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Kras mutations and p53 overexpression in pseudomyxoma peritonei: association with phenotype and prognosis

Shreya Shetty, MBBS,[a] *Peter Thomas, PhD,*[a,*] *Bala Ramanan, MBBS,*[a] *Poonam Sharma, MD,*[b] *Venkatesh Govindarajan, PhD,*[a] *and Brian Loggie, MD*[a]

[a] *Department of Surgery, Creighton University Medical Center, Omaha, NE*
[b] *Department of Pathology, Creighton University Medical Center, Omaha, NE*

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ABSTRACT

Background: Little information exists on *Kras* mutations and p53 overexpression in pseudomyxoma peritonei (PMP). These genetic alterations are associated with poorer prognoses in colorectal cancer. We postulated that these mutations might be more frequent in high-grade (HG) PMP (peritoneal mucinous carcinomatosis) *versus* low-grade (LG) PMP (disseminated peritoneal adenomucinosis/peritoneal mucinous carcinomatosis), for which survival differences are well documented.

Methods: We collected data retrospectively on patients with PMP of appendiceal origin tested for *Kras* mutation (commercial assay) and p53 overexpression (immunohistochemistry). We used Fisher's exact test, chi-square test, and Kaplan-Meier survival curves for analysis.

Results: Of 64 cases with *Kras* mutations, 25 were classified as LG and 39 as HG PMP. Median age at diagnosis was 53 ± 11.5 y. We detected *Kras* mutations in 37 of 64 patients (57.8%). In LG PMP, 15 of 25 (60%) were *Kras* mutant *versus* 22 of 39 (56.4%) in HG PMP ($P = 0.80$). Nearly 89% of mutations were seen in codon 12. We noted overexpression of *p53* in 44.3% (86 of 194) of patients overall, which was significantly different between LG PMP and HG PMP: 35.5% (37 of 104) *versus* 54.4% (49 of 90), respectively ($P = 0.009$). *Kras* mutations did not affect prognosis. Overexpression of p53 was associated with a worse outcome.

Conclusions: *Kras* mutation and p53 overexpression rates are comparable to those of colorectal adenomas and mucinous colorectal cancer. Codon 12 mutations may be associated with mucin production. *Kras* mutation status is not prognostic for overall survival. Overexpression of p53 was significantly correlated with female sex, higher-grade disease, and worse survival.

1. Introduction

Pseudomyxoma peritonei (PMP) refers to the progressive intra-abdominal accumulation of mucinous, jellylike material. This is associated with mucin-producing, neoplastic, epithelial implants along peritoneal surfaces and involves the omentum and organ surface areas. It is a rare condition with an estimated incidence of 1–2 per million per year [1]. Although considered a benign entity, it is well acknowledged that it progresses into massive intra-abdominal fluid collections, causing bowel obstruction and nutritional compromise. Pseudomyxoma peritonei also has the potential in some

* *Corresponding author.* Departments of Surgery and Biomedical Sciences, Creighton University, 2500 California Plaza, Criss III Room 254, Omaha, NE 68178. Tel.: +1 402 280 3921; fax: +1 402 280 1878.
E-mail address: peterthomas@creighton.edu (P. Thomas).
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patients to exhibit frank malignant behavior, as seen in peritoneal carcinomatosis of colorectal origin. The most common origin for PMP is the appendix [1–3].

Pseudomyxoma peritonei has been histologically classified into the following groups: disseminated peritoneal adenomucinosis (low grade [LG]), with scant mucinous epithelium and abundant extracellular mucin; disseminated peritoneal adenomucinosis with intermediate features (intermediate grade I), characterized by largely LG-grade appearing lesions with focal areas of well-differentiated carcinoma; and peritoneal mucinous carcinomatosis (high grade [HG]), with frank atypia, mucinous, gland-forming epithelium [4]. According to the *World Health Organization Classification of Tumors of the Digestive System, Fourth Edition*, an LG and HG system of nomenclature should be used [5]. The histological groups of disseminated peritoneal adenomucinosis and disseminated peritoneal adenomucinosis with intermediate features are thus classified as LG PMP, and peritoneal mucinous carcinomatosis is classified as HG PMP [6,7]. Studies by Bradley *et al* [6] have shown a worse prognosis among HG PMP, with a median 5-y survival of 37.7% compared with 62.5% in LG PMP.

