ORIGINAL ARTICLE

Effect of Subtype of K-*Ras* Mutation on Survival in Resected Pancreatic Adenocarcinoma

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ABSTRACT

Objective The purpose is to determine if the different Kras mutations found in pancreatic ductal adenocarcinoma (PDA) confer different survivals after adjuvant gemcitabine, docetaxel and capecitabine (GTX) therapy, a regimen that affects Kras signaling. **Methods** We evaluated the survival with the type of Kras mutation in 53 patients who had resected PDA. All patients were treated either with neo-adjuvant or adjuvant GTX therapy. The types and frequencies of Kras mutations in our PDA patients were compared to those in the literature for PDA, lung cancer and colon cancer. **Results** We found that 79% of our patients had a mutation in Kras at codon 12, with replacement of the glycine for either aspartic acid (47%), valine (19%), arginine (9%) or cysteine (4%). Serine and alanine substitutions and codon 13 mutations were not found. The frequency of Kras mutations detected in PDA differs markedly from those found in lung and colon cancer. Our PDA patients with aspartic acid or valine substitutions for glycine 12 had more relapses (p=0.026). **Summary** The types and frequencies of Kras mutations are different in PDA from those observed in lung cancer or colon cancer. PDA patients with aspartic acid and valine have a poor survival, but it is not clear if all Kras mutations are equally detrimental as other mutation had fewer relapses. Larger sample sizes are needed to know different amino acid substitutions in Kras result in different responses or survivals with GTX or other therapy.

INTRODUCTION

K-*ras* is activated when bound to GTP and inactivated by hydrolysis to GDP. K-*ras* has a slow intrinsic rate of lysis of the bound GTP, requiring an accessory protein to augment its catalytic activity. The accessory proteins are called regulators of G protein signaling (RGS) also known as GTPase activating protein (GAP) [1].

In wild-type K-*ras*, codons 12 (GGT) and 13 (GGC) code for glycine. Wild-type K-*ras* is mutated at codon 12 in over 70% of PDA's, which replaces the glycine for another amino acid with a side-chain. Mutating the first or second guanosine of the codon 12 yields 6 potential mutations. The 6 possible amino acid substitutions arginine (R), cysteine (C), serine (S), valine (V), alanine (A), and aspartic (D) should confer different degrees of allosteric inhibition of the RGS protein's augmentation of K-*ras* hydrolysis of GTP [2]. Thus, the rate of catalysis of GTP should differ among the different mutations, conferring different durations of signaling activity of GTP-K-*ras*. Different durations of signaling may lead to different clinical outcomes. We analyzed our non-metastatic pancreatic cancer patients, all treated with the same GTX regimen, for the frequency and type of K-*ras* mutation and the effect of the mutation on survival.

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METHODS

This data collection was approved by the IRB of Columbia University.

We reviewed sixty-five patients who underwent resection for localized pancreatic adenocarcinoma for K-*ras* mutation. All were treated with adjuvant or neoadjuvant GTX chemotherapy at Columbia University Medical Center from 2006 to 2014. Patients presenting with arterial involvement or those with R1 resection (<2 mm margin of resection) without prior RT received localized radiation therapy with GX [3]. The resected tissue was assayed for K-*ras* mutation by PCR as a routine pathology procedure. Patients were followed for recurrence and survival. Upon relapse, most patients resumed GTX, and FOLFIRINOX following progression on GTX. Of the 65 patients, 55 patients with k-*ras* data were included in this analysis. K-*ras* data was not available on 10 patients.

We reviewed the literature for the type of K-*ras* mutation in PDA and representative articles for patients with lung and colon cancers.

STATISTICS

The data analysis included descriptive statistics for all variables. Frequency tables were used to evaluate the distributions of categorical and discrete variables. Chi

Square and Mann-Whitney U tests were performed to compare the difference between groups. Overall survival (OS) and Disease free survival (DFS) were performed with Kaplan-Meier method and compared with log rank test. Cox proportional hazard regression model and odd ratio were used to evaluate the prognostic value of K-*ras* status. Significant values were considered when $p<0.05$. Statistical analyses were performed using the SPSS® statistical program (V 21; SPSS, Chicago, Ill).

