at the time of mRCC diagnosis were evaluated. The patients were grouped into favorable (>12 months/>3 years), intermediate (3–12/12–36 months), and poor groups according to their PFS/OS (<3 months/<12 months). We identified any significant therapeutic targeted genes relating to the survival with a significance at p<0.050.

Results: The first line therapeutic response showed 1.8% complete remission, 14.2% partial response, 42.9% stable disease, and 41.1% progressive disease. Among the overall TGS results, the cumulative effect of *CDH1*, and/or *PTK2* genes significantly reflected the therapeutic responses in terms of PFS/OS; *CDH1* and *PTK2* mutations were associated with poor prognostic outcomes (p<0.050). Among only triplet-quality check passed tissues, the *SGO2*, *BRAF*, *URB1*, and *NEDD1* mutated genes significantly correlated with OS. Regarding metastasis, patients with liver metastasis had the worst OS (p=0.050). The combinational mutation number from these two candidate genes in the liver metastatic samples with mutated *EGFR2* and *FABP7* also showed a significantly worse OS than those with other metastatic lesions (p<0.050).

Conclusions: This study reports several significant mutated genes related to the survival prognosis in mRCC patients treated with first-line TT.

Keywords: Cancer mutated gene; Clear-cell metastatic renal cell carcinoma; Overall survivals; Targeted gene sequencing; Targeted therapy

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INTRODUCTION

Renal cell carcinoma (RCC) accounts for 2% to 3% of all cancers worldwide. The incidence of RCC has increased by 2% over the last two decades, with the highest prevalence in Western countries [1]. The prognosis of patients diagnosed with RCC in terms of survival largely depends on multiple factors including tumor, nodes, and metastases (TNM) staging, in which patients with localized RCC have an overall survival (OS) of >90% with adequate therapy. The standard therapy for localized RCC is curative nephrectomy, including partial nephrectomy. However, approximately one third of surgically removed RCCs progress to local recurrence or metastatic RCC (mRCC); one third of patients with RCC have metastases at the time of diagnosis, resulting in a dismal 5-year OS of <20% [1-3].

Advances in genomic technologies have facilitated the development of multiple targeted agents based on tumor microenvironment and genetic profile. These targeted therapy (TT) approaches have resulted in substantial improvements in the survival of patients with mRCC by about 20% to 50% compared with the survival outcomes following conventional cytokine therapy; thus, TTs have now become the standard care for mRCC [2,3]. Various attempts have been made to find optimal markers for predicting survival prognoses and therapeutic responses according to the metastatic organs and their associated prognoses based on genomic profiles [4-8].

Targeted gene sequencing (TGS) of tissue samples is a common analytical tool to identify significant genetic biomarkers related to therapeutic responsiveness and survival prognosis in mRCC and other cancers [7-9]. Furthermore, genetic sequencing of specific metastatic organs has been used to find predictive genetic markers relating to risk of metastasis [10]. Neagu et al. [8] reported targetable signaling pathways in brain metastasis from various cancers, including RCC.

Based on this concept, we aimed to discover potential genetic markers closely related with metastatic sites of RCC. Toward this end, we sequenced 88 genes from the NCC kidney cancer panel to retrospectively analyze matched primary kidney and metastatic tumor tissue samples of patients with mRCC treated with first-line TT. We assessed gene mutations that were significantly associated with metastasis at different organs and their prognostic value with respect to metastatic OS (metOS), defined as the time since diagnosis of metastasis to death, and ability to differentiate RCC metastasis to different sites following treatment.

MATERIALS AND METHODS

1. Patients and clinical parameters

Between January 2005 and December 2017, 66 patients with mRCC were enrolled prospectively and 205 metastatic specimens were collected. After evaluating medical records and pathologist's review, and the exclusion criteria, such as a lack of follow-up history at this institution, absence of paired normal and cancer tissue samples, lack of complete medical records, and disagreement with enrollment in the study for TGS, only 56 (84.8%) patients with mRCC harboring 168 (82.0%) metastasectomy tissue lesions were finally enrolled in the study. The 56 patients' medical records including OS and TT were evaluated with the TGS. Twenty-nine (51.8%) patients received only TT, and 27 (48.2%) patients first received cytokine therapy and then TT.