Kras mutations (codons 12 and 13) and their impact on systemic chemotherapy options for metastatic colorectal cancer (CRC) have been extensively studied [8–12]. In many cases of PMP, surgical treatment (cytoreductive surgery and/or heated intraperitoneal chemotherapy) alone is either not warranted or not sufficient and systemic chemotherapy may be necessary. Mutations in p53, a tumor suppressor gene on chromosome 17p, and overexpression of the protein have been described in various malignancies including CRC [13,14]. Assuming that appendiceal tumors are a subset of CRC, as defined by their location, histology, and embryology, we investigated the frequency of *Kras* mutations and p53 overexpression in our set of PMP patients with appendiceal primaries, by conducting a retrospective chart review. We surmised that the frequency of *Kras* mutations and p53 overexpression would be similar to that seen in CRC.

The presence of *Kras* mutations in CRC is associated with a poor prognosis [15–17]. Similarly, p53 overexpression is associated with aggressive behavior and worse prognosis in CRC [18–22]. We postulated that patients with HG PMP with demonstrated worse prognosis [4] would show higher frequencies of *Kras* mutations and p53 overexpression compared with LG PMP.

2. Methods

2.1. Patient data

We collected data retrospectively from a chart review of all patients with PMP of appendiceal origin treated by a single surgeon at our institution from 2005 to 2011. Of 229 patients with a diagnosis of PMP, we tested 194 for p53 overexpression. We tested 64 patients for *Kras* mutations. No patients in this series were lost to follow-up and we observed all for at least 1 y. We reviewed clinicopathological findings and confirmed the pathological diagnosis by reviewing hematoxylin-eosin slides.

2.2. Mutation analysis

We performed a mutational analysis by manually microdissecting tumor tissue from 4-μm-thick unstained paraffin sections, using an adjacent hematoxylin-eosin–stained section as a guide. We isolated DNA from the tumor tissue using the DNeasy tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. We performed detection of codon 12 and 13 mutations in exon 1 of the *Kras* gene using shifted termination assay technology (Muctector 2; TrimGen Genetic Diagnostics, Inc, Sparks, MD). The assay can detect 12 common *Kras* mutations in codons 12 and 13. In the shifted termination assay reaction, mutant target sequences are identified and the detection primers are selectively extended with labeled nucleotides. The mutation signal is enriched by repeating the extension 20 times. This signal is then further amplified by an enzymatic reaction followed by colorimetric detection. The codon 12 mutations included in this assay are GGT to AGT, CGT, TGT, GAT, GCT, and GTT. The codon 13 mutations are GGC to AGC, CGC, TGC, GAC, GCC, and GTC. Shifted termination assay technology can detect mutations present in as few as 1% of cells.

2.3. Immunohistochemistry

We detected expression of p53 in tumor cells by immunohistochemistry. We stained 4-μm paraffin sections for p53 immunoperoxidase stain using p53-Ab antibody (Thermo-Scientific; Labvision, Kalamazoo, MI). Overexpression of p53 was reported if >10% of the nuclei stained positive for p53. Other studies have used higher percentages to determine P53 positivity, but the 10% cutoff rate is standard in our hospitals.

