# *KRAS* Mutation is a Local Tumour Event and Not a Field Change in Pancreatobiliary Tumours

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*Authors' contributions*

*This work was carried out in collaboration between all authors. Author CSW designed the study, gained ethics approval and funding, performed the statistical analysis, wrote the protocol and revised the manuscript. Author MDC coordinated the study, recruited patients, wrote the first draft, revised the manuscript, managed the analyses and performed the literature searches. Author DYC recruited patients and was involved in the analyses and was involved in the drafting and revisions. Author AR performed the pathological study and analyses and was involved in the drafting and revisions. Author GC conducted the genomic analyses of the specimens and was involved in the drafting and revisions. Authors CPT, ELN, JWC, PMD, and MEBS participated in the study design, reviewed and revised the manucript and literature searches. All authors read and approved the final manuscript.*

**Research Article**

## ABSTRACT

**Background:** *KRAS* mutation (KRM) is the earliest, most common mutation in pancreatic cancer. Accurate assessment of tumour KRM status in pancreatobiiary tumours is relevant in an era of targeted molecular therapies.
**Aim:** To assess KRM in tumour and non-tumourous margin tissue in patients undergoing a pancreatic resection.
**Study Design:** Original research, retrospective review of prospectively collected specimens.

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**Place and Duration of Study:** Patients who had undergone pancreaticoduodenectomy and distal pancreatic resection at the Royal Adelaide Hospital from 2011-2012 were consented for the study.

**Methods:** Patient demographics, background history and tumour details were collated. Tumour tissue and margin areas were macrodissected from FFPE tissue sections following identification by a pathologist. DNA was prepared from the tissue using the QIAamp FFPE Tissue kit (Qiagen GmbH, Hilden Germany). KRM at codons 12 and 13 was assessed using SNaPShot ™ (Applied Biosystems, Warrington UK) in tumour tissue and non-tumourous margin tissue.

Fourteen patients were included in the study. The median age of the patients in the study was 68 (range 57-86) years. The M : F ratio was 8 : 6.

**Results:** Twelve patients had adenocarcinomas (5 pancreatic; 4 ampullary, 3 biliary) and two had benign mucinous tumours. Six patients with adenocarcinomas had KRM (5@codon 12 and 1@codon 13). Margin tissue was negative for KRM in all the tested patients (p<0.016 Fisher) particularly, in those with tumour KRM.

Tumours with KRM were associated with larger tumours 30(22-65) mm vs 20(15-35) mm [median(range)](p = .045 − MW-U). Nodal disease occurred in 6/6 with KRM vs 2/6 without KRM (p = .61 − Fisher).

**Conclusions:** KRM is a local tumour event and not a field change. This suggests that testing for KRM should be reliant on tumour tissue and not surrounding normal margin tissue. KRM was associated with larger malignant tumours and a trend towards nodal disease.

## PRESENTED AT

1) The Australian Health and Medical Research Congress (AHMRC) 2012, Adelaide, Australia.
2) The Australian and New Zealand Hepatobiliary (ANZHPBA) Meeting 2012, Queensland, Australia.

## 1. INTRODUCTION

The *KRAS* oncogene encodes a protein responsible for signaling in the mitogen activated (MAP)-kinase pathway of intracellular signal transcription. *KRAS* mutation results in continual activation of the protein with consequent continual stimulus for cellular proliferation. *KRAS* mutation (KRM) is the earliest and most common mutation in pancreatic cancer [1,2]. *KRAS* point mutations at codon 12 are present in approximately 85-95% of pancreatic cancers [3,4,5]. The mutations are generally a single amino acid substitution in codon 12 or less frequently codon 13. A single amino acid substitution from glycine (G) to aspartic acid (D) at codon 12 is seen in up to 95% (60-95%) of pancreatic ductal cancers [6,7].

The aim of this study was to assess KRM in resected pancreatobiliary tumours and the non-tumourous margin tissue such that molecular changes within the tumour and surrounding tissue could be mapped. This was to assess if genomic alterations reflected a field change within the entire pancreas or if these changes occurred only in tumour tissue.

