Original Article
EGFR, KRAS and BRAF mutations in Chinese patients with clear cell renal cell carcinoma

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Abstract: The EGFR-KRAS-BRAF-MEK-ERK (MAPK) pathway plays an important role in most cancers with close association of EGFR, KRAS or BRAF mutations with carcinogenesis and drug resistance. EGFR have been developed for molecular targeted therapy of various cancers. However, it remains to be determined whether aberration of this pathway is essential for the survival of clear cell renal cell carcinoma (CCRCC) and whether EGFR tyrosine kinases inhibitors (TKIs) could benefit CCRCC patients. In this study, we detect EGFR, KRAS and BRAF mutations in CCRCC, investigate the potential of EGFR TKIs for the treatment of CCRCC patients, and summarize clinicopathological characteristics of CCRCC carrying EGFR, KRAS and BRAF mutations. EGFR, KRAS and BRAF mutation in 100 CCRCC from China were detected by high resolution melting analysis. Positive mutations were confirmed using direct sequencing. The cases with EGFR, KRAS and BRAF mutations were further assessed by immunohistochemistry (IHC). The diagnosis of CCRCC was confirmed by IHC staining of CK (Pan), EMA, Vimentin, CD10, Desmin and SMA, and the activation of MAPK pathway was assessed by IHC staining of EGFR, KRAS, BRAF and p-BRAF. One KRAS while no EGFR or BRAF mutations were identified in these 100 CCRCC patients. The case with KRAS mutation was histologic grade G4, tumor stage III and TNM stage III (T3N0M0). Lung metastasis was found four months after surgery and the patient died two and a half months after metastasis. The tumor with KRAS mutation was positive for CK (Pan), EMA, Vimentin, CD10, but negative for desmin and SMA, and positive for KRAS, BRAF, and p-BRAF, but negative for EGFR. To conclude, our results support EGFR, KRAS and BRAF mutations are rare in CCRCC, EGFR TKIs may be not suitable for the majority of CCRCC patients and CCRCC patients with the tumor carrying KRAS mutation might have poor prognosis.

Keywords: Clear cell renal cell carcinoma, EGFR, KRAS, BRAF, mutation

Introduction

Clear cell renal cell carcinoma (CCRCC) is the most common cancer of kidney and accounts for 75% of all renal cell carcinomas (RCC). The incidence of CCRCC has been increasing over the past several years. Surgical resection is the main treatment module for CCRCC and the majority of patients will obtain good prognosis after surgery. However one-third of patients will develop metastatic lesions with a 5-year survival rate less than 10%. Traditional cytotoxic chemotherapy is difficult to kill the tumor cells of CCRCC due to anatomical and physiological characteristics of kidney. Therefore, it is very important to elucidate the molecular biology and identify effective therapeutic strategies for CCRCC.

Some genetic and epigenetic events contribute to the occurrence and development of CCRCC, including loss-of-function mutations of Von Hippel-Lindau (VHL) gene [1]. Inactivation of VHL leads to stabilization and accumulation of transcription factor hypoxia inducible factor alpha (HIFα), which in turn accelerates development of CCRCC by upregulating a series of proteins [2]. However, VHL mutation rate is only 24%-45% in CCRCC, which suggests the mutations of other important genes might exist in CCRCC. The EGFR-KRAS-BRAF-MEK-ERK (MAPK) pathway regulates multiple cellular functions including growth, proliferation, and apoptosis. It has been well documented MAPK pathway is upregulated and molecular targeting this pathway is a promising strategy for the development of targeted medicine of many

Table 1. Relationship of EGFR, KRAS and BRAF mutations to clinicopathological features of CCRCC patients

Variables	n	EGFR status	KRAS status	BRAF status
Gender				
Male	72	0	1	0
Female	28	0	0	0
Age (Median 61)				
≤61	50	0	0	0
>61	50	0	1	0
Tumor size (Average 5.5 cm)				
≤7 cm	69	0	0	0
>7 cm	31	0	1	0
Histologic grade				
G1-G2	75	0	0	0
G3-G4	25	0	1	0
Tumor stage				
T1-T2	81	0	0	0
T3-T4	19	0	1	0
TNM stage				
I-II	73	0	0	0
III-IV	27	0	1	0

cancers including non-small cell lung cancer, breast cancer, gastric cancer and so on [3-5]. Previous studies have confirmed that EGFR, KRAS and BRAF are the most frequently altered genes in cancers. Whether these three genes play an important role in CCRCC remains elusive.