2.4. Statistical analysis

We used Fisher's exact test and the chi-square test to look for correlations between *Kras* mutation/p53 overexpression status and clinicopathological factors including age, sex, and histology of the tumor. We used the Kaplan-Meier method to estimate the overall survival (OS) (interval between diagnosis and death at last follow-up) in both subsets of patients, which we then compared using the log-rank test (SPSS 19 for Windows software; SPSS, Inc, Chicago, IL). A two-sided P value <0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological features

We tested for *Kras* mutations in 64 patients (Table 1),51.6% of whom were male (33 of 64) and 48.4% of whom were female (31 of 64). Median age at diagnosis was 53 ± 11.5 y. Among these patients, 39% (25 of 64) were classified as LG and 61% (39 of 64), including 21 patients with the SRC variant, were HG. Of 194 patients tested for p53 overexpression (Table 2), 49.5% were male (96 of 194) and 50.5% were female (98 of 194). Median age at diagnosis was 51 ± 12 y. We saw LG PMP in 53.6% of patients (104 of 194), and 46.4% (90 of 194) were HG PMP.

Table 1 – Clinicopathological associations of *Kras* mutation.

Patient data	Total ($n=64$)	$Kras^{mut}$ ($n=37$) (57.8%)	$Kras^{wild}$ ($n=27$) (42.2%)	P value
Age (y) at diagnosis (mean ± standard deviation)	52 ± 12	54 ± 12	48 ± 9	0.03
Sex				
Male	33 (52%)	20 (54%)	13 (48%)	0.8
Female	31 (48%)	17 (46%)	14 (52%)	
Grade				
LG	25 (39%)	15 (41%)	10 (37%)	0.8
HG	39 (61%)	22 (59%)	17 (63%)	

Overall, we detected *Kras* mutations in 57.8% of patients (37 of 64). Of the 35 patients with specific mutation data available, 88.6% (31 of 35) were in codon 12 and 11.4% (4 of 35) were in codon 13 (Table 3). We found *Kras* mutations in 60.6% of male patients (20 of 33) and 54.8% of female patients (17 of 31) ($P=0.8$). The mean age of patients with and without *Kras* mutations was 54 ± 12 and 48 ± 9 y, respectively ($P=0.025$). In LG PMP, 60% (15 of 25) were $Kras^{mut}$, *versus* 56.4% (22 of 39) in HG PMP ($P=0.8$). As Table 2 shows, we noted p53 overexpression in 44.3% of patients (86 of 194). We saw a gender difference in that 36.5% of male patients (35 of 96) and 52% of female patients (51 of 98) overexpressed p53 ($P=0.03$). There was no correlation between age and p53 overexpression. There was a significant difference in p53 overexpression between LG PMP (37 of 104 [35.6%]) and HG PMP (49 of 90 [54.4%]) ($P=0.009$). We noted no correlation between *Kras* mutation rates and rates of *p53* overexpression in the 62 patients tested for both.

3.2. *Survival*

Survival analysis did not show a difference in overall survival between patients with and without *Kras* mutations (median follow-up, 39 mo) (Fig. 1). Overexpression of p53 was associated with a significantly worse OS (median survival, 89 ± 12 mo *versus* 71 ± 12 mo) ($P=0.04$) (median follow-up, 47 mo) (Fig. 2). However, an analysis further stratified into LG PMP and HG PMP showed no statistically significant difference (Fig. 3).

4. Discussion

Little is known about *Kras* mutations and overexpression of p53 in PMP, although a large body of work exists about these in CRC. Mucinous appendiceal neoplasms are considered to be the most common origin of PMP [2,3,23]. Embryologically, the appendix and the cecum are derived from the same diverticulum of the caudal limb of the midgut loop. It is supplied by the appendiceal artery, a branch of the ileocolic artery. Incipient taenia coli (characteristic of the colon) are present in the longitudinal muscle coat at the base of the appendix. The mucous membrane is lined by columnar epithelium and resembles that of the rest of the colon. The main differences are the presence of lymphoid tissue in the submucous coat and the obvious differences in diameter: 1–2 cm compared with a mean diameter of 7.5 cm of the cecum [24]. Their shared embryonic origin, anatomy, and histology suggest the possibility that appendiceal neoplasms may be considered a subset of CRC. Our aims were to determine the frequency and clinicopathological distribution of *Kras* mutations and p53 overexpression in PMP, and to compare them to those of CRC.