2. Clinical outcome and risk classification

The OS and progression-free survival (PFS) were defined as the time interval between the diagnosis of metastasis and death/disease progression after failure of first-line TT according to the RECIST criteria v 1.1 at the time of enrollment (https://recist.eortc.org). The patients were grouped into favorable (>12 months/>3 years), intermediate (3–12/12–36 months), and poor groups based on their PFS/OS (<3 months, <12 months) after first-line TT according to the Memorial Sloan Kettering Cancer Center (MSKCC) and Heng/International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) criteria based on our previously published papers using IMDC and MSKCC criteria for mRCC treated with TT [11,12]. The IMDC and MSKCC had some differences in their constituting parameters. Besides the same Karnofsky performance status <80%, time from diagnosis to treatment interval <1 year, anemia, and hypercalcemia, the IMDC had neutrophilia and thrombocytosis, whereas the MSKCC had serum lactate dehydrogenase [11]. The therapeutic response was estimated to group into good response (complete remission [CR]+partial response [PR]) and bad response (stable disease [SD]+progressive disease [PD]) according to the RECIST v1.1. Groups (favorable vs. poor groups and good vs. bad groups) were also compared for the survival prognoses and therapeutic responses in terms of their mutated genes.

The follow-up protocol and patient evaluation, which included laboratory and imaging evaluations, were conducted as previously described [9]. The choice of first-line targeted agent and its regimen was at the discretion of the treating urologist according to each patient's pathology and coverage by the National Health Insurance System. First-line TT comprised sunitinib, sorafenib, pazopanib, or temsirolimus;

all TTs were administered orally or intravenously according to the regimen recommended by the international European Association of Urology and National Comprehensive Cancer Network guidelines (available at: www.nccn.org/patients for patients) [2,3].

3. Analysis of targeted gene sequencing data

All the tissue samples from the 56 mRCC patients' 168 tissue blocks were obtained from either nephrectomized, metastisectomized, or biopsied tissue samples and subjected to TGS having 88 targeted genes after distinctively categorized the tissue samples from the obtained organ lesions. Those tumor and normal samples from each mRCC tissue block were marked and obtained triplet tumor specimens by a 20-years experienced uro-pathologist (W.S.P.) including triplet tumor samples and one paired adjacent normal sample after passed their quality checks for TGS. Of the 56 patients, 18 (32.1%) patients had all triplet blocks passed for quality check, whereas 21 (37.5%) and 17 (30.4%) patients had only two or one tissue block passed (Supplementary Fig. 1).

In total, 88 targeted genes were selected from the National Cancer Center (NCC) Kidney Cancer Panel (Supplementary Table 1) [9]. We sequenced and analyzed the TGS of the kidney cancer panel sequencing data using our custom pipeline. Sequencing data were aligned onto the human reference genome (GRCh 38); somatic point mutations were then detected by MuTect2, and gene annotation was performed by Oncotator [10]. To establish a normal reference panel, we aggregated and used following databases: Korean whole-exome sequencing study (KoEX) [13], Korean Genome Project [14], 1000 genomes project, The Exome Aggregation Consortium (ExAC) [15], and the genome aggregation database (gnomAD) [16]. We identified any significant therapeutic targeted genes associated with the therapeutic responsiveness to TT after comparing mutational burdens between all positive for three blocks and one or two positive blocks with a significance of p-value<0.050. Those significant genes found in the mutational analysis were defined when the genes were identified positive from each of the doublet and triplet specimens. Therapeutic targetable genes were selected by a custom script.

4. Statistical analysis

Baseline characteristics of intermediate-risk patients according to the prognostic mRCC criteria based on the MSKCC/IMDC criteria are expressed as frequency with percentage for categorical variables and median with range or mean with standard deviation for continuous variables. The bioinformatics analyses of the TGS results were performed by two medical bioinformaticians (J.P. and D.H.) in 2019 after completion of survival follow-up of the 56 patients. The spreading metastatic patterns and survival curves among the 32 patients' either two or three different metastatic organs were analyzed. In addition, we also used the quality-passed singlet normal-tumor paired TGS expression data for validating those found candidate genes whether those genes were still significant in the rest of singlet tumor samples. The Kaplan–Meier method was used to compute probabilities of survival and the log-rank test was used to compare survival curves according to metastatic organs. Genes with the strongest predictive potential for OS among the 88 genes in the panel were then screened out. In all statistical analyses, $p<0.050$ was considered to represent a statistically significant effect; all analyses were performed using R Foundation for Statistical Computing (version 3.6.2, R Development Core Team, 2013; http://www.r-project.org).

5. Ethics statement

This study was approved by the Institutional Review Board of the National Cancer Center (approval no. NCC 2017-0045, 2021-0147). Patients' written informed consent was obtained prospectively. The enrolled patients' medical records were also obtained a prospectively registered RCC registry database of the institution. This study was conducted in accordance with the Declaration of Helsinki.