EGFR is a transmembrane glycoprotein with receptor tyrosine kinase (RTK) activity and regulates cell proliferation, differentiation, and apoptosis. EGFR mutations increase its kinase activity, which leads to upregulated activation of several downstream signaling pathways including AKT and MAPK. EGFR mutations have been found in many malignancies such as lung, colorectum, head and neck cancers [6-8]. EGFR tyrosine kinases inhibitors (TKIs) such as gefitinib or erlotinib bind the tyrosine kinase domain of EGFR by competing with ATP. Accumulating data indicate tumors carrying EGFR mutations are sensitive to EGFR TKIs especially in non-small cell carcinoma (NSCLC) [9, 10]. Recently, EGFR mutation has drawn great attention in the potential use of EGFR TKIs for other cancers treatment. Nevertheless, there are few reports about EGFR mutations and therapeutic potential of EGFR TKIs in CCRCC.

In addition, KRAS, a downstream molecule of EGFR, plays a 'molecular switch' role in MAPK signaling pathway, and KRAS mutations lead to active downstream molecules even if EGFR is inhibited by EGFR TKIs. KRAS mutations are critical in EGFR TKIs resistance and high frequency of KRAS mutations is found in many malignant tumors cancers such as colorectal cancer [11], NSCLC [12] and pancreatic cancer [13]. However, the status of KRAS mutations in CCRCC is still controversial. A previous study from Italy reported that KRAS mutations rate were high [14], while studies from Germany [15], France [16] and Austria [17] showed that KRAS mutation could not be detected in CCRCC. In CCRCC of Chinese, whether KRAS mutation exists is unknown.

Furthermore, similar to KRAS mutation, BRAF mutations lead to constitutive activation of the downstream molecules in MAPK pathway and are commonly found in melanoma, thyroid papillary carcinoma and colorectal-cancer [18-20], however, BRAF mutation status in CCRCC remains unclear and there is no report on BRAF mutation in CCRCC from China.

In this study, we detected the mutation status of EGFR, KRAS and BRAF genes in 100 CCRCC to estimate the applicability of EGFR TKIs in CCRCC patients, and profiled the clinicopathological characteristics of CCRCC carrying EGFR, KRAS or BRAF mutations.

Materials and methods

Patients and samples

A total of 100 CCRCC tissues were obtained from the Dalian Friendship Hospital of Northern China from 2003 to 2015. Among the 100 CCRCC patients, 72 were male and 28 were female with a median age of 61 years (range 26-86 years) and an average maximum diameter of 5.5 cm (range 1.3-17 cm). This study was approved by The Ethics Committee of the Dalian Friendship Hospital. Informed consent of all patients was obtained. None of the patients received chemotherapy and radiotherapy for cancer before surgical treatment. The diagnoses were certified by two experienced pathologists according to standard

Table 2. EGFR, KRAS and BRAF primers

Exon	Forward	Reverse
EGFR		
19	5'-TGGATCCCAGAAGGTGAGAA-3'	5'-AGCAGAAACTCACATCGAGGA-3'
21	5'-CGCAGCATGTCAAGATCA-3'	5'-CCTCCTTACTTTGCCTCC-3'
KRAS		
2	5'-AGGCCTGCTGAAAATGACTG-3'	5'-TCAAAGAATGGTCCTGCACC-3'
BRAF		
15	5'-TAGTAACTCAGCATCTCAGG-3'	5'-CTCTTCATAATGCTTGCTCTGATAgg-3'

criteria. Histologic grade and TNM stage was determined according to Fuhrman criteria [21] and 2006 WHO TNM staging system [22]. Clinicopathological features of the patients are shown in **Table 1**. These specimens were diagnosed as G1 and G2 (n=75), G3 and G4 (n=25); T1 and T2 (n=81), T3 and T4 (n=19); TNM I and TNM II (n=73), TNM III and TNM IV (n=27).

Genomic DNA extraction

Paraffin embedded sections of 5 μm thickness were stained with H&E, followed by localization of tumor-rich regions under a microscope. Tumor-rich regions were isolated for DNA extraction. Genomic DNA was extracted by TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing, China) according to manufacturer's instructions and kept at 4°C till use.