RESULTS

We had information on K-*ras* status in 55 patients 28 neoadjuvant patients and 27 adjuvant patients. Twenty-nine patients were male (53%) and 26 were female (47%) with a mean of age of 63 (range; 38-78) years at the time of diagnosis **(Table 1)**. Of the 55 patients, 29 patients recurred. The median follow up time was 34.3 months (range; 7-97.7 months). Among those who received neoadjuvant treatment, only three had more than one positive lymph node. Seventeen (68%) of the adjuvant patients and 20 (71%) of the neoadjuvant patients had R0 resections and the rest had R1 resections. R0 was defined by a minimum of a 2 mm margin. Thirty-four patients received GX/RT: ten patients with R1 resections treated adjuvantly, twenty-two patients with arterial involvement treated neoadjuvantly and 2 patients with R1 resections following neoadjuvant GTX.

The distribution of the K-*ras* mutations in our patients is seen in **Table 1**. There is no difference between the adjuvant and neoadjuvant groups with respect to age, gender, wild type, mutations in K-*ras*, and recurrence. There were 10 dead (35%) in neoadjuvant group and 7 dead (26%) in adjuvant group ($p=0.56$). The median survival of the neoadjuvant group is 54. 7 months (95% CI; 41–70). The adjuvant group has been evaluated for a shorter period of time making the median survival indeterminate; but, so far the survival curves of the two groups appear similar.

Only 11 patients (20%) had wild type K-*ras*. Of the 44 patients (80%) with K-*ras* mutations, the two most common mutations were in the second guanosine of codon 12. This changed the glycine to aspartic acid (GAT) in 27 (61%) patients or to valine (GTT) in 11 (25%) patients. There were only 2 patients (5%) with a cysteine substitution (TGT) and 5 patients (11%) with an arginine substitution (CGT). Serine (AGT) and alanine (GCT) substitutions were not found. All K-*ras* mutations occurred at codon 12. No patient had a mutation at codon 13. The DNA sequence at codon 61 was not assessed.

The survival based on the type of K-*ras* mutation is seen in **Figure 1**. Valine and aspartic acid substitutions appear to have the same survival. The median survival for those with an aspartic acid substitution is 54.6 months (95% CI; 45-64). Patients with aspartic acid or valine have a trend to a shorter survival (OS; 54.7 months, 95% CI; 44.8-64.5) compared to wild-type K-*ras* or substitutions to arginine or cysteine where the median survival has not been reached ($p=0.09$) **(Figure 2).** Also patients with aspartic acid or valine substitutions appear more likely recur (OR; 1.353, 95% CI: 0.774-2.360) and die (OR; 1.520, 95% CI; 0.623-3.711) compared to the others. Early deaths from surgical mortality or medical complications, not progressive pancreatic cancer, and the small sample size limit the statistical analysis.

DISCUSSION

Kirsten *ras* (K-*ras*) is a small GTP binding protein with GTPase activity [23]. K-*ras* undergoes an activating conformational change when it binds GTP. This conformational change exposes a binding site for bringing other proteins together to effect signal transduction. K-*ras* is active as long as it binds GTP. While K-*ras* has intrinsic GTPase properties, the rate of hydrolysis is slow. Two additional

Table 1. Comparison of patient demographics.

	Neoadjuvant	Adjuvant	Stats
Age mean (range)	63(45-78)	64 (38-78)	0.667(U)
Gender (F/M)	15/13	11/16	0.422(x^2)
K*ras* (pos/neg)	22/6	22/5	1.00 (x^2)
K*ras* subtype			
Aspartic	13	12	
Valine	4	7	
Cysteine	1	1	
Arginine	4	2	
Serine	0	0	
Recurrence	14	15	0.789(x^2)
Death	10	7	0.563 (x^2)
OS	54.7mon	Not reached	P=1.57
Total	**28**	**27**	