RESULTS

1. Overall patients' clinicopathological characteristics

The female-to-male gender ratio, mean age, and body mass index (BMI) among the 56 patients were 13:43, 51.4±9.86 years, and 23.58±2.47 kg/cm^2, respectively. The median PFS and OS for first-line TT were 8.7 and 42.0 months, respectively. The metastatic type was 31 synchronous and 25 metachronous mRCC. Forty-four patients underwent nephrectomy including 19 that underwent cytoreductive nephrectomy with metastasectomy. Sixteen, ten, and twenty-seven patients underwent additional metastasectomy, radiation therapy, and cytokine therapy, respectively. The clinical T stage comprised of T1-2 69.6%, T3-4 25.0%, Tx 5.4%, and N stage of 26.8% of N1. The histopathology showed clear cell 50.0%, non-clear cell 1.8%, and mixed 48.2%. MSKCC and Heng risk criteria showed 28.6%/66.1%/5.3% and 26.8%/57.1%/16.1% for favorable, intermediate, and poor risk groups, respectively. The first line therapeutic response showed 1.8% CR, 14.2% PR, 42.9% SD, and 41.1% PD (Table 1). The rest of the information was presented in Table 1 including the median disease-

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Table 1. The patients' overall baseline characteristics table (n=56)

Characteristic	Total
Sex (female:male)	13:43 (23.2:76.8)
Age (y)	51.41±9.86
Body mass index (kg/m^2)	23.58±2.47
Metastatic type	
Synchronous	31 (55.4)
Metachronous	25 (44.6)
Purpose of nephrectomy	
Curative radical nephrectomy without metasectomy	25 (44.6)
Cytoreductive nephrectomy with metasectomy	19 (33.9)
Metasectomy	16 (28.6)
Radiation therapy	10 (17.9)
Anticancer drug (cytokine: TKIs)	27:29 (48.2:51.8)
Clinical tumor (tumor, nodes, and metastases)	
cT1-2	39 (69.6)
cT3-4	14 (25.0)
cTx	3 (5.4)
cN0	15 (26.8)
cN1	15 (26.8)
cNx	26 (46.4)
cM1	22 (39.3)
cMx	26 (46.4)
Memorial Sloan Kettering Cancer Center	
Favorable	16 (28.6)
Intermediate	37 (66.1)
Poor	3 (5.3)
Heng/IMDC	
Favorable	15 (26.8)
Intermediate	32 (57.1)
Poor	9 (16.1)
Therapeutic response	
Complete remission	1 (1.8)
Partial response	8 (14.2)
Stable disease	24 (42.9)
Progressive disease	23 (41.1)
Disease-free interval (mo)	14 (1–179)
PFS from targeted therapy (mo)	5 (1–60)
OS from RCC diagnosis (mo)	64 (4–211)
OS from metastasis diagnosis (mo)	22 (3–164)

Values are presented as number (%), mean±standard deviation, or median (range).
TKI, tyrosine kinase inhibitor; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium; PFS, progression-free survival; OS, overall survival; RCC, renal cell carcinoma.

free interval time, PFS, OS, and metOS of patients in the liver, bone, and other metastatic groups. Survival was the lowest for patients with liver metastases in all categories.

2. Correlation between OS and mutations in mRCC patients of triplet and doublet-passed tissues

The 39 patients' triplet and doublet-passed tissues were evaluated to show that *BCR* (n=39), *KMT2C* (n=39), *TFE3* (n=37), *ARID1A* (n=32), and *TFEB* (n=27) were the most frequently mutated genes. Among them, the presence of mutated *BRAF* (Log-rank p<0.001, hazard ratio [HR]: 4.259), *NEDD1* (Log-rank p=0.019, HR: 2.429), *SGO2* (Log-rank p=0.013, HR: 4.298), and *URB1* (Log-rank p=0.002, HR: 3.485) genes had worse OS. The combinational stratification of these four genes showed the single mutated gene had worse OS (single gene mutation HR: 2.765, double gene mutation HR: 7.803) than absence of the four mutated genes in mRCC patients (Fig. 1).

The *BCR*, *KMT2C*, *TFE3*, *A RID1A*, *TFEB*, *BRAF*, *NEDD1*, *SGO2*, and *URB1* genes were analyzed for their relationship with the OS according to the age. The patients were divided by the mean age (51.4±9.86). However, two aged-groups of either lower or higher than the mean age showed insignificant differences of gene expressions (p>0.050).

3. Discovery of genes associated with OS of first-line therapeutic response in mRCC

We showed a grouping of therapeutic responses based on RECIST criteria v1.1 and identified relapse according to mutations of targeted genes (Table 2 and Supplementary Fig. 2).