High resolution melting analysis (HRMA)

Polymerase chain reactions (PCRs) were performed using a 96-well PCR plate on the gradient Eppendorf Mastercycler (Eppendorf, Germany). The primers of EGFR, KRAS and BRAF are listed in **Table 2**. Reaction mixture in a 10 μL final volume contained the following: for exon 19 of EGFR, 1×PCR buffer (Takara, China), 0.2 mM of dNTPs (Takara, China), 0.5 μM of each primer, 0.5 U of HotStart Taq (Takara, China), 1×LC Green Plus (Biochem, USA), 5 ng genomic DNA; for exons 21 of EGFR, 1×PCR buffer (Takara, China), 2.5 mM $MgCl_2$, 0.2 mM of dNTPs, 0.5 μM of each primer, 0.5 U of Taq DNA polymerase (Takara, China), 1×LC Green Plus (Biochem, USA), 5 ng of genomic DNA; for exon 2 of KRAS, 1×PCR buffer (Takara, China), 0.2 mM of dNTPs, 0.5 μM of each primer, 0.5 U of Taq DNA polymerase (Takara, China), 1×LC Green Plus (Biochem, USA), 5 ng of genomic DNA; for exon 15 of BRAF, 1×PCR buffer (Takara, China), 2 mM $MgCl_2$, 0.2 mM

of dNTPs, 0.5 μM of each primer, 0.5 U of Taq DNA polymerase (Takara, China), 1×LC Green Plus (Biochem, USA), 5 ng of genomic DNA. Cycling conditions for exon 19 of EGFR were as follows: 60 cycles of 98°C for 10 s, 53°C for 10 s, 72°C for 20 S. Cycling conditions for exon 21 of EGFR as follows: 60 cycles of 98°C for 10 s, 58°C for 10 s, 72°C for 20 s. Cycling conditions for exon 2 of KRAS were as follows: 60 cycles of 98°C for 10 s, 61°C for 10 s, 72°C for 20 S. Cycling conditions for exon 15 of BRAF were as follows: 60 cycles of 98°C for 10 s, 60°C for 10 s, 72°C for 20 S. PCR for each specimen and corresponding wild type specimen was performed in triplicate for mutation screening. PCR products were scanned by LightScanner (Idaho Technology, USA).

DNA sequencing

All positive samples identified by HRMA were further confirmed by DNA sequencing. Samples with potential exon 2 of KRAS mutation were amplified using the primers described in HRMA. Reaction mixture a 60 μL final volume contained 1×PCR buffer (Takara, China), 0.2 mM of dNTPs, 0.5 μM of each primer, 0.5 U of Taq DNA polymerase (Takara, China), 1×LC Green Plus (Biochem, USA), 30 ng of genomic DNA. The reaction conditions were as follows: 60 cycles of 98°C for 10 s, 61°C for 10 s, 72°C for 20 S. Purified PCR products were sequenced in Takara.

Immunohistochemistry

Using immunohistochemistry, the diagnosis associated proteins including CK (Pan), EMA, Vimentin, CD10, Desmin and SMA, and MAPK pathway associated proteins including EGFR, KRAS, BRAF and p-BRAF were detected in CCRCC with EGFR, KRAS or BRAF mutation. The steps of immunohistochemical staining were operated according to the instruction manual. The antibodies used in this study are listed in **Table 3**. Primary antibodies were replaced by PBS in the negative control.

Table 3. The detailed information of the antibodies used in immunohistochemistry

| Name	Clonality	Clone number	Dilution	Manufacturers, country
CK (Pan)	Monoclonal	MX005	Without	Mai Xin, China
EMA	Monoclonal	E29	Without	Mai Xin, China
Vimentin	Monoclonal	V9	Without	Mai Xin, China
CD10	Monoclonal	MX002	Without	Mai Xin, China
Desmin	Monoclonal	D33	Without	Mai Xin, China
SMA	Monoclonal	1A4	Without	Mai Xin, China
EGFR	Monoclonal	EGFR.113	Without	Mai Xin, China
KRAS	Monoclonal	F 132-62	1:50	Abcam, England
BRAF	Monoclonal	F-7	1:250	Santa Cruz, America
p-BRAF	Monoclonal	EPR2207	1:100	Abcam, England
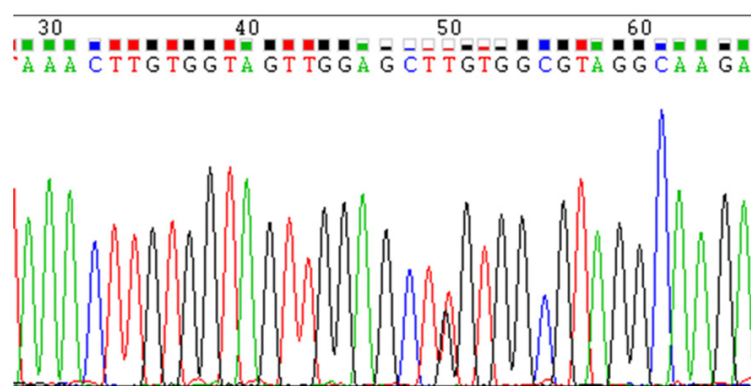
Figure 1. DNA sequencing showed KRAS mutation in CCRCC. g.10570G>T, c.34 G>T, heterozygous, p.G12C.