The frequency of *Kras* mutations in our patients with PMP of appendiceal origin was 57.8%. This frequency is similar to the 55% reported in an earlier study that included 30 appendiceal carcinomas [25]. A more recent study by Austin *et al* [26] reported a *Kras* mutation rate of 61.3% in patients with peritoneal carcinomatosis of appendiceal origin. This is slightly higher than the rates observed in various studies in CRC, which range from 36% to 50% [10,16,27–29].

Mutations of *TP53* gene and overexpression of the protein p53 are frequent in CRC and range from 5% to 30% in colorectal adenomas [30–34] and to 50% to 75% in adenocarcinomas [33–35]. Appendiceal neoplasms have infrequent *TP53* gene mutations [25,36,37] and a lower rate of loss of the allele 17p, which is the location of the *TP53* gene [3]. Our data demonstrated a frequency of p53 overexpression (44.3%) between the two groups in CRC that was slightly higher than the rates reported in appendiceal neoplasms. This higher rate could be because our laboratory reported overexpression of p53 if >10%

Table 2 – Clinicopathological association of p53 overexpression.

Patient data	Total ($n=194$)	p53 overexpressed ($n=86$) (44.3%)	p53 not overexpressed ($n=108$) (55.7%)	P value
Age (y) at diagnosis (mean ± standard deviation)	51 ± 12	51 ± 12	50 ± 12	0.56
Sex				
Male	96 (49%)	35 (41%)	61 (56%)	0.03
Female	98 (51%)	51 (59%)	47 (44%)	
Grade				
LG	104 (54%)	37 (43%)	67 (62%)	0.009
HG	90 (46%)	49 (57%)	41 (38%)	
Kras mutation				
$Kras^{mut}$	36 (19%)	14 (16%)	22 (20%)	1.00
$Kras^{wild}$	26 (13%)	10 (12%)	16 (15%)	

Table 3 – Type of *Kras* mutation.

Codon	Number of mutations ($n = 35$) (%)	Type of mutation (n)	LG PMP ($n = 14$)	HG PMP ($n = 21$)
12	31 (88.6%)	Gly to Asp (20)	10	10
		Gly to Val (8)	2	6
		Gly to Cys (3)	1	2
13	4 (11.4%)	Gly to Asp (2)	0	2
		Gly to Arg (1)	1	0
		Gly to Cys (1)	0	1

Amino acid abbreviations: GLY = glycine; Asp = aspartic acid; Val = valine; Cys = cysteine; Arg = arginine.

of tumor nuclei stained for p53, as opposed to >50% reported by Kabbani *et al* [25].

The age and sex of the patient did not correlate with the presence of mutated *Kras* gene. Similar findings have been reported in an earlier smaller study of appendiceal carcinoma [25] as well as CRC [28,29,38]. Our data suggest a correlation between female gender and p53 overexpression, contrary to findings in earlier studies in CRC [39], which found no such correlation between p53 overexpression and gender.

Most appendiceal epithelial neoplasms are mucin-producing tumors. A study by Szych *et al* [3] on synchronous ovarian mucinous neoplasms and appendiceal mucinous neoplasms found *Kras* mutations in 100% of tumors producing ascites and only in 69% of tumors that did not produce ascites. Studies in colorectal cancers have shown trends toward *Kras* mutations occurring with greater frequency in mucinous tumors compared with nonmucinous tumors [40–42]. Similar findings have also been reported in ovarian cancers [43]. In particular, codon 12 mutations in CRC have been shown to be associated with a mucinous phenotype, and codon 13 mutations with more aggressive biology and worse outcome [44]. Our data showed a high frequency of *Kras* mutations in the LG PMP group, which is associated with greater mucin production. Codon 12 was affected in 88.6% (31 of 35) of the mutations *versus* 11.4% (4 of 35) seen in codon 13 (Table 3). These findings