Groups (favorable vs. poor and good vs. bad) were compared in terms of their mutated genes. First, comparative analysis of mutated genes between two extreme survival groups, the poor (n=5 for PFS and n=7 for OS) and favorable (n=14 for PFS and n=20 for OS) groups, showed the presence of either *CDH1* (HR: 4.217 for PFS and HR: 3.355 for OS) or *PTK2* (HR: 3.458 for PFS), which significantly differentiated the PFS and OS in patients who received first-line TT (log-rank test: p<0.050). As for the 27 patients who first received cytokine therapy followed by first-line TT, only the presence of *ATK3* mutations (HR: 8.67 for PFS) significantly differentiated PFS and OS (log-rank test p<0.050; Supplementary Fig. 3). Lastly, analysis of the combinational mutation of *CDH1*, and/or *PTK2* on PFS and OS indicated that combinations of mutations could also be used to significantly differentiate PFS and OS (p<0.050; Fig. 2). Accumulation of all five mutated genes had HR 15.555 compared to that of either one or none of the mutated genes (p<0.050). Particularly, the combined *PTK2* and *CDH1* mutated genes had worse HR (11.992 and 8.944, respectively) for OS than none and one mutated gene, respectively (p<0.050; Fig. 3). Therefore, *CDH1* and *PTK2* mutated genes were significant therapeutic responsive genes with worse prognostic outcomes for first-line TT.

