







KRAS, BRAF, PIK3CA mutation frequency of radical prostatectomy samples and review of the literature

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ABSTRACT

Objective: The molecular basis of prostate cancer is highly heterogeneous. Our study aimed to perform the mutation analysis of *KRAS*, *BRAF*, *PIK3CA*, and immunohistochemical (IHC) evaluation of EGFR, HER2, p16, and PTEN to demonstrate new areas for targeted therapies.

Methods: A total of 24 prostatectomy samples diagnosed with adenocarcinoma were analyzed by microarray hybridization. Also, these samples were IHC stained for EGFR, HER2, P16, and PTEN. The cases were divided into two groups based on low and high Gleason scores. All findings were compared with the clinicopathological parameters of the patients.

Results: While *KRAS* mutation was in 3/24 (12.5%) of our cases, *BRAF* and *PIK3CA* mutations were not detected. There was no significant difference between the groups in terms of *KRAS* mutation frequency. HER2 was immunohistochemically negative in all samples. There was no correlation between EGFR, P16 immunopositivity, and clinicopathological features.

Conclusion: *KRAS* mutation frequency is similar to those in Asian populations. *BRAF* and *PIK3CA* mutation frequencies have been reported in the literature in the range of 0–15% and 0–10.4%, respectively, consistent with our study findings. HER2 immunopositivity is a controversial issue in the literature. EGFR and p16 expressions may not correlate with the stage.

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Introduction

Prostate cancer (PCa) is the second most common malignancy in males except for non-melanoma skin cancer in the world. It is the fifth cause of cancer deaths worldwide [1]. Although localized PCa patients benefit from androgen ablation therapy, the disease may turn into an androgen-independent state and, the risk of metastasis-recurrence increases in advanced stages. Therefore, a better understanding of the molecular basis of PCa is required to create new treatment areas. Oncogenic mutations affecting signal transduction pathways in the cell are directly related to carcinogenesis. KRAS/MAPK and PI3K/AKT pathways are the most important mechanisms in intracellular signal transduction. Molecules and genes in these pathways at different phases have been shown as targets in cancer pathogenesis. In this context, mutations of the *BRAF*, *KRAS*, and *PIK3CA* genes were identified with varying frequencies in many cancer types [2–10]. BRAF and PI3K inhibitors have taken place in treatment schemes at various stages; studies for KRAS still

continue at changing clinical and laboratory stages [11–13]. In PCa, no targeted gene therapy has yet been approved [14]. *KRAS* and *BRAF* mutation frequencies in PCa were reported in up to 40% and 15% in the literature, respectively [15–63]. The PI3K/AKT signaling pathway is frequently affected in PCa. Deregulations in this pathway may base on different causes such as *PIK3CA* mutations, *PIK3CA* amplifications, or deletions of *PTEN*, an inhibitor of the pathway. *PIK3CA* mutations have been reported in up to 10.4% of PCa in various studies [29,31,33,38,42–52,57,64–76].

EGFR (ErbB1) is crucial for stimulating both the RAS/MAPK and PI3K/AKT signaling pathway. While EGFR is defined especially in castration-resistant PCa, HER2 (ErbB2) has yielded conflicting results [77–79]. Although there have been targeted therapy studies for ErbB receptors in PCa, it also brings problems like target therapy resistance [80–81]. In patients with targeted therapy resistance to ErbB receptors, signaling pathway actors in the later stages of signalling, such

as RAS/MAPK, may be actual targets in the treatment approach. Besides, RAS/MAPK and PI3K/AKT pathways are known to interact with the CDK/Cyclin D complex inhibited by p16 [82]. Therefore, identifying the expressions of EGFR, HER2, p16, and PTEN together with the mutations in these pathways will guide in understanding PCa carcinogenesis.

In this study, we analyzed the genetic alterations of *KRAS*, *BRAF*, and *PI3KCA* mutations. We compared the genetic findings, immunoexpression profiles with clinical features to determine diagnostically relevant alterations and compared these findings with those of other studies.

Methods

Tissue samples

The study was performed based on the tenets of the Helsinki Declaration and according to approval by the local Ethics Committee of the Duzce University, School of Medicine (protocol number: 2018/93). It was also supported financially by the SRP unit (Scientific Research Projects: 2018.04.02.796) of Duzce University. Thirty-five radical prostatectomy samples diagnosed with PCa in our department between 2012–2018 were evaluated. The samples that did not contain sufficient tumoral tissue for genetic evaluation and the cases without clinical information were excluded from the study (Figure 1). A total of 24 cases were subdivided into two groups as Gleason score = 6 (Grade Group 1, GS-L) and Gleason score ≥ 9 (Grade Group 5, GS-H). Age, preoperative PSA level, Gleason score, stage, surgical margin positivity, extraprostatic extension, lymphovascular invasion, perineural invasion, family

history, and biochemical recurrence status of selected cases were compiled. Formalin-fixed paraffin-embedded (FFPE) blocks that included the most adequate tumoral tissue were selected for the PCR and IHC method. FFPE blocks that had large tumor tissue and no necrosis or bleeding were preferred.