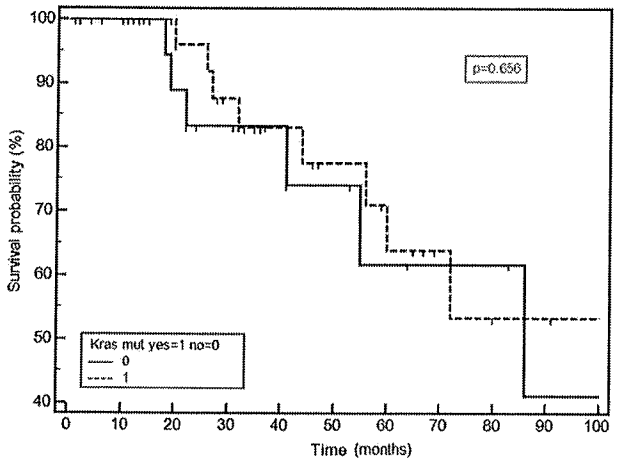

Fig. 1 – Kaplan-Meier curves comparing overall survival in $Kras^{mut}$ *versus* $Kras^{wild}$.

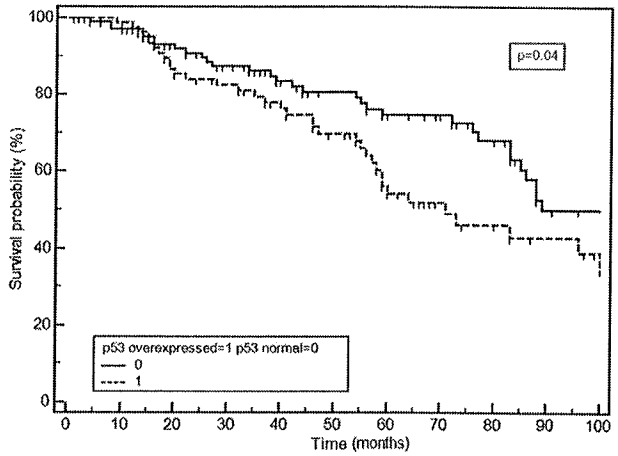

Fig. 2 – Kaplan-Meier curves comparing overall survival in patients with and without overexpression of p53.

support the suggestion [3] that *Kras* mutations, specifically mutations in codon 12, appear to be associated with mucin production [44].

Right-sided CRC and appendiceal neoplasms share a common embryology, anatomy, and histology. Stang and Kluttig found similar surface area–adjusted rates of carcinomas in the appendix and the ascending colon, which hints at a similar etiology [45]. The average age at diagnosis in appendiceal neoplasms is younger at 52 ± 11.5 y in our data, compared with CRC (71.3 y) [46]. The frequency of *Kras* mutations, which occur earlier in tumorigenesis, is comparable to that found in CRC. The frequency of *TP53* gene mutations and p53 overexpression, which occur later in tumorigenesis [28,47] is lower in colorectal adenomas (5%–32%) [30–32] and is comparable to our finding of 38%. Mucinous appendiceal neoplasms have a lower rate of loss of

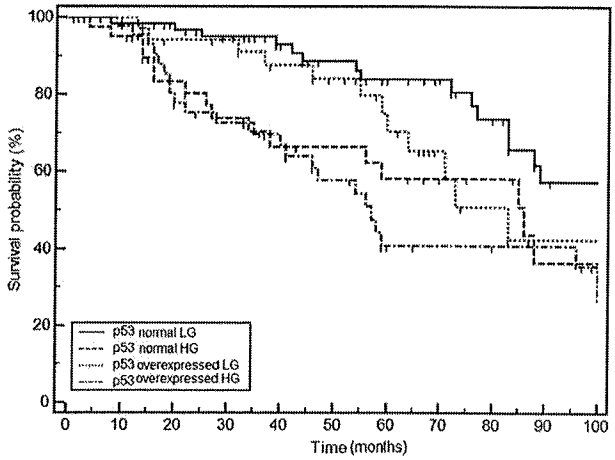

Fig. 3 – Kaplan-Meier curves comparing overall survival in patients with and without overexpression of p53 further stratified by grade of PMP. The P value of survival difference between p53 overexpressors and p53 normal in LG PMP is 0.11, and in HG PMP is 0.40.