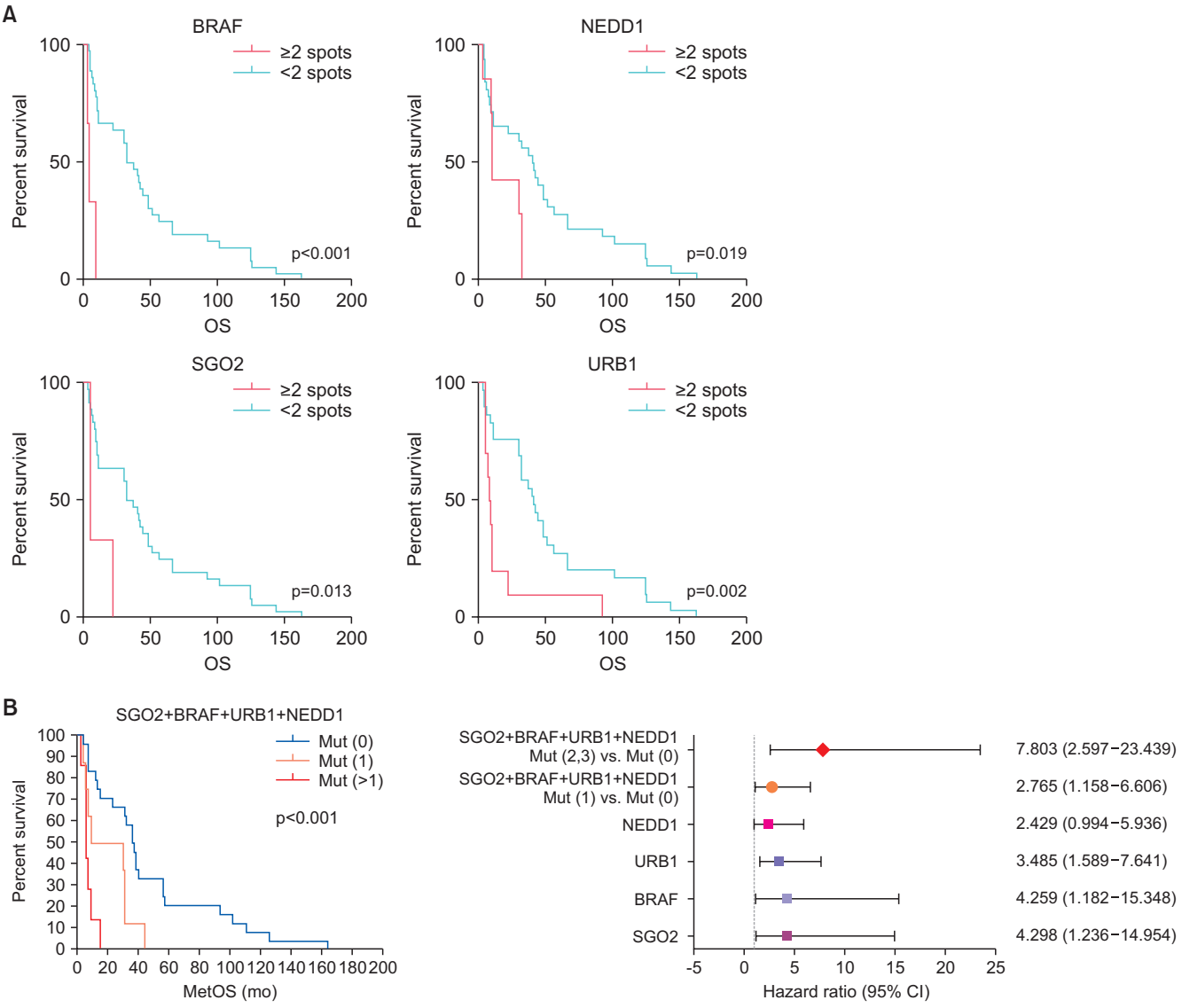

Fig. 1. Kaplan–Meier overall survival (OS) curves according to the presence of each four mutated genes and combined four mutated genes. (A) OS curve (group ≥2 spots and others) of each gene BRAF, NEDD1, SGO2 and URB1. (B) OS curve of combined four genes (Number of mutations: 0, 1, and ≥1) and 95% confidence intervals (CIs) of four genes and combined mutated genes. Mut, mutation; MetOS, metastatic OS.

4. Identification of association between mutated genes and metastatic sites in mRCC

In total, 32 patients were evaluated based on their metastatic tissue samples. The median age, BMI, and sex ratios of these patients were 60.5 (56–65) years, 45.6 (19.4–29.3) kg/m^2, and 5/27 (15.6%/74.4%), respectively. There were 6, 9, and 17 cases of liver, bone, and other metastases (12 lung, 3 lymph node, 1 brain, and 1 skin metastatic lesions), respectively (Table 2, Supplementary Fig. 4). The patients were equally distributed across clinical TNM stages, except for few patients at stage cT4 (Table 2).

Overall, patients with liver metastasis had the worst OS (p=0.050). Among the 88 targeted genes, *EGFR* (log-rank test p=0.008; Fig. 4A) and *FABP7* (log-rank test p=0.023; Fig. 4B) could significantly differentiate metOS among different metastatic lesions. The combined mutation number from these two candidate genes in the liver metastatic samples also showed a significantly worse OS than those with other metastatic lesions (log-rank test p=0.006, HR: 5.11; Fig. 4C). Hazard ratio (confidence interval) of EGFR, FABP and combined EGFR and FABP7 among metastasis sites is 14.300 (1.481–138.100), 5.384 (1.202–24.100), and 5.114 (1.277–24.400) (Fig. 4D). *EGFR* and *FABP7* mutations are, therefore, candidate markers to significantly predict metOS in patients with liver metastasis from mRCC, further highlighting potential treatment targets.

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Table 2. Baseline characteristics in patients of triplet and doublet-passed tissues (n=32)

Characteristic	Liver metastasis (n=6)	Bone metastasis (n=9)	Other metastasis (n=17)
Sex (female/male)	1/5	1/8	3/14
Age (y)	53.17±13.03	53.00±8.38	54.82±10.75
Body mass index (kg/m^2)	21.74±3.17	23.41±2.82	23.73±2.68
Tumor (tumor, nodes) staging			
cT1-2	1	7	11
cT3-4	5	2	5
cTx	2	1	6
cN0	2	2	6
cN1	2	3	3
cNx	3	5	10
Disease-free interval (mo)	2 (0–9)	3 (0–76)	4 (0–87)
Progression-free survival (mo)	2 (0–9)	8 (0–60)	3 (0–36)
OS (mo)	11 (6–31)	33 (6–144)	38 (5–163)
Metastatic OS (mo)	8 (6–24)	32 (6–111)	20 (3–164)

Values are presented as mean±standard deviation or median (range).
OS, overall survival.