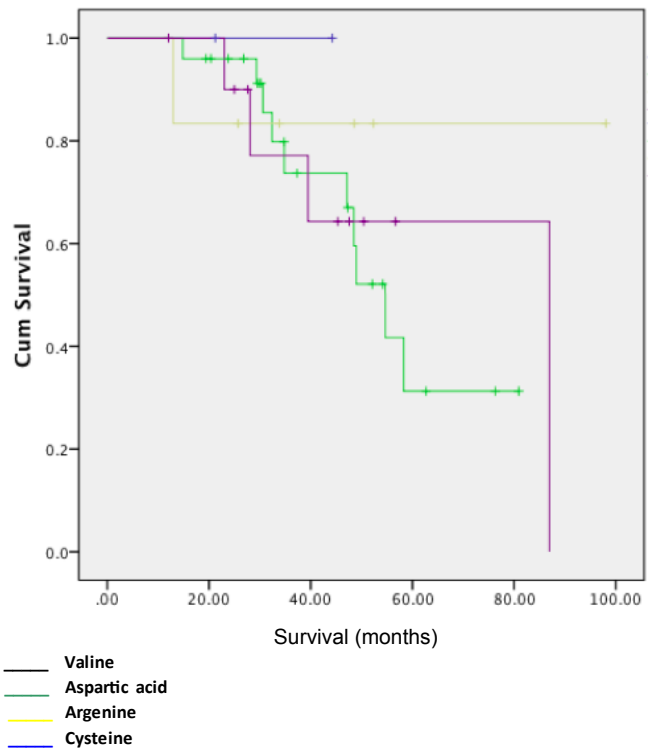

Figure 1. Survival based on type of K-ras mutation. Survival for valine and aspartic acid mutations are superimposed for the first 50 months. The late death for valine is due to new primary--adenocarcinoma of the lung. Survival for argenine and cystine mutations separate from those of valine and aspartic acid by 35 months.

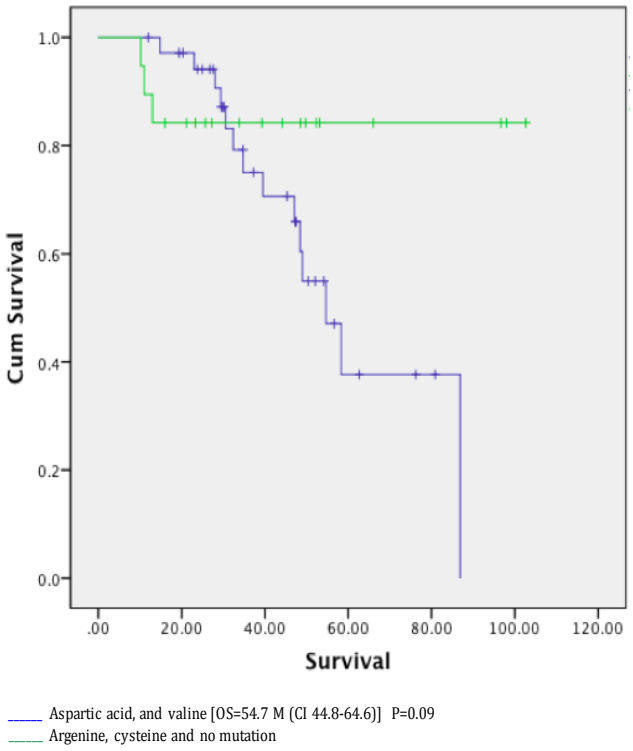

Figure 2. Survival aspartic acid and valine vs wild and other mutations. The long-term survival for aspartic acid and valine mutations in K-ras appears worse than the survival for wild-type, cysteine or argenine mutations.

proteins—the guanosine exchange factors (GEP's) and RGS's--assist in the control of this important molecule. Guanosine exchange factors are needed to remove GDP from inactive K-*ras* so it can be re-activated by binding GTP. RGS proteins augment lysis of bound GTP to GDP to inactivate K-*ras* faster, thus decreasing the duration of active K-*ras*.