Detection of mutations

Tumoral tissue of 5–10 μm from the selected FFPE blocks was manually removed from the surrounding intact tissue elements by macrodissection. Following the QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) protocol, deparaffinization and DNA extraction were performed. After extraction, DNA values were measured with DNA-50 value in Colibri Nanodrop device. The PCR process was performed on the VERITI device, following the Multiplex PCR protocol of the *KRAS*/*BRAF*/*PIK3CA* Array (RANDOX) kit. Microarray Hybridization, imaging, and result acquisition procedures of PCR products were performed in Evidence Investigator (RANDOX) device by following the Microarray Hybridization, imaging, and result acquisition protocols of EV3799A/B *KRAS*/*BRAF*/*PIK3CA* Array kit. In this study, 20 different point mutations on the *KRAS* (codon 12, codon 13, codon 61, codon 146), *BRAF* (V600E), and *PIK3CA* (codon 542, codon 545, codon 1047) genes were analyzed at the same time.

Immunohistochemical analysis

Five-micrometer sections were taken on positively charged slides. The sections were deparaffinized in a 75-degree oven for 1 h. After deparaffinization, tissues stained with EGFR (Genemed Biotechnologies, clone

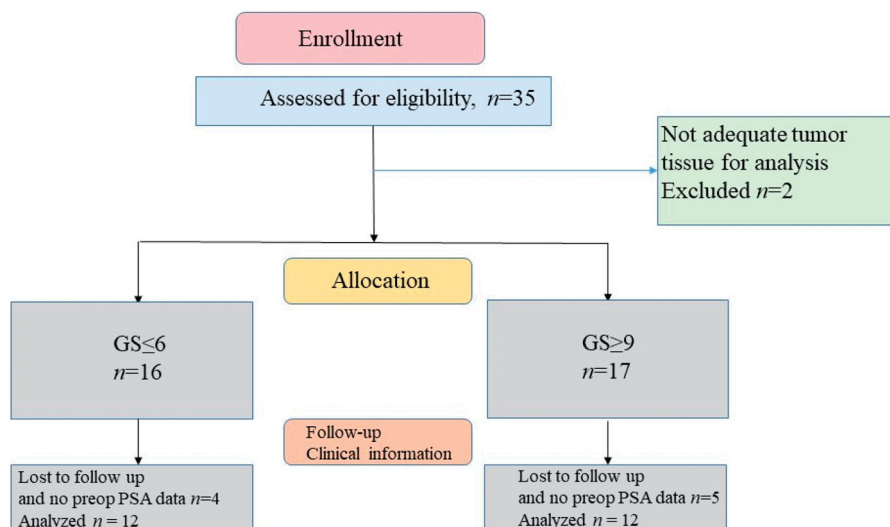


Figure 1. Selection of cases.

31G7), Cerb-B2 (Biocare Medical, clone EP1045Y), p16 (Biocare Medical, clone G175-405), and PTEN (Thermo, clone 17.A) antibodies by Ventana Benchmark XT brand automatic IHC stainer.

IHC evaluation

IHC staining intensity was scored between 0 and 3+ for P16 and EGFR. It was scored as 0 (no staining), 1+ (weak), 2+ (medium) and 3+ (strong). Also the degree of staining was scored between 0 and 3 according to the percentage of stained cells 0, <30%, 30%–60%, > 60% for P16; 0, <10%, 10%–50%, > 50% for EGFR, as previously reported [83–84]. The cases were scored between 0 and 9 by obtaining a multiplicative staining score. A range of 7 to 9 was considered strong staining, a range of 4 to 6 was moderate staining, a range of 1 to 3 was considered weak staining.

Statistical analysis

Appropriate descriptive statistics (mean, standard deviation, minimum, maximum, percentage) of all data in the study were calculated. Relationships between categorical variables were examined using the Pearson chi-square, Fisher Freeman Halton (*post hoc* Bonferroni test), and Fisher exact tests. To examine the relationships between variables, contingency (*rc*) coefficient, point biserial (*rp*), and Kendall tau b (*rk*) correlation coefficients were calculated according to the types of variables. SPSS 22.0 (Statistical Package for the Social Sciences) program and special syntaxes were used for statistical evaluations. A *p*-value of < .05 was considered statistically significant.

Results

Twelve of the 24 individuals had a Gleason score of ≤ 6 (GS-L), while 12 had a Gleason score of ≥ 9 (GS-H). The mean age of the patients was 65.4±7.9 years (Min-Max: 43–79), and the preoperative mean PSA value was 6.4±3.1 (Min-Max: 0.48–13.32).

A statistically significant difference was found between GS-L and GS-H groups in terms of pT (*p* = .005). Stages and TNM status are presented in Table 1. There was no significant difference between the lymph node metastasis status and the rates of distant metastasis according to the groups (*p* = .999 for each). According to the groups, there was no significant difference between the rates of vascular invasion (*p* = .590). The rate of perineural invasion (33.3%) in individuals in the GS-L group was significantly lower

Table 1. Stage and TNM status of patients according to the groups.