## 2. METHODOLOGY

The study was approved by the Royal Adelaide Hospital Human Research Ethics Committee. We recruited patients from the Royal Adelaide Hospital Hepatopancreatobiliary Surgery Unit, using our web-based database. Patients who had undergone pancreaticoduodenectomy and distal pancreatic resection at our institution from 2011-2012 were identified. Patient demographics, background history and tumour details were collated. Tumour tissue and non-tumourous margin tissue was selected by a single pathologist.

Tumour tissue and margin areas were macrodissected from FFPE tissue sections following identification by a pathologist. DNA was prepared from the tissue using the QIAamp FFPE Tissue kit (Qiagen GmbH, Hilden Germany). Plasma DNA was isolated from 3mL K2EDTA plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen GmbH, Hilden Germany). KRM at codons 12 and 13 was assessed using a primer extension assay SNaPshot $^{TM}$ (Applied Biosystems, Warrington UK). SNaPshot $^{TM}$ uses fluorescent dideoxynucleotides (ddNTPs) as the detection nucleotides. The region of interest of the *KRAS* gene is amplified by polymerase chain reaction (PCR) and the resultant product is purified by the removal of excess nucleotides and primers. For the primer extension step, detection primers and SNaPshot reaction mix, containing fluorescent ddNTPs, buffer and enzyme, which are then added to the purified PCR product. Each detection primer is extended by a single fluorescent nucleotide which, following alkaline phosphatase purification is detected by capillary electrophoresis. The results are analysed using fragment analysis software.

Statistical analysis was performed using the Fischer exact test for categorical outcomes and the Mann-Whitney U test (MW-U) for continuous variables. A $p< .05$ (2-sided) was taken as the least of statistical significance.

## 3. RESULTS

Fourteen patients were recruited and consented for the study. Of these, 13 patients had undergone a pancreaticoduodenectomy and one patient had a distal pancreatectomy. The median age of the patients in the study was 68 (range 57-86) years. The M : F ratio was 8 : 6. Of the 14 patients, 12 patients had adenocarcinomas (5 pancreatic; 4 ampullary, 3 biliary cancers) and 2 patients had benign mucinous tumours (BMT) (Table 1).

I. TUMOUR KRM STATUS
    Six patients (6/12) with adenocarcinomas had KRM in their tumour tissue (5 in codon 12 and 1 in codon 13). The mutations and base substitutions are delineated in Table 1.
II. NON-TUMOROUS MARGIN KRM STATUS
    Non-tumourous margin tissue was negative in all patients with cancer regardless of their tumour KRM status. ($p = .014$ – Fisher).
III A) TUMOUR SIZE
    Malignant tumours with KRM were significantly larger than wild type tumours, 30 (22-65) mm vs 20 (15-35) mm [median (range)] ($p = .045$ – MW-U).
III B) NODAL STATUS
    Tumours with KRM were all node positive compared to wild type tumours 6/6 vs 2/6 ($p= .06$ – Fisher).
III C) VASCULAR INVASION
    Vascular invasion was noted in 4/6 with KRM compared to 2/6 with wild type.
III D) PERINEURAL INVASION
    Perineural invasion occurred in 5/6 in each group.