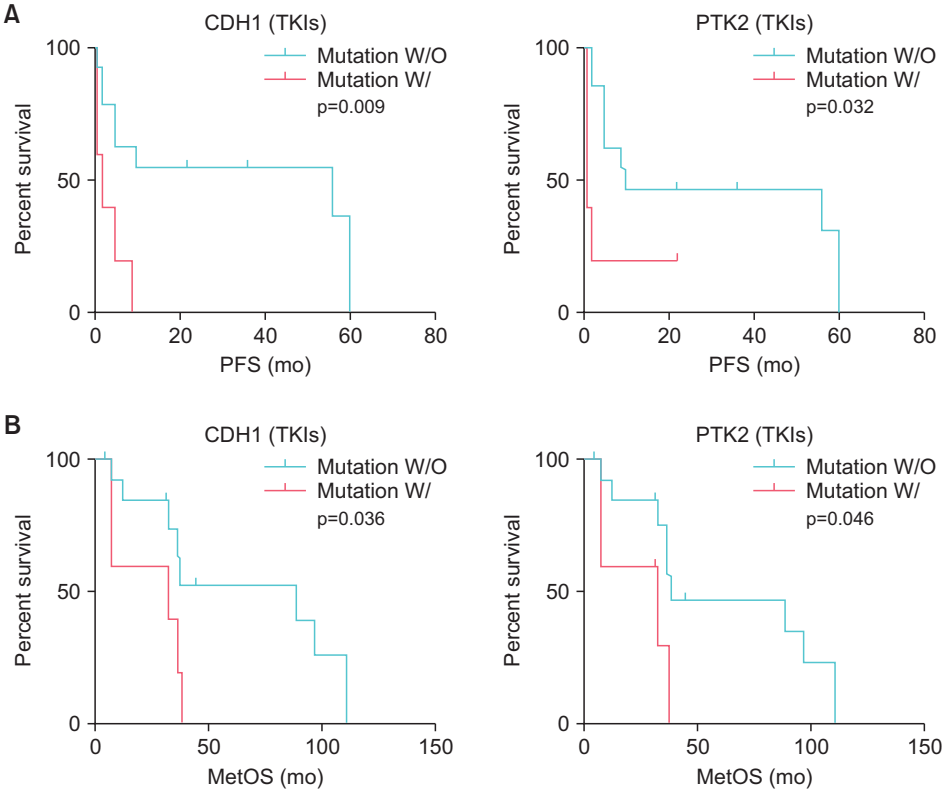

Fig. 2. The Comparison of progression-free survival (PFS) and overall survival (OS) curves according to the presence of either *CDH1* and *PTK2* gene mutation in patients with metastatic renal cell carcinoma treated with targeted therapy. (A) PFS curves of each two genes. Blue line shows no mutation group and red line shows mutated group. p-values are CDH1: 0.009 and PTK2: 0.032. (B) OS curves of two genes. Blue line shows no mutation group and red line shows mutated group. p-values are CDH1: 0.036 and PTK2: 0.046. W/, with; W/O, without; TKI, tyrosine kinase inhibitor; MetOS, metastatic OS.

DISCUSSION

RCC is a heterotropic, heterogenic cancer characterized by histological and molecular heterogeneity, posing a substantial problem for clinical management [17]. Intratumor heterogeneity may foster tumor adaptation and therapeutic failure with variable responses to TT [18]. Diverse therapeutic and diagnostic concepts have been developed to overcome the heterogeneity of mRCC using genetic sequencing approaches, including single-cell RNA sequencing to optimize a combinatorial therapeutic strategy [19], TGS with pan-cancer panel [20], and genomically annotated risk model and classification [21].

Advances in genomic sequencing technique have played pivotal role in improving the survival prognosis of patients with mRCC by developing TT. Recently, the introduction of

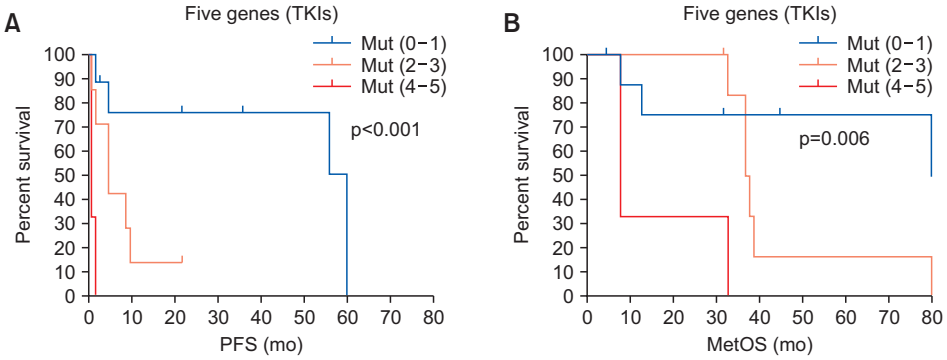

Fig. 3. Progression-free survival (PFS) (A) and overall survival (OS) (B) curves according to the combinations of the presence of these *CDH1*, *PTK2*, *FABP7*, *MAPK14*, *STAG2* gene mutations. Blue lines are survival curves of a group having number of mutations from 0 to 1, orange lines are survival curves of a group having number of mutations from 2 to 3 and red lines are survival curves of a group having number of mutations from 4 to 5. A p-value of PFS and OS is <0.001 and 0.006, respectively. Mut, mutation.