Results

EGFR mutation detection

Among the 100 CCRCC patients, no one was found to have sequence variations in EGFR exon 19 and 21 as detected by HRMA (**Table 1**).

BRAF mutation detection

None of the 100 CCRCC patients was revealed to harbor sequence variations in BRAF exon 15 as detected by HRMA (**Table 1**).

KRAS mutation detection

In the 100 CCRCC cases, one mutation in KRAS exon 2 was found by HRMA. The mutation was confirmed by DNA sequencing (**Figure 1**). The mutation was a substitution of glycine by cysteine at codon 12 (34 G>T, G12C). The patient was a 77 years old man. Maximum diameter of the tumor was 14 cm. The histologic grade was G4 according to Fuhrman differentiation (**Figure 2A**). The tumor infiltrated renal adipose capsule and there was a tumor thrombus in inferior vena cava. The tumor stage was III. No metastasis was found before operation and TNM stage was III (T3N0M0). He did not receive radiotherapy, chemotherapy or molecular targeted therapy before radical nephrectomy. Lung metastasis was found four months after operation. The patient died two and a half months after metastasis. The correlation between KRAS mutation and clinicopathological features is summarized in **Table 1**.

Immunohistochemistry analysis

Immunohistochemistry analysis demonstrated the tumor with KRAS mutation was positive for CK (Pan), EMA (**Figure 2B**), Vimentin (**Figure 2C**) and CD10, but negative for Desmin and SMA proteins, and positive for KRAS (**Figure 2D**), BRAF (**Figure 2E**) and p-BRAF (**Figure 2F**), but negative for EGFR proteins.

Discussion

In MAPK pathway, EGFR, KRAS and BRAF are the most important components, mutations of them result in activation of a series of protein kinases, leading to enhanced cell growth, proliferation and survival. In the past two decades, accumulating evidence has shown that EGFR, KRAS or BRAF mutation occur in lots of cancers, and KRAS or BRAF mutations contribute to the response of patients to tyrosine kinases inhibitors such as gefitinib or erlotinib. The MAPK signaling pathway is one of the main target pathways for the currently molecular targeted therapy of cancers. However, it remains to be determined whether aberration of this pathway is essential for the survival of CCRCC, and whether EGFR tyrosine kinases inhibitors
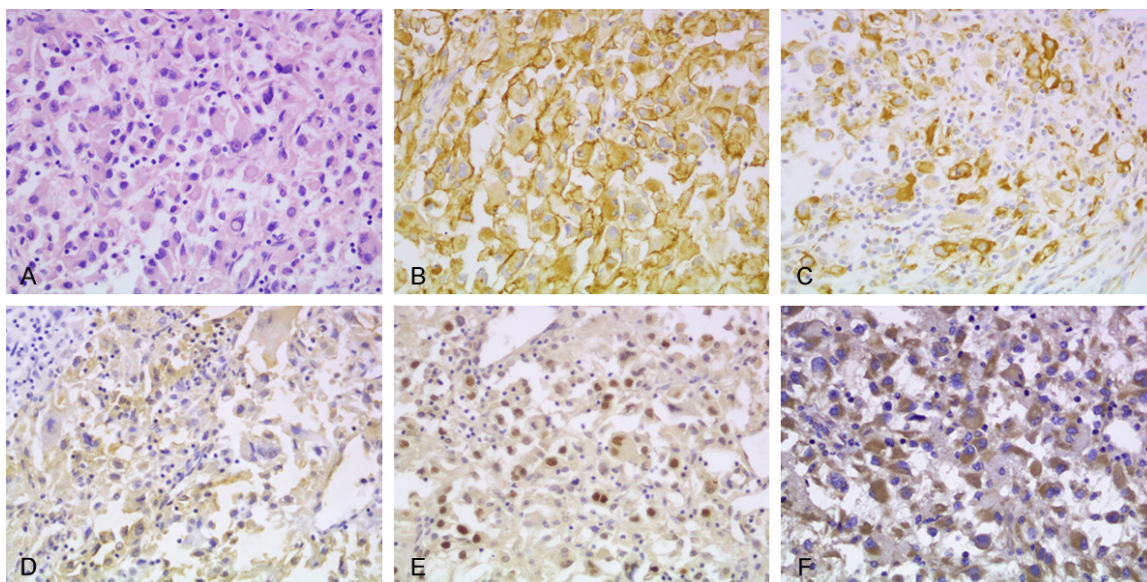
Figure 2. Immunohistochemical analysis of the CCRCC with KRAS mutation. A: H&E staining. B: EMA staining. C: Vimentin staining. D: KRAS staining. E: BRAF staining. F: p-BRAF staining. Magnification, ×400.