	GS-L (Gleason score ≤ 6) <i>n</i>	GS-H (Gleason score ≥ 9) <i>n</i>
T		
pT2	12 (100.0%)	5 (41.7%)
pT3a	0	2 (16.7%)
pT3b	0	5 (41.7%)
N		
N0	11 (91.7%)	10 (83.3%)
N1	1 (8.3)	2 (16.7%)
M		
M0	11 (91.7)	11 (91.7%)
M1	1 (8.3)	1 (8.3%)
Stage		
I	10 (83.3)	0
IIA	1 (8.3)	0
IIIC	0	9 (75.0%)
IVA	0	2 (16.7%)
IVB	1 (8.3)	1 (8.3%)

than the rate of perineural invasion in individuals in the GS-H group (83.3%) (*p* = .036). While no periprostatic extension was observed in GS-L individuals, there was a periprostatic extension in 58.3% of GS-H individuals (*p* = .005). There was a significant difference between the rates of seminal vesicle invasion according to the groups (*p* = .014). No significant difference was found between the rates of surgical margin positivity, biochemical recurrence, and family history according to the groups (*p* = .214, *p* = .590, *p* = .193, respectively).

KRAS mutation was detected in 3/24 (12.5%) of our PCa cases, while *BRAF* and *PIK3CA* mutations were not found in any of the cases. 2 of the *KRAS* mutations were at codon 12, and 1 of them was at codon 61 localization. All cases with *KRAS* mutations were Gleason score 3+3=6 and their pathological stages were pT2. While lymph node involvement, distant metastasis, vascular invasion and periprostatic extension were not observed in 3 of these 3 cases, perineural invasion was detected in 1 case. There was no significant difference between the rates of *KRAS* mutation according to the groups. In addition, there were no correlation between *KRAS* mutation and age (*rp* = -.249 *p* = .240), preoperative PSA (*rp* = -.359 *p* = .085), Gleason grade (*rk* = -.364 *p* = .075), pT (*rk* = -.233 *p* = .250), stage (*rk* = -.381 *p* = .050), lymph node metastasis (N) (*rc* = .141 *p* = .999), distant metastasis (M) (*rc* = .113 *p* = .999), vascular invasion (*rc* = .167 *p* = .999), perineural invasion (*rc* = .188 *p* = .550), periprostatic extension (*rc* = .236 *p* = .530), seminal vesicle invasion (*rc* = .213 *p* = .546), surgical margin positivity (*rc* = .188 *p* = .550), biochemical recurrence (*rc* = .167 *p* = .999) and family history (*rc* = .292 *p* = .209).