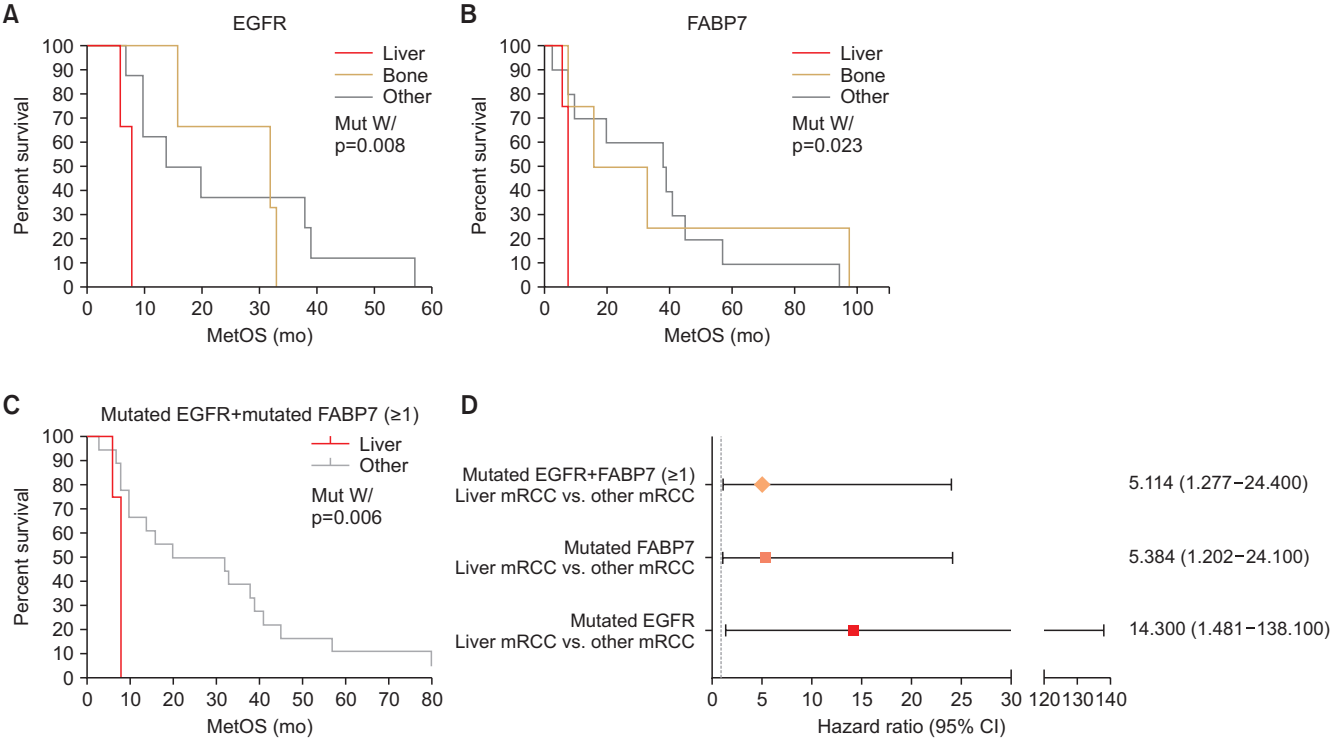

Fig. 4. Correlation between metastatic sites and hazard ratio having 95% confidence intervals (CIs) of *EGFR* and *FABP7* gene mutation with metastatic renal cell carcinoma (mRCC). (A) Overall survival (OS) curves of patients having EGFR gene mutations on metastatic sites (liver, bone, and others). A p-value is 0.008. (B) OS curves of patients having FABP7 gene mutations on metastatic sites (liver, bone, and others). A p-value is 0.023. (C) OS curves of patients having EGFR as well as FABP7 gene mutations on metastatic sites (liver, bone, and others). A p-value is 0.006. (D) A hazard ratio (CI) of EGFR gene mutations, FABP7 gene mutations, and EGFR+FABP7 genes mutations between liver and other metastasis is 14.300 (1.481–138.100), 5.384 (1.202–24.100), and 5.114 (1.277–24.400). Mut, mutation; W/, with; MetOS, metastatic OS.