(TKIs) could be used for CCRCC patients. Moreover, the mutation rates of EGFR, KRAS and BRAF of CCRCC in Chinese are unclear. With the advance of precision medicine for cancer patients, it is urgent to determine EGFR, KRAS and BRAF mutation status in CCRCC and provide the genetic basis for clinician to choose the right drugs for the right patients.

EGFR is a tyrosine kinase receptor belonging to the ErbB family and plays a key role in multiple cellular processes. EGFR mutations can cause constitutive activation downstream proteins, and patients with EGFR mutations in NSCLC are sensitive to EGFR TKIs. The current available data show EGFR mutation rate is relatively low in CCRCC. Sakaeda et al [23], and Szymańska et al [16] tested 19 and 50 CCRCC cases respectively, and none of them found EGFR mutation. However, Ivantsov et al reported four EGFR mutations in 336 CCRCC cases with the frequency of 1.2% [24]. Similar to these previous studies, we found no EGFR mutation in 100 CCRCC samples. Our findings demonstrated EGFR mutation is very low in Chinese CCRCC, which suggests EGFR-TKIs may be not suitable for most Chinese CCRCC patients. However, Iyevleva et al reported one CCRCC patient carrying an EGFR mutation was sensitive to gefitinib treatment [25], indicating the very few CCRCC patients might benefit from EGFR-TKIs.

High KRAS mutation rates have been found in various malignant tumors. The KRAS mutation frequency is 15-32% in NSCLC [26, 27], 35-40% in colorectal cancer [28], 0-21% in gastric cancer [29]. Current data show that KRAS mutations in CCRCC are variable among different populations. In a study from Italy, Raspollini et al detected KRAS mutation in 11 CCRCC of T1a stage and found all cases carried KRAS mutation [14]. However, no KRAS mutation in CCRCC tumors was found in 63 cases from Germany [15], 50 tumors from France [16], and 12 tumors from Austria [17]. In our study, there was one KRAS mutation in 100 CCRCC with mutation rate of 1%. KRAS mutation in human cancers takes place primarily at codon 12 and 13 in exon 2. The common types include G12D, G12V, G12A, G12C, G12S, G12R, G13D and G13A, which are usually detected in pancreatic and colorectal cancers. In CCRCC, KRAS mutations were founded only in one study. The mutations occurred at codon 12 and 13 in exon 2 with the mutations types of G12S, G12V, G12C, G12D and G13D, G13S [14]. In this study, the mutation type was G12C, similar to previous report of Raspollini [14]. Our results suggest that KRAS mutation frequency of CCRCC from Chinese mainland is similar to that in Germany, France and Austria. But KRAS mutation rate was found to be higher in Italy than that in other countries. This discrepancy might be due to geographic and racial factors.

BRAF is one of the most important pro-onco-genes. The mutation rate is approximately 8% in total human tumors [30, 31]. BRAF mutations exist in melanoma, thyroid carcinoma and colorectal cancers most commonly, but relatively low in other carcinomas. Similar to the report of Gattenlöhner [15], we did not detect BRAF mutation in CCRCC. Therefore, BRAF mutation may be very rare in CCRCC.

In conclusion, the very low frequency of EGFR, KRAS and BRAF mutations suggests EGFR; KRAS and BRAF mutations may be not required for the initiation, development and survival of most CCRCC. However, KRAS mutations lead to constitutive activation of downstream signal molecules and are associated with poor response to EGFR TKIs in carcinomas. Since EGFR TKIs have the possibility to be applied for CCRCC patients carrying EGFR mutations [25], and KRAS mutations do exist in CCRCC though with a relatively low rate, KRAS mutation must be detected before EGFR TKIs therapy.

In our study, the patient, with the tumor carrying KRAS mutation, was a 77-year-old man, who was diagnosed as CCRCC in right renal with the maximum tumor diameter of 14 cm in December 2003. The age of the patient was significantly older than the mean 61 years in our study and a previous report of 62.2 years [32]. The tumor maximum diameter was also bigger than the average maximum diameter of 5.5 cm in our study and another study of 5.3 cm [32]. These suggest CCRCC patients with KRAS mutation might be older and with larger tumor size. Histopathology analysis by H&E staining showed the tumor was architecturally diverse with solid and acinar patterns. There were 20% region with rhabdoid patterns in solid features region, demonstrating as large epithelioid cells, eccentric nuclei and large and paranuclear intracytoplasmic hyaline globules (**Figure 2A**). The rhabdoid cells were positive for CK (pan), EMA, Vimentin and CD10 proteins, negative for Desmin and SMA proteins. These results proved rhabdoid cells also were CCRCC. Agaimy et al reported KRAS mutations existed in pancreatic undifferentiated rhabdoid carcinoma and the mutation rate was 54% [33]. In our study, the tumor with rhabdoid feature also carried KRAS mutation similar to the study of Agaimy et al. The tumor with KARS mutation expressed KRAS, BRAF and p-BRAF proteins but not EGFR protein, suggesting

KRAS mutation activates expression of KRAS and downstream proteins without EGFR protein in MAPK pathway leading to promote tumor cells proliferation.