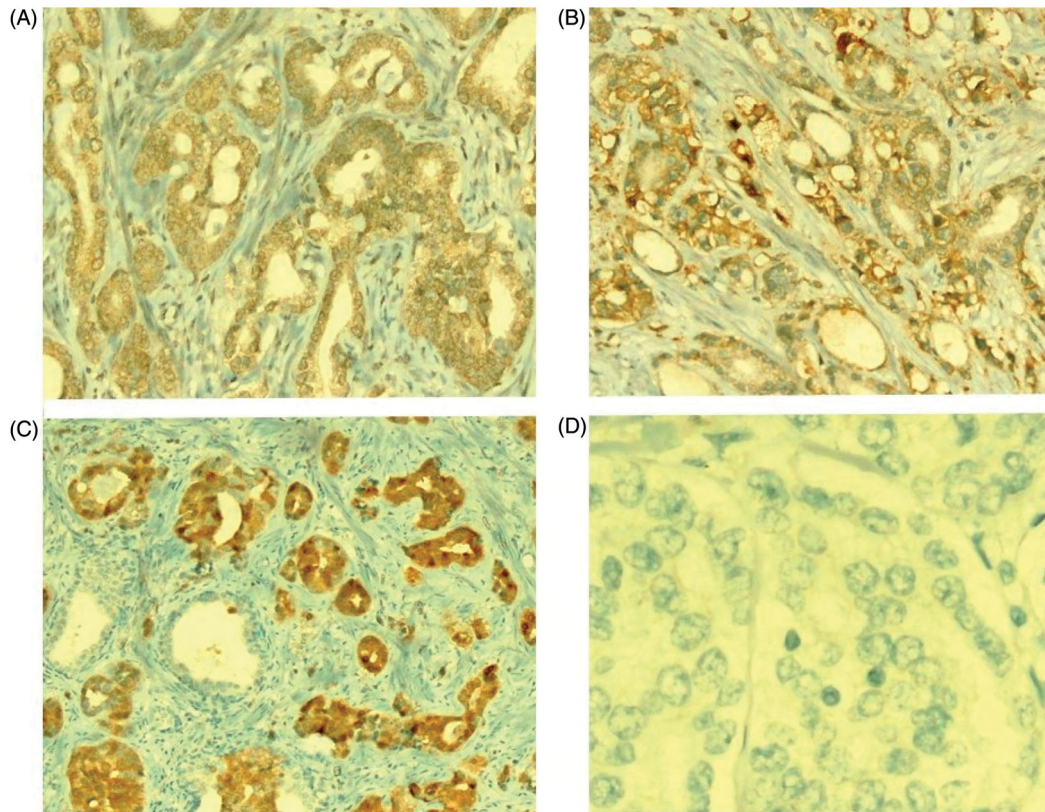


Figure 2. (A) Weak staining with EGFR in neoplastic glands (EGFRx200). (B) EGFR strong staining (EGFRx200). (C) Intense and strong staining with P16 in tumoral glands, negativity in the benign gland adjacent (P16 × 200). (D) No staining in neoplastic glands with HER2 (HER2 × 400).

Immunohistochemically, there was no staining for HER2 in either group (Figure 2(D)). While most (62.5%) of the cases showed weak staining for EGFR, 8 cases (33.4%) had a moderate or strong expression (Figure 2(A,B)). p16 were immunopositive for 83.3% of cases (Figure 2(C)). There was no significant difference in EGFR and P16 immunopositivity between the groups. Detailed IHC staining and mutation positivity status in the GS-L and GS-H groups are shown in Table 2. PTEN analysis was not performed for technical reasons.

There were no correlation between EGFR immunopositivity and age ($rk = .160$ $p = .349$), preoperative PSA ($rk = .295$ $p = .080$), Gleason grade ($rk = .328$ $p = .098$), pT ($rk = .165$ $p = .399$), stage ($rk = .206$ $p = .274$), lymph node metastasis (N) ($rk = -.216$ $p = .284$), distant metastasis (M) ($rk = .197$ $p = .329$), vascular invasion ($rk = -.201$ $p = .319$), perineural invasion ($rk = .290$ $p = .151$), periprosthetic extension ($rk = .127$ $p = .528$), seminal vesicle invasion ($rk = .189$ $p = .350$), surgical margin positivity ($rk = -.179$ $p = .374$), biochemical recurrence ($rk = .146$ $p = .469$), presence of family history ($rk = .175$ $p = .395$).

Also no significant relationship was observed P16 immunopositivity vs. age ($rk = .062$ $p = .707$),

Table 2. KRAS, BRAF and PIK3CA mutation and HER2, EGFR, and p16 expression status of cases according to groups.

	GS-L <i>n</i>	GS-H <i>n</i>	Total <i>n</i>	<i>p</i> [*]
KRAS				
mutation	3 (25%)	0	3 (12.5%)	.217 ^{&}
BRAF				
mutation	0	0	0	–
PIK3CA				
mutation	0	0	0	–
HER2				
positivity	0	0	0	–
EGFR				
weak	9 (75%)	6 (50%)	15 (62.5%)	.271 [§]
moderate	2 (16.7%)	5 (41.7%)	7 (29.2%)	
strong	0	1 (8.3%)	1 (4.2%)	
P16				
weak	3 (25%)	6 (50%)	9 (37.5%)	.350 [§]
moderate	6 (50%)	4 (33.3%)	10 (41.7%)	
strong	0	1 (8.3%)	1 (4.2%)	

^{*}Exact *p* value, [&]Fisher Exact test, [§]Fisher Freeman Halton test.

preoperative PSA ($rk = .039$ $p = .810$), Gleason grade ($rk = .088$ $p = .649$), pT ($rk = .102$ $p = .591$), stage ($rk = .126$ $p = .492$), lymph node metastasis (N) ($rk = .330$ $p = .092$), distant metastasis (M) ($rk = .055$ $p = .780$), vascular invasion ($rk = -.024$ $p = .901$), perineural invasion ($rk = .006$ $p = .975$), periprosthetic extension ($rk = .253$ $p = .196$), seminal vesicle invasion ($rk =$

.182 $p = .353$), surgical margin ($rk = -.240$ $p = .221$), biochemical recurrence ($rk = .081$ $p = .678$), presence of family history ($rk = -.080$ $p = .691$).

Discussion

The molecular basis of PCa has not been elucidated for many years. Although much progress has been made in recent years, it remains complex. The main difficulty in understanding the molecular basis of PCa is heterogeneity. In this study, we analyzed the intracellular signal transduction mechanisms of both PI3K and MAPK pathways at the same time. We evaluated the frequency of *KRAS*, *BRAF*, and *PIK3CA* mutation frequency and immunohistochemical expression molecules belonging to these two different pathways in PCa.