the allele 17p, which is the location of the *TP53* gene [3]. In addition, a lower rate of p53 overexpression has been demonstrated in mucinous and right-sided CRC (16%–25% and 52%, respectively) compared with distal CRC (73%) [33,35,48–50]. Our data show a significantly higher rate of p53 overexpression (54.4%) in HG PMP compared with LG PMP (35.6%), which correlates with the comparatively aggressive behavior of the former. These facts support the hypothesis that appendiceal cancers are an earlier manifestation of CRC that acquire the early mutations along the adenoma-carcinoma sequence, including *Kras* [28,29]; but because of the limited size and volume of the appendix, they progress sooner to obstruction, rupture, or invasion than in the larger and more distensible colon. If located in the colon, they progress silently, gain the subsequent mutations along the adenoma-carcinoma sequence and manifest as frankly malignant tumors. Along with the similarities between malignant appendiceal neoplasm and mucinous CRC, differences include microsatellite instability characteristics that are frequent in CRC, particularly in hereditary non polyposis colorectal cancer, but are infrequent in mucinous appendiceal neoplasms (Loggie BW, unpublished manuscript) [25]. Most appendiceal cancers have mucinous characteristics and are LG, whereas a minority of CRC is classified as mucinous.

The SRC variant in adenocarcinomas, including the colon and appendix, is associated with a more aggressive biology and shorter survival [51]. In our study, we found the frequency of $Kras^{mut}$ to be lower in this subset compared with the other groups, which is similar to findings reported in SRC CRC [40,52]. Together with the finding of 0% $Kras^{mut}$ in the goblet cell carcinoid/adenocarcinoid group of appendiceal neoplasms [36,53], this appears to indicate a lower mutation rate in the more aggressive subsets of the disease. One explanation for the apparent lower rates of *Kras* mutations in HG PMP could be the presence of mutations other than those at codon 12 and codon 13 [54] that commercial assays do not generally detect.

Kras mutations have been shown to be a marker of poor survival in CRC, and are associated with a twofold increase in risk of cancer mortality [12,16,17,55]. In our study, there was no difference between OS rates in $Kras^{mut}$ *versus* $Kras^{wild}$ (Fig. 1). These results are similar to findings by Kabbani *et al* [25] in appendiceal neoplasms, Gillern *et al* [56] in colorectal cancer with peritoneal carcinomatosis, and Dobrzycka *et al* [43] in ovarian cancers. Reports of an association between p53 overexpression and survival are varied. Some studies show aggressive tumor biology and poor prognosis associated with p53 overexpression [18–22]. Others show an association of absent or low p53 expression with worse prognosis [17,57]. We found a significant difference ($P = 0.04$) in OS between patients who overexpressed p53 and those who did not (Fig. 2). p53 overexpression status within the grades showed no statistically significant difference (Fig. 3). However, this was a univariate analysis; a multivariate analysis with other potential risk factors may give different results.

This study was a retrospective study conducted by chart review and analysis of existing clinical reports of patients seen at our center over the past decade. We analyzed data from 64 patients tested for *Kras* mutations and 194 patients for p53 mutations. This constitutes a large sample size for a rare disease such as PMP. Our numbers are definitely comparable to other studies [58–60] on mucinous appendiceal neoplasms, in which sample sizes have varied from 21 to 134.

In conclusion, in our study, the rate of *Kras* mutations (57.8%) as well as p53 overexpression (44.3%) in mucinous appendiceal neoplasms is similar to those reported for CRC. *Kras* mutations do not appear to affect prognosis in these patients. Higher rates of *Kras* mutations in codon 12 may be associated with a mucinous phenotype, which is a characteristic of PMP [44]. Our data also provide correlative evidence to support this idea, in that 89% of our PMP patients have *Kras* mutations in codon 12.

Overexpression of p53 is associated with significantly worse OS and is seen more frequently in HG PMP.

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