The relationship between KRAS mutations and prognosis of carcinoma patients has been a controversial issue. Most reports showed that KRAS mutations were significantly associated with shorter disease-free survival or recurrence-free survival period compared to patients with wild-type KRAS in NSCLC [34], colon adenocarcinoma [35] and so on. On the contrary, other researchers founded KRAS mutations were not poor prognosis factors in NSCLC [36], Vater cancer [37] and pancreatic cancer [38]. In the study of Kulemann et al, pancreatic carcinoma patients with KRAS mutation in circulating tumor cells had a substantially better survival than patients with wild type KRAS [39]. In the study of Raspollini, KRAS mutation was detected only in T1a stage CCRCC [14]. However in our study, tumor with KRAS mutation had high histologic grade and tumor stage. The tumor infiltrated into renal adipose capsule. There was a tumor thrombus in inferior vena cava. Lung metastasis was found soon after surgery. Survival time of the patient was only six and a half months. The result suggests the prognosis of CCRCC patients with the tumor carrying KRAS mutation may be very poor. Nevertheless, due to the low frequency of KRAS mutation and small number of patients screened up to now in CCRCC, the relationship between KRAS mutation and prognosis of CCRCC patients remains to be determined through a large cohort of patients in multiple centered studies.

In summary, EGFR, KRAS and BRAF mutations are very rare in CCRCC. EGFR, KRAS and BRAF are not the main genes during the occurrence and progress of CCRCC and the majority of CCRCC patients may not benefit from EGFR TKIs. However, the CCRCC patients with the tumor carrying KRAS mutation may have poor prognosis.

Disclosure of conflict of interest

None.

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References

[1] Nickerson ML, Jaeger E, Shi Y, Durocher JA, Mahurkar S, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Mukeria A, Holcatova I, Schmidt LS, Toro JR, Karami S, Hung R, Gerard GF, Linehan WM, Merino M, Zbar B, Boffetta P, Brennan P, Rothman N, Chow WH, Waldman FM, Moore LE. Improved identification of von Hippel-Lindau gene alterations in clear cell renal tumors. Clin Cancer Res 2008; 14: 4726-4734.

[2] Peter E Clark. The role of VHL in clear-cell renal cell carcinoma and its relation to targeted therapy. Kidney Int 2009; 76: 939-945.

[3] Russo A, Franchina T, Ricciardi GR, Picone A, Ferraro G, Zanghì M, Toscano G, Giordano A, Adamo V. A decade of EGFR inhibition in EGFR-mutated non small cell lung cancer (NSCLC): Old successes and future perspectives. Oncotarget 2015; 6: 26814-26825.

[4] Slattery ML, Lundgreen A, John EM, Torres-Mejia G, Hines L, Giuliano AR, Baumgartner KB, Stern MC, Wolff RK. MAPK genes interact with diet and lifestyle factors to alter risk of breast cancer: the breast cancer health disparities. Study Nutr Cancer 2015; 67: 292-304.

[5] Lin Y, Wu Z, Guo W, Li J. Gene mutations in gastric cancer: a review of recent next-generation sequencing studies. Tumour Biol 2015; 36: 7385-7394.

[6] Schmid K, Oehl N, Wrba F, Pirker R, Pirker C, Filipits M. EGFR/KRAS/BRAF mutations in primary lung adenocarcinomas and corresponding locoregional lymph node metastases. Clin Cancer Res 2009; 15: 4554-4560.

[7] Borràs E, Jurado I, Hernan I, Gamundi MJ, Dias M, Martí I, Mañé B, Arcusa A, Agúndez JA, Blanca M, Carballo M. Clinical pharmacogenomic testing of KRAS, BRAF and EGFR mutations by high resolution melting analysis and ultra-deep pyrosequencing. BMC Cancer 2011; 11: 406.

[8] Smilek P, Neuwirthova J, Jarkovsky J, Dusek L, Rottenberg J, Kostrica R, Srovnal J, Hajduch M, Drabek J, Klozar J. Epidermal growth factor receptor (EGFR) expression and mutations in the EGFR signaling pathway in correlation with anti-EGFR therapy in head and neck squamous cell carcinomas. Neoplasma 2012; 59: 508-523.