In the literature, the frequency of *KRAS* mutations in PCa is reported between 0% and 40% [15–52]. *KRAS* mutations have yielded variable results in different populations depending on the ethnic origins of the populations. *KRAS* mutation frequencies are reported to be higher in Asian populations than in Western populations. Shen et al. [30] reported the frequency of *KRAS* mutation as 9.1% in the study performed on 121 patients in the Chinese population. Similarly, Cho et al. [28] and Salmaninejad et al. [40] reported *KRAS* mutation frequency as 7.3% and 5.7% in Korean and Iranian patients, respectively. Similar to these studies, the frequency of *KRAS* mutations in the current study was detected to be 12.5%. As shown in Table 3, *KRAS* mutation frequency is reported to be as low as 0, 3% in the USA, and several Western societies, unlike Asian populations [29,31,33,37,38]. We think these differences are mainly related to ethnic origin. However, Watanabe et al. [22] found the frequency of *KRAS* mutations in Japanese patients as 14.2%, in Hawaiian Japanese as 5%. The rate is somewhat low in Hawaiian Japanese compared to this study and other studies in Japanese patients. This result suggests that environmental and epigenetic factors may affect the *KRAS* gene. Also, the significantly low *KRAS* mutation frequency in studies in the United States may be because of its relatively low proportion due to the excess of non-genetic factors in the etiology of PCa.

Two studies have analyzed *KRAS* mutations in PCa in Turkey. Vural et al. [41] reported a rate of 8.8%, similar to the current study. Besides, gene regions in which *KRAS* mutations occurred (exon 2, codon 12/13; exon 3, codon 61) were similar to our study. Silan et al. [36] reported *KRAS* mutation as 40% in the study that included PCa patients diagnosed in the middle

Anatolian region. This is the highest rate ever reported in the literature. The relatively high number of Gleason score ≥ 7 cases in that study or the regional/environmental changes within the country may have caused the difference. Also, an automatic reverse hybridization method was used in that study, while the microarray hybridization method was used in ours. Another remarkable finding in the study of Silan et al. is the combined codon 12–13 mutations in 9 cases (30%). Another combined mutation was reported by Konishi et al. in the literature [24]. In that study, a combined 13–61 mutation was observed in 1 case (11.1%). In our study, no combined mutation was found.

Ras expression has been observed to increase the ability of malign transformation and resistance to castration in PCa cell lines (LNCaP). Bakin et al. showed that increased RAS expression and sustained activation of MAP kinase decreased androgen requirement for growth, PSA expression, and tumorigenesis [85–87]. It was also suggested that the *KRAS* mutation in PCa increases the susceptibility to bone metastasis through CD24a expression [88]. In parallel with these studies, Anwar et al. [20] found that the frequency of *KRAS* mutations in patients with stage III and IV was two times higher than in patients with stage I and II. Moreover, all cases with *KRAS* mutation were poorly differentiated PCa in the study of Suzuki et al. [23]. On the other hand, there was no significant relationship between *KRAS* mutation status vs. stage, high Gleason score, or metastatic disease in the current study as in the studies of Shen et al. [30] and Cho et al. [28]. Additionally, survival analysis could not be performed because all our patients except one were alive.

BRAF is another target of the MAPK signaling pathway in carcinogenesis. *BRAF* mutation rates in PCa have yielded different results in various studies. Although results reaching 15% were reported in several studies in Asian populations, in many other studies in Western countries and Asia, no mutations were detected consistent with our study (Table 3) [28–34,38,40,42,44–46,48,53–63].

According to The Cancer Genome Atlas (TCGA) data, the frequency of *BRAF* mutation in PCa is specified as 1.4% [89]. These mutations are several activating mutations other than the canonical V600E. Cho et al. [28] reported the *BRAF* mutation rate as 10%. Likewise, most of the mutations detected in that study were V600A and V600M variants, which are rare and were not evaluated by us. Considering only V600E mutation positivity in that study, it shows a low rate (1%) similar to ours. In a recent study, Suh et al. [61]

Table 3. Continued.

		KRAS	BRAF	PIK3CA	n	+ codon	method
26	Salmanijad 2015	5.7%	0%	—	35	KRAS 12, 13	PCR/Sanger sequencing
27	Vural 2018	8.8%	—	—	45	KRAS Exon 2, exon 3*	PCR/High resolution DNA melting analysis
28	Martinez-Gonzalez 2018	28.3%	5%	3.3%	60	KRAS 12, 13 BRAF V600E PIK3CA	ARMS (Amplification Refractory Mutation System) PCR/Custom qBiomarker Somatic Mutation PCR Arrays Probe based analysis
29	Jarattiridrisi 2018	0.7%	—	0%	15	Q546P, H1047Y #	Whole genome sequencing
30	Ikeda 2019	2.8%	0%	10.4%	67	#	NGS (Next Generation Sequencing)
31	Toomey 2020	0%	0%	4.8%	83	#	PCR/Mass spectrometry based single nucleotide polymorphism genotyping technology (Agena MassArray technology)
32	Cohen 2003	—	0%	—	17	—	PCR/Restriction enzyme analysis, direct sequencing
33	Burger 2006	—	0%	—	79	—	Allele-specific PCR
34	Liu 2009	—	0%	—	93	—	Direct sequencing
35	Köllerman 2010	—	0%	—	43	—	PCR/High resolution DNA melting analysis
36	Robinson 2015	—	0.6%	%4	150	#	PCR/Sequencing
37	Beltran 2016	—	1.2%	—	81	#	Whole exome sequencing
38	Cohn 2017	—	0%	—	23	—	Whole exome sequencing
39	Jafarian 2018	—	4%	—	100	BRAF V600E	PCR/Direct Sanger Sequencing PCR/Direct Sequencing
40	Suh 2020	—	15%	—	20	BRAF K601E**	tNGS (targeted Next Generation Sequencing)
41	Kasajima 2020	—	4.7%	—	21	BRAF K601E	Nucleotide sequencing
42	Müller 2007	—	—	0%	12	—	PCR/SSCP ve automatic nucleotide sequencing
43	Sun 2009	—	—	3%	40	PIK3CA E545A	PCR-SSCP
44	Boormans 2010	—	—	0%	63	—	Direct sequencing
45	Beltran 2013	—	—	4%	45	#	PCR/Automatic sequencing
46	Barbieri 2013	—	—	4%	112	#	tNGS
47	Millis 2016	—	—	3.4%	173	PIK3CA E545K H1047R***	Exome sequencing NGS
48	Kumar 2016	—	—	-1.8	54	—	Whole exome sequencing aCGH (Microarray Based Comparative Genomic Hybridization) RNA transcript profiling
49	Huang 2017	—	—	<3%	102	#	Exome sequencing
50	Rescigno 2018	—	—	6%	418	#	MiSeqTM based targeted next generation sequencing
51	Dawson 2020	—	—	4.5%	1010	#	NGS