While there are 6 potential mutants at codon 12, only 3 are commonly found in PDA. Eighty-percent involve a transversion of the second guanosine to either adenosine (50%) or thymidine (30%) **(Table 2).** Transition of the second guanosine to cytosine (0.2%) is either a rare event or it may not be mutagenic. Transition of the first guanosine to cytosine (12%) is more common than transversion of the first guanosine to adenosine (1%) or thymidine (5%). This distribution of K-*ras* mutation differs markedly from the mutation frequency in lung cancer or colon cancer. In lung cancer, 44% of K-*ras* mutations are transversions of the first guanosine, but almost exclusively to thymidine not adenosine **(Table 2)**. In colon cancer, mutations in codon 13 are almost all transversions to the bulky aspartic acid amino acid and account for 20% of the K-*ras* mutations. Codon 13 mutations are quite rare in pancreatic cancer. Whether by transversion or transition, mutations in K-*ras* that confer a very small side-chain on the new amino acid like serine-OH--or alanine--CH_3--are uncommon in all three malignancies—0 to 2.7% of all cancers. This suggests that smaller mutations may not, or only weakly, inhibit lysis of GTP.

Even though some mutagenic agents may have a predilection for a guanosine depending on the adjacent nucleotides [24], the distribution of the mutations among pancreatic, lung and colon cancers is unlikely to be attributable to the mutagenic agent. While the major mutagen in cigarette smoke is found in the pancreatic duct fluid of smokers [25], the prevalent cysteine substitution found in lung cancer is uncommon in PDA. Thus, either there is a very specific mutagen for each cancer or involvement or other factors which may account for the frequency of types of K-*ras* mutations. These other factors include expression of DNA repair enzymes and/or selection by the expressed RGS protein.

The RGS proteins have a common motif - an arginine finger that promotes de-phosphorylation of GTP [1]. A bulky mutation at amino acids 12, 13 or 61 interferes with the ability of the arginine finger of the RGS to augment GTP lysis. The carboxyl group of aspartic acid or the two methyl groups of valine are bulkier than the hydroxyl group of serine. While there is a paucity of data on the RGS proteins in most cancers, RGS 16 is up-regulated early in PDA, perhaps in an attempt to regulate mutant K-*ras* [26]. RGS 17 is up-regulated early in lung cancer [27]. Thus, differential tissue expression of RGS proteins could account for the observed frequency distributions of K-*ras* mutants among PDA, lung cancer, and colon cancer. Different mutations in K-*ras* have been shown to have an effect on survival in other malignancies. In lung cancer, Sun *et al*. [19] noted that those with valine substitutions had a longer survival than those with aspartic or cysteine substitutions. As there are on average over 360 mutations in non-small cell lung cancer, other mutations could be confounding the effect on survival [28]. In colon cancer, Al-Mulla *et al*. [29] suggested that valine substitutions do worse than others, but only 26 cases of Duke's D carcinomas were analyzed. In PDA, there are many fewer mutations than in lung cancer, on average less than 70 [30]. K-*ras* mutated pancreatic cancers have a shorter survival than wild type K-*ras* when erlotinib is part of the regimen [24, 25]. However, the reports did not analyze the specific types of mutation to see if all mutations confer the same prognosis.

In our PDA patients treated with GTX, there is a trend for survival to negatively correlate with the "bulkiness" of the amino acid replacing glycine **(Figure 1)**. This trend was not statistically significant; but, most patients are still alive and early post-operative deaths confound the survival data. With a longer time to follow these patients, the survival differences may reach statistical significance for aspartic acid or valine as compared with other substitutions **(Figure 2)**. Given the frequency of the various K-*ras* mutations, at least 200 patients, all treated with the same regimen, are needed to assess the statistical significance of each mutation on survival.

SUMMARY

The frequency of specific K-*ras* mutations differs among different types of cancers. Bulky substitutions of

Table 2. K-ras mutatuion in pancreatic, lung and colon cancer.