[9] Wang H, Huang J, Yu X, Han S, Yan X, Sun S, Zhu X. Different efficacy of EGFR tyrosine kinase inhibitors and prognosis in patients with subtypes of EGFR-mutated advanced non-small cell lung cancer: a meta-analysis. J Cancer Res Clin Oncol 2014; 140: 1901-1910.

[10] Lilenbaum R, Horn LA. Management of EGFR mutation-positive non-small cell lung cancer. J Natl Compr Canc Netw 2016; 14: 672-676.

[11] Bagci B, Sari M, Karadayi K, Turan M, Ozdemir O, Bagci G. KRAS, BRAF oncogene mutations and tissue specific promoter hypermethylation of tumor suppressor SFRP2, DAPK1, MGMT, HIC1 and p16 genes in colorectal cancer patients. Cancer Biomark 2016; 17: 133-143.

[12] Lee SE, Lee SY, Park HK, Oh SY, Kim HJ, Lee KY, Kim WS. Detection of EGFR and KRAS mutation by pyrosequencing analysis in cytologic samples of non-small cell lung cancer. J Korean Med Sci 2016; 31: 1224-1230.

[13] Saiki Y, Horii A. Molecular pathology of pancreatic cancer. Pathol Int 2014; 64: 10-19.

[14] Raspollini MR, Castiglione F, Martignoni G, Cheng L, Montironi R, Lopez-Beltran A. Unlike in clear cell renal cell carcinoma, KRAS is not mutated. Virchows Arch 2015; 467: 687-693.

[15] Gattenlöhner S, Etschmann B, Riedmiller H, Müller-Hermelink HK. Lack of KRAS and BRAF mutation in renal cell carcinoma. Eur Urol 2009; 55: 1490-1491.

[16] Szymańska K, Moore LE, Rothman N, Chow WH, Waldman F, Jaeger E, Waterboer T, Foretova L, Navratilova M, Janout V, Kollarova H, Zaridze D, Matveev V, Mates D, Szeszenia-Dabrowska N, Holcatova I, Bencko V, Le Calvez-Kelm F, Villar S, Pawlita M, Boffetta P, Hainaut P, Brennan P. TP53, EGFR, and KRAS mutations in relation to VHL inactivation and lifestyle risk factors in renal-cell carcinoma from central and eastern Europe. Cancer Lett 2010; 293: 92-100.

[17] Betge J, Pollheimer MJ, Schlemmer A, Hoefler G, Langner C. Gastric cancer and concomitant renal cancer: a systematic immunohistochemical and molecular analysis. Oncol Rep 2011; 26: 567-575.

[18] Ascierto PA, Kirkwood JM, Grob JJ, Simeone E, Grimaldi AM, Maio M, Palmieri G, Testori A, Marincola FM, Mozzillo N. The role of BRAF V600 mutation in melanoma. J Transl Med 2012; 10: 85.

[19] Puxeddu E, Durante C, Avenia N, Filetti S, Russo D. Clinical implications of BRAF mutation in thyroid carcinoma. Trends Endocrinol Metab 2008; 19: 138-145.

[20] Cushman-Vokoun AM, Stover DG, Zhao Z, Koehler EA, Berlin JD, Vnencak-Jones CL. Clinical utility of KRAS and BRAF mutations in a cohort of patients with colorectal neoplasms

submitted for microsatellite instability testing. Clin Colorectal Cancer 2013; 12: 168-178.

[21] Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982; 6: 655-653.

[22] Eble JN, Sauter G, Epstein JI, Sesterhenn IA. Tumors of the urinary system and male genital organs. In: Eble JN, Sauter G, Epstein JI, Sesterhenn IA, editors. French: International Agency for Research on Cancer; 2004. pp. 16-19.

[23] Sakaeda T, Okamura N, Gotoh A, Shirakawa T, Terao S, Morioka M, Tokui K, Tanaka H, Nakamura T, Yagi M, Nishimura Y, Yokoyama M, Okumura K. EGFR mRNA is upregulated, but somatic mutations of the gene are hardly found in renal cell carcinoma in Japanese patients. Pharm Res 2005; 22: 1757-1761.

[24] Ivantsov AO, Imianitov EN, Moiseenko VM, Matsko DE, Artem'eva AS. Expression of Ki-67, p53, bcl-2, estrogen receptors alpha in patients with clear cell renal carcinoma and epidermal growth factor receptor mutation. Arkh Patol 2011; 73: 6-7.

[25] Iyevleva AG, Novik AV, Moiseyenko VM, Imyanitov EN. EGFR mutation in kidney carcinoma confers sensitivity to gefitinib treatment: A case report. Urol Oncol 2009; 27: 548-550.