(continued)

Table 3. Continued.

		KRAS	BRAF	PIK3CA	n	+ codon	method
52	Schultz Loda Sander 2015	#	2.4%	-1.8%	333	BRAF Non-V600Ee noncanonical PIK3CA E545K, Q546K, N345I, C420R, E474A, 1 case combined E542A-N345I	Whole exome sequencing
53	Hovelson 2015	#	#	#	114	#	NGS
54	Grasso 2015	#	-1.8%	-1.8%	53	BRAF V600insHT	NGS qRT-PCR
55	Iacono 2016	-	-	#	60	#	NGS
56	Seed 2017	-	-	#	110	#	Target amplicon sequencing (Illumina MiSeq platform) Exome sequencing
57	Barata 2018	#	#	#	66	#	aCGH
58	Mitri 2018	#	#	#	10	#	tNGS
59	Hussain 2018	-	-	-3.75%	80	#	Whole genome sequencing (Illumina NextSeq)
60	Abida 2019	#	#	#	429	#	Targeted exome sequencing Whole genome sequencing
61	Zhao 2020	-	#	-	100	#	Transcriptome sequencing Whole genome bisulfate sequencing Deep whole genome and transcriptome sequencing
62	Crumbaker 2020	#	#	#	13	#	Deep whole genome sequencing
63	Current	12.5%	0%	0%	24	KRAS 12, 61	PCR/Microarray hybridization (Randox Molecular Diagnostics)

#The detailed codon information and rate of specific mutations are not available especially in NGS-based studies since numerous genetic changes including amplification, deletion, or mutation in multiple gene regions were analyzed simultaneously.

*Codon 12 and 13 are in exon 2; codon 61 is in exon 3. Exon data was given in the article but detailed codon information was not available.

**K601E mutation was found in two of three cases. It was not available codon data of the other case with BRAF mutation.

***The most common codons.

reported the *BRAF* mutation rate in locally advanced PCa as 15%, which is the highest frequency in the literature. We think that the high *BRAF* mutation rate in that study resulted from the new generation sequencing (NGS) method. As a matter of fact, 2/3 of the detected *BRAF* mutations are K601E changes. We examined the V600E mutation in exon 15, which is a hotspot class I mutation of *BRAF*. Therefore, other mutations in exon 15 and mutations in exon 11 were not included in the study. V600E mutation is a hotspot mutation whose oncogenic potential is well known. *BRAF* mutations other than V600E are categorized as class II (kinase-active non V600E), class III (RAS dependent), and variants of unknown significance (VUS). Class II *BRAF* mutations show moderate/high kinase activity, while class III mutations lead to weak or no kinase activity [90]. With the NGS method, many gene regions, including VUS, can be analyzed simultaneously. However, NGS is not cost-effective for many institutions, and it is a disadvantage in that it provides results that do not reflect the pathogenic potential exactly due to random genetic variants.

In the report of Jafarian et al., which is one of the studies only V600E mutation was examined, mutation frequency was found to be 4% [60]. The mean age of patients in that study was higher than in the current

study (71.83; 65.4, respectively), and all patients with mutations were between the ages of 70 and 90 years. Also, Gleason score > 6 cases consist of more than 80% of all cases in that study, while 50% in ours. On the other hand, Agell et al. reported the *BRAF* mutation frequency as 0%, in which a Gleason score > 6 cases predominant serial, in line with our study [33].