PDL1/PD1/CTLA4-based immune-checkpoint inhibitors has opened a new therapeutic era for mRCC. However, there still remains a high frequency of therapeutic resistance to TT and immune checkpoint inhibitors; no diagnostic guidelines have been established for enabling the best genomic analyses of mRCC from tissue biopsies to guide these treatments in a clinical setting [2,3]. Our research team has already shown the limitations of the current single biopsy-based diagnosis method for mRCC due to the intratumoral and intertumoral heterogeneity of mRCC [9], in accordance with previous studies [22,23]. In this study, triplet primary-matched-metastatic tumor samples showed a 37.5% success rate in the quality check from 1,168 blocks [1]. Additionally, only 30.4% had doublet cancer samples pass the quality check, resulting in a total of 67.9% passed quality checked samples.

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A new concept for sampling and analysis has been introduced which uses multiple primary and metastatic tumor sampling methods with "cocktail concepts," mixing together samples from blood and tissues to identify biomarkers [24].

Beside intratumoral heterogeneity and sampling quality check, the results of this study have elucidated potential prognostic indicators among the 88 cancer genes. Genetic analysis of samples that passed the quality checks enabled us to identify genetic mutations significantly related to survival prognosis [25]. For the 67.9% of tissue samples passed for either doublet or triplet paired samples, the significant genes were genes associated with cancer development: *BRAF*, *NEDD4*, *SGO2*, and *URB1* for OS ($p<0.05$). Conversely, the entire set of tissues including any singlet, quality passed matched-tissues from 168 tissues indicated the *CDH1*, and/or *PTK2* were significantly related to the PFS and OS. In particular, *PTK2* mutations were significantly related to therapeutic response to first-line TT according to the responsive groups from RECIST criteria ($p<0.05$). Moreover, the endothelium-associated *ATK3*, which inhibits vascular tumor growth [26], was the only significant gene identified from tissues of 32 patients with a prior history of cytokine therapy. We have, thus, demonstrated that candidate genes for indicating prognosis vary depending on a patient's therapeutic background, the specific type of tissue sample, and the number of quality-passed multiregional samples with intra- and intertumoral heterogeneity. These factors should be used to inform further large-scaled studies to validate these candidate genes and identify candidate genes which can indicate prognosis regardless of sample quality and biopsy location. The genes identified in this study for mRCC may act as potential new therapeutic targets; indeed, some of these genes have already been identified as therapeutic targets in other cancers [25,27].

We further evaluated the association of gene mutations relating to the metastatic survival prognosis including metastatic sites and lesions. The metastatic tissues were used for TGS to evaluate any potential mutated genes relating to PFS/OS. Tissue samples from 32 patients were genetically sequenced, and the TGS reports showed that the combined mutation number from mutated *EGFR2* and *FABP7* genes were significantly associated with poorer survival. Additionally, these two genes were also associated with a significantly worse OS than those for other types of metastatic lesions (log-rank test $p=0.006$, HR: 5.11), especially in liver metastasis which our previous researchers identified as the worst metastatic sites influencing the poor survival [11]. Among the many indicators used in prognostic risk classification of mRCC, liver metastasis is clinically and widely reported as a predictor of poor outcome for patients with mRCC [4,11]. As the most frequent organ with RCC metastasis was the lung, followed by bone (20%–35%), lymph nodes, liver, adrenal gland, and brain, it is very important to detect metastatic lesions as early as possible. This is especially true for poor prognostic organs, which are associated with a substantial decrease in survival rate, with a median survival rate of 6 months and a 50% mortality rate in a metastatic setting [11,28].

The candidate genes identified in this study could act as biomarkers during first-line TT in mRCC and contribute toward improving the potential for early detection of therapeutic failure and metastatic progression. The clinical applications of these candidates should be further evaluated; to date, there are no established biomarkers available for predicting therapeutic responses to TT during mRCC treatment. Collection of a larger number of samples to validate these findings using a TGS cancer panel could potentially enable a personalized therapeutic strategy for each patient applicable to a clinical setting, enabling improved monitoring of disease progression and better survival outcomes. For example, *EGFR* and *FABP7* identified as genes associated with liver metastasis outcomes in this study have already been examined in previous studies [25,27]; thus, these genes could be indicators of liver metastasis during TT and considered as target genes for currently available TT [16-18,29]. Because no potential candidate marker for liver metastasis in mRCC has been reported to date, this study provides the first evidence to suggest two potential genetic markers associated with metastasis after first-line TT, regardless of the mechanism of action of the targeted agents.

CONCLUSIONS

The study showed for the first time several candidate genes with mutations significantly related to the survival prognoses in Korean patients with mRCC treated with first-line TT using mRCC tissues. These candidate genes should be further validated in a future, large-scaled study, with prospective collection of blood and tissue samples from patients with mRCC.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

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SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.4111/icu.20210341.

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