[26] Yu HA, Sima CS, Shen R, Kass S, Gainor J, Shaw A, Hames M, Iams W, Aston J, Lovly CM, Horn L, Lydon C, Oxnard GR, Kris MG, Ladanyi M, Riely GJ. Prognostic impact of KRAS mutation subtypes in 677 patients with metastatic lung adenocarcinomas. J Thorac Oncol 2015; 10: 431-437.

[27] Dumenil C, Vieira T, Rouleau E, Antoine M, Duruisseaux M, Poulot V, Lacave R, Cadranel J, Massiani MA, Wislez M. Is there a specific phenotype associated with the different subtypes of KRAS mutations in patients with advanced non-small-cell lung cancers? Lung Cancer 2015; 90: 561-568.

[28] Zlobec I, Kovac M, Erzberger P, Molinari F, Bihl MP, Rufle A, Foerster A, Frattini M, Terracciano L, Heinimann K, Lugli A. Combined analysis of specific KRAS mutation, BRAF and microsatellite instability identifies prognostic subgroups of sporadic and hereditary colorectal cancer. Int J Cancer 2010; 127: 2569-2575.

[29] Victor T, Du Toit R, Jordaan AM, Bester AJ and van Helden PD. No evidence for point mutations in codons 12, 13, and 61 of the ras gene in a high-incidence area for esophageal and gastric cancer. Cancer Res 1990; 50: 4911-4914.

[30] Kamata T, Pritchard C. Mechanisms of aneuploidy induction by RAS and RAF oncogenes. Am J Cancer Res 2011; 1: 955-971.

[31] Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949-954.

[32] Kruck S, Eyrich C, Scharpf M, Sievert KD, Fend F, Stenzl A, Bedke J. Impact of an altered Wnt1/β-catenin expression on clinicopathology and prognosis in clear cell renal cell carcinoma. Int J Mol Sci 2013; 14: 10944-10957.

[33] Agaimy A, Haller F, Frohnauer J, Schaefer IM, Ströbel P, Hartmann A, Stoehr R, Klöppel G. Pancreatic undifferentiated rhabdoid carcinoma: KRAS alterations and SMARCB1 expression status define two subtypes. Mod Pathol 2015; 28: 248-260.

[34] Ulivi P, Chiadini E, Dazzi C, Dubini A, Costantini M, Medri L, Puccetti M, Capelli L, Calistri D, Verlicchi A, Gamboni A, Papi M, Mariotti M, De Luigi N, Scarpi E, Bravaccini S, Turolla GM, Amadori D, Crinò L, Delmonte A. Nonsquamous, non-small-cell lung cancer patients who carry a double mutation of EGFR, EML4-ALK or KRAS: frequency, clinical-pathological characteristics, and response to therapy. Clin Lung Cancer 2016; 17: 384-390.

[35] Lee DW, Kim KJ, Han SW, Lee HJ, Rhee YY, Bae JM, Cho NY, Lee KH, Kim TY, Oh DY, Im SA, Bang YJ, Jeong SY, Park KJ, Park JG, Kang GH, Kim TY. KRAS mutation is associated with worse prognosis in stage III or high-risk stage II colon cancer patients treated with adjuvant FOLFOX. Ann Surg Oncol 2015; 22: 187-194.

[36] Geles A, Gruber-Moesenbacher U, Quehenberger F, Manzl C, Al Effah M, Grygar E, Juettner-Smolle F, Popper HH. Pulmonary mucinous adenocarcinomas: architectural patterns in correlation with genetic changes, prognosis and survival. Virchows Arch 2015; 467: 675-686.

[37] Kwon MJ, Kim JW, Jung JP, Cho JW, Nam ES, Cho SJ, Kim JS, Park HR, Min SK, Seo J, Min KW, Kim DH, Jeon JY. Low incidence of KRAS, BRAF, and PIK3CA mutations in adenocarcinomas of the ampulla of Vater and their prognostic value. Hum Pathol 2016; 50: 90-100.

[38] Zhou L, Baba Y, Kitano Y, Miyake K, Zhang X, Yamamura K, Kosumi K, Kaida T, Arima K, Taki K, Higashi T, Imai K, Hashimoto D, Yamashita

Y, Chikamoto A, Beppu T, Tan X, Baba H. KRAS, BRAF, and PIK3CA mutations, and patient prognosis in 126 pancreatic cancers: pyrosequencing technology and literature review. Med Oncol 2016; 33: 32.

[39] Kulemann B, Liss AS, Warshaw AL, Seifert S, Bronsert P, Glatz T, Pitman MB, Hoeppner J. KRAS mutations in pancreatic circulating tumor cells: a pilot study. Tumour Biol 2015; 37: 7547-7554.