Genetic changes have been identified at almost every step of the PI3K pathway in PCa carcinogenesis [91]. Some of those are the increased PI3K synthesis by *PIK3CA* mutations, functional losses in PTEN protein suppressing AKT, or increased AKT synthesis by mutations in the *PTEN* gene [44,92]. Besides, the synergistic interaction with the RAS signaling pathway is also important in the effect of this pathway on carcinogenesis. RAS effect is minimal in stimulating the PI3K pathway under normal conditions. However, oncogenic RAS is an effective activator of this pathway. PI3K pathway deregulation was reported at a rate of 42% in localized PCa, and 100% in metastatic PCa. This suggests that the pathway also plays a role in castration resistance [31]. *PIK3CA* is the gene encoding the catalytic subunit of PI3K. In the literature, the frequency of *PIK3CA* mutations in PCa has been reported between 0% –10.4% (Figure 3). MacConaill et al. [29], Taylor et al. [31], and Toomey et al. [45] found the

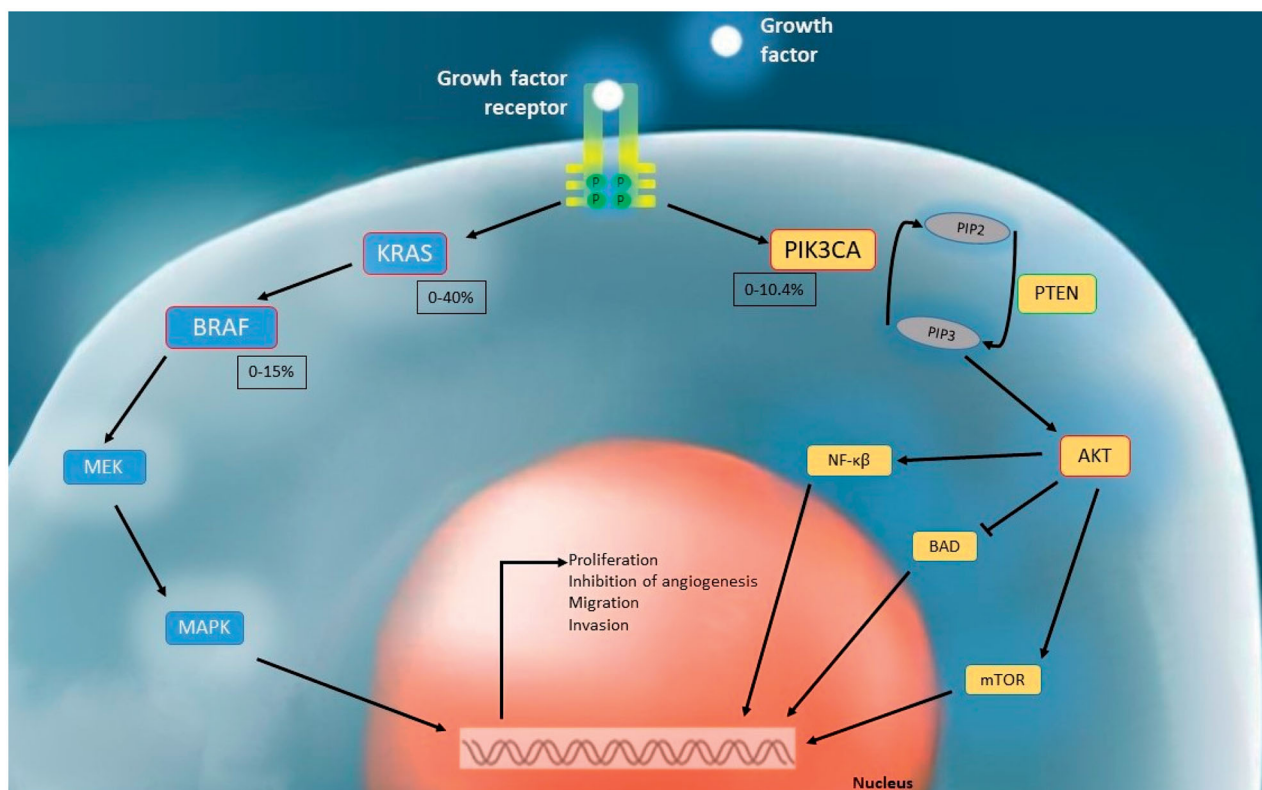


Figure 3. Interaction of intracellular signal transduction pathways and mutation frequency ranges detected in *KRAS*, *BRAF* and *PIK3CA* in human PCa tissues in the literature.

PIK3CA mutation at a rate of 2.1% in 95 patients, 1.2% in 156 patients, 4.8% in 83 patients, respectively. The mutation rates are slightly higher than in the current study in these three studies, which included a relatively large number of cases. This may be due to the mass spectrometry-based method with high-throughput genotyping (OncoMap, iPLEX, and Agena, respectively) in all three studies. In addition, fresh tumor samples were analyzed in the study of MacConaill [29] et al. Additionally, Barnett et al. [38] detected *PIK3CA* mutation in 2 of 40 patients by the mass spectrometric method. While one of these mutations is the H1047R mutation that we analyzed in the current study, the other is the Q546F mutation that we did not.