**Table 1. Demographics, tumour characteristics, and KRM status in tumour and non-tumour margin**

| No | Age | Gender | Operation | Tumour | Histology | Differentiation | Tumour Size (mm) | Lymph node status | Vascular invasion | Perineural invasion | K-Ras Mutation | |
|----|-----|--------|-----------|--------|-----------|-----------------|------------------|-------------------|-------------------|---------------------|----------------|---|
| | | | | | | | | | | | Tumour KRM (point base substitution) | Non-tumour margin KRM |
| 1 | 70 | M | PD | Ampulla | Adenocarcinoma | Well | 12 | 0/9 | N | N | No | No |
| 2 | 73 | M | PD | Ampulla | Adenocarcinoma | Mod-poor | 20 | 0/3 | Y | Y | No | No |
| 3 | 56 | M | PD | Bile duct | Adenocarcinoma | Mod | 16 | 0/6 | N | Y | No | No |
| 4 | 86 | M | PD | Bile duct | Adenocarcinoma | Well | 20 | 0/19 | N | Y | No | No |
| 5 | 74 | M | PD | Pancreas | Adenocarcinoma | Well | 35 | 6/27 | Y | Y | No | No |
| 6 | 70 | F | PD | Pancreas | Adenocarcinoma | Poor | 25 | 3/15 | N | Y | No | No |
| 7 | 57 | F | PD | Bile duct | Adenocarcinoma | Well | 22 | 1/11 | N | Y | c.34G>C (p.Gly12Arg) | No |
| 8 | 67 | F | PD | Ampulla | Adenocarcinoma | Mod-poor | 28 | 1/15 | N | N | c.38G>A (p.Gly13Asp) | No |
| 9 | 77 | M | PD | Ampulla | Adenocarcinoma | Poor | 30 | 3/15 | Y | Y | c.35G>A (p.Gly12Asp) | No |
| 10 | 64 | F | PD | Pancreas | Adenocarcinoma | Well | 30 | 17/18 | Y | Y | c.35G>T (p.Gly12Val) | No |
| 11 | 63 | M | PD | Pancreas | Adenocarcinoma | Poor | 65 | 1/16 | Y | Y | c.35G>A (p.Gly12Asp) | No |
| 12 | 69 | F | PD | Pancreas | Adenocarcinoma | Well | 35 | 4/9 | Y | Y | c.34G>C (p.Gly12Arg) | No |
| 13 | 62 | M | DP | Pancreas | Benign mucinous tumour | Well | 40 | 0/12 | N | N | No | No |
| 14 | 59 | F | PD | Pancreas | Benign mucinous tumour | Well | 60 | 0/7 | N | N | No | No |

*PD: Pancreaticoduodenectomy; DP: Distal pancreatectomy*

## 4. DISCUSSION

KRM was negative in non-tumorous margin tissue in all our patients, including patients who had KRM in tumour tissue. This sheds light on the mapping of molecular changes within pancreatic tumour tissue and the surrounding pancreatic parenchyma. The genomic mutations are therefore a local event within tumour tissue and not a widespread phenomenon.

In our series, tumours with KRM were larger and all had nodal disease. Fifty-percent of our patients had KRM in their tumour tissue. These results are comparable to another study that included periampullary tumours in addition to pancreatic cancers. Their overall incidence of KRM was 55% [8]. Oliveira-Cunha et al reported that in a hundred patients in their series with pancreatic and periampullary cancers, the incidence of KRM was 41.2%, and they went on to say that the true incidence of KRM may be far less common that previously reported [9]. They also found no correlation to survival.

It is interesting that although KRM is thought to occur in almost all pancreatic cancers, as an iniator to cancer progression or "sine quo non", studies have found prognostic significance of KRM in pancreatic cancer [2,10]. One study found that their incidence of KRM in their series of 272 pancreatic adenocarcinoma was 53.8%, and KRM was associated with poor survival [10]. KRM in inoperable pancreatic cancer has been reported to be independent negative prognostic factor and is associated with reduced survival [11]. It may well be that these observations support that perhaps the incidence of KRM is lower than previously thought.

Our assessment of margin tissue involved peritumoural normal margin tissue. Given that genomic mutation is a local tumour event, then a margin with positive KRM may have clinical relevance. Kim et al found KRM in 53% of their histologically negative margin tissue and found that this was associated with poor prognosis [12]. This is markedly different from our 0% in our margin assessment for important reasons. We evaluated histologically normal margin tissue for assessment of KRM, whereas Kim et al assessed histologically "negative" margin tissue. Their negative margin tissue was negative for cancer, but included pre-malignant lesions from low-grade to high-grade pancreatic intraepithelial neoplasia or PanIN, which account for this higher incidence of KRM and the discrepancy without findings.

Accurate assessment of KRM is imperative in an era of growing use of selective, targeted molecular and chemotherapeutic agents [13]. KRM status in lung and colorectal cancer, has led to advances in the management of these cancers, with personalised therapy based on KRM status [14,15]. This has included improved survival in patients with wild-type KRAS colorectal cancer undergoing anti-EGFR treatment [16]. The future holds promise for pancreatobiliary malignancies and potential treatment if we can extrapolate these advances to our practice.

## 5. CONCLUSIONS

KRM is a local event in pancreatobiliary tumours. KRM in our study was associated with larger malignant tumours and a trend towards nodal involvement. Accurate assessment of tumour KRM depends on study of tumour tissue as it is a local event.

## CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for this study.

## ETHICAL APPROVAL

The institutional ethics committee approved the study.

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## COMPETING INTERESTS

Authors have no competing interests to declare.

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