Pancreatic cancer	Wild(%) Gly	CGT(%) aRg	TGT(%) Cys	AGT(%) Ser	GTT(%) Val	GCT(%) Ala (A)	GAT(%) Asp (D)	Codon 13
Burmer [4] (n=20)	4 (20)	8 (40)	2 (10)	0	4 (20)	1 (10)	1 (10)	
Capella [5] (n=68)	17 (25)	5 (7)	6 (9)	1	14 (21)	0	24 (35)	1--D
Grunwald [6] (n=63)	16 (25)	15 (24)	0	0	15 (24)	1 (1)	16 (25)	
Mariyama [7] (n=12)	4 (33)	2 (17)	0	0	2 (17)	0	4 (33)	
Motojuma [8] (n=41)	5 (12)	4 (10)	1 (2.5)	0	7 (17.5)	0	24 (60)	
Nagata [9] (n=40)	3 (8)	2 (15)	1 (3)	0	9 (24)	0	24 (63)	1--D
Smit [10] (n=30)	2 (7)	1 (3)	10 (33)	0	8 (23)	0	9 (30)	
Hruban [11] (n=82)	14 (17)	8 (10)	3 (4)	0	24 (29)	0	33 (40)	
Wei [12] (n=30)	5 (17)	4 (13)	4 (13)	0	11 (43)	0	5 (17)	1--C
Kitago [13] (n=13)	1 (8)	0	0	0	6 (46)	0	6 (46)	
Kulmann [14] (n=25)	12 (48)	0	0	0	1 (4)	0	10 (40)	--
Kim [25] (n=135)	65 (48)	7 (5)	3 (2)	0	19 (14)	0	41(30)	0
Schultz [16] (n=160)	34 (21)	13(8)	0	6 (4)	46 (29)	0	60 (38)	1-D
Shin [17] (n=234)	108(46)	13 (5.6)	4 (1.7)	1 (0.4)	34(14.5)	0	73(31)	1-S
Sherman (n=55)	11(20)	5 (9)	2 (4)	0	11 (20)	0	27 (48)	0
Total (n=1099)	301(30)	87(7.7)	36 (4.5)	8 (0.8)	213 (21)	2 (0.2)	357(35)	5(0.5)
% of mutant		12%	5%	1%	30%	0.20%	50%	0.70%
Lung Cancer	**Wild(%)**	**CGT(%)**	**TGT(%)**	**AGT(%)**	**GTT(%)**	**GCT(%)**	**GAT(%)**	**Codon 13(%)**
Dogan [18] (n=2529)	1859 (74)	8(0.3)	261 (10)	8 (0.3)	141(60)	74(30)	114 (50)	8 (0.3)
Sun [19] (n=632)	595 (94)	0	9 (1.4)	1 (0)	10 (1.6)	3(0.1)	33 (5)	1--LYS
Tsao [20] (n=253)	134 (53)	4 (1.6)	51 (20)	3 (0.1)	29 (19)	8 (2.3)	10 (0.7)	6 (0.2)
Total (n=3414)	2588 (76)	12(0)	321(9)	12(0)	180 (5)	85(2)	157(5)	15 (0)
% of mutant		1.6	44	1.6	25	12	22	2
Colon Cancer	**Wild(%)**	**CGT(%)**	**TGT(%)**	**AGT(%)**	**GTT(%)**	**GCT(%)**	**GAT(%)**	**Codon 13(%)**
Brink [21] (n=737)	465 (73)	0	16 (2.2)	16(2.2)	68(93)	16(2.2)	74(10)	ASP(D),56(7.7)
Neumann [22] (n=1018)	621 (71)	0	32(3.1)	27(2.7)	86(8.5)	23(2.3)	142 (14)	79(7.8)
Total (n=1755)	1086 (72)	0	48 (2.7)	47 (2.4)	154 (8.8)	39 (2.2)	216 (13)	135 (13)
% of mutant		0	7	7	23	6	32	20

Wild GGT=Glycine (G)--H; CGT=Arginine (R) C-C-C-NH-C$(NH)_2$; TGT=Cysteine (C) C-SH; AGT=Serine (S) C-OH; GTT=Valine (V) C-$(CH3)_{2;}$ GCT=Alanine (A) CH_3; GAT=Aspartic (D) C-COOH

glycine to aspartic acid or valine may drive the PDA while the rare, less bulky substitutions to serine or alanine may not cause cancer. In our small series, there is a trend for patients with mutations in K-*ras* causing aspartic acid or valine substitutions to have a higher recurrence rate and a shorter survival.

More analyses of the specific K-*ras* mutations and RGS protein expression on treatment response and survival are needed to confirm our observations.

Conflict of interest

The authors have no conflict of interest to declare.

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