Similar to our study, Agell et al. found no mutation in 102 patients [33]. Likewise, Jaratlerdrishi achieved a *PIK3CA* mutation rate of 0% in 15 cases with the whole genome sequencing method [43]. Boorman et al. [66] had the same result as ours by automatic sequencing from fresh tumor tissues in 63 patients. On the other hand, Beltran et al. [67] detected the *PIK3CA* mutation rate as 4% in 45 patients. The difference between this result and ours is likely to be since the number of metastatic PCa in that study is almost twice that of localized PCa. We think that the proportionally lower number of metastatic PCa in our study had a negative effect on the *PIK3CA* mutation profile. Indeed, Dawson et al. detected *PIK3CA* frequency as 4.5% by NGS in 1010 cases [72]. Approximately half of the tissues consist of metastatic biopsies in that large sample analysis.

It is thought that both intertumoral and intratumoral heterogeneity plays a role in the molecular heterogeneity of PCa. This is because the tumor consists of more than one clone developing with a step-by-step branched progression through its evolutionary process [93–94]. We believe that intratumoral heterogeneity may also have a role in the low results of *BRAF* and *PIK3CA* in our study.

EGFR is the main trigger of activation of the MAPK and PI3K signaling pathways. EGFR stimulation also activates the androgen receptor even in the absence of androgen hormone [95]. Even more, castration leads to an increase in EGFR expression [96]. Supporting this crosstalk between AR and EGFR, several studies have found a significant relationship between EGFR expression and PCa progression [96–102]. In this study, no correlation was found in this direction. This result may be due to differences in immunohistochemical interpretation. EGFR immunostaining was strongly associated with advanced tumor stage, high Gleason grade, and

preoperative PSA according to the study of Schlomm et al. However, as shown in the same study, EGFR expressions were not correlated with EGFR copy number changes in PCa [77,97,103].

HER2 overexpression/gene amplification, a predictive parameter in breast cancer and is involved in treatment, has yielded variable results in PCa. We have no HER2 positive case. In the literature, studies have reported low or no HER2 expression/amplification levels in parallel with our study, as well as studies reporting high rates up to 100% [77,101,104–108]. Kankaya et al. reported HER2 expression as 72.9%, while they found HER2 gene amplification as 4.05% in the same study. A meta-analysis of 5976 PCa cases reported an association between HER2 overexpression with mortality and recurrence [109]. However, it has been observed that HER2 target therapies alone have a limited antitumor effect on PCa [81,110]. Combination therapies or immunotherapy options can be promising for a specific subpopulation.

p16, a major member of the cell cycle control mechanism, interacts with various mitogenic signaling pathways through cyclin D. It has a role in inhibiting the MAPK signaling pathway. p16 immunohistochemical expression has been reported between 23% and 100% in PCa in the literature [111–115]. Evaluation only nuclear staining positively may be the reason for the low rate of 23% in the study of Vlachostergios PJ et al. The rate was %86 in our study. Many other studies that reported similar to ours have considered both cytoplasmic and nuclear positivity [111–113]. Cut-off value variabilities, immunohistochemical technics, or fixation differences are the other points that might cause different results. A recent analysis based on TCGA data showed a reverse association with p16 expression and Gleason score [116]. The reason for not reaching such a result in our study may be the small sample size. Another point we were surprised that there was no association between *KRAS* mutation positivity and p16 expression levels.

PI3K pathway dysregulations are very common in PCa carcinogenesis. *PTEN* deletions and inactivations are blamed for the majority of dysregulations of this pathway [44,46,57]. In our study, we could not evaluate *PTEN* due to technical reasons.

Unlike PCa, almost no *KRAS* or *BRAF* mutations were detected in benign prostate hyperplasia (BPH) in several studies in the literature [20,21,25,35,60]. In fact, in a recent NGS study, no evidence of driver genomic alterations was found in BPH cases, inconsistent with a neoplastic process. Even they observed multiple signatures related to the inactivation of *KRAS* signaling [117].

Best of our knowledge, this is the first report that analyses *KRAS*, *BRAF*, and *PIK3CA* mutations simultaneously in PCa from Turkey. The small number of cases and the relatively narrow gene mutation panel for *BRAF* and *PIK3CA* are among the limitations of our study. In addition, the proportionally low number of metastatic PCa in our study is another limitation.

Our study provides new data to the literature as a basis for pharmacological target therapy research. We analyzed the various targets of two main signal transduction pathways, MAPK and PI3K, in the cell. In the patient group with *KRAS* mutation, targeted *KRAS* inhibitor agents that are in the research stage may be a ray of hope in treatment. Although the *BRAF* V600E and *PIK3CA* mutations were determined to be 0% in our study, consistent with many studies in the literature, there are also studies that determined different rates than ours. To more clearly determine the significance of these pathways in PCa, studies are needed in large series with predominantly advanced cases. The use of *BRAF* and PI3K inhibitors, which are approved in the treatment of different types of cancer and with satisfactory results may potentially prevent organ loss and increase the quality of life of patients with PCa.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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