

# Values of mutations of K-ras oncogene at codon 12 in detection of pancreatic cancer: 15- year experience

De-Qing Mu, You-Shu Peng, Qiao-Jian Xu

**De-Qing Mu, You-Shu Peng**, Department of Surgery of the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou 310009, Zhejiang Province, China

**Qiao-Jian Xu**, Undergraduate Medical College of Zhejiang University, Hangzhou 310009, Zhejiang Province, China

**Correspondence to:** Dr. De-Qing Mu, Department of surgery of the Second Affiliated Hospital, Medical College of Zhejiang University, Hangzhou 310009, Zhejiang Province, China. samier-1969@163.com

**Telephone:** +86-571-87783762

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## Abstract

**AIM:** To summarize progress in the study of K-ras gene studies in pancreatic cancer and its potential clinical significance in screening test for early detection of pancreatic cancer, and to differentiate pancreatic cancer from chronic pancreatitis in recent decade.

**METHODS:** Literature search (MEDLINE 1986-2003) was performed using the key words K-ras gene, pancreatic cancer, chronic pancreatitis, and diagnosis. Two kind of opposite points of view on the significance of K-ras gene in detection early pancreatic cancer and differentiation pancreatic cancer from chronic pancreatitis were investigated. The presence of a K-ras gene mutation at codon 12 has been seen in 75-100% of pancreatic cancers, and is not rare in patients with chronic pancreatitis, and represents an increased risk of developing pancreatic cancer. However, the significance of the detection of this mutation in specimens obtained by needle aspiration from pure pancreatic juice and from stools for its utilization for the detection of early pancreatic cancer, and differentiation pancreatic cancer from chronic pancreatitis remains controversial.

**CONCLUSION:** The value of K-ras gene mutation for the detection of early pancreatic cancer and differentiation pancreatic cancer from chronic pancreatitis remains uncertain in clinical practice. Nevertheless, K-ras mutation screening may increase the sensitivity of FNA and ERP cytology and may be useful in identifying pancreatitis patients at high risk for developing cancer, and as an adjunct with cytology to differentiate pancreatic cancer from chronic pancreatitis.

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## INTRODUCTION

Cytology, for detection of pancreatic cancer is limited for the definitive diagnosis by a low sensitivity and accuracy<sup>[1-4]</sup>. K-ras oncogene as a cytological adjunct can be date back to ten years ago<sup>[5]</sup>. K-ras oncogene has been found to be activated by specific point mutations restricted to condon 12 in 75 to 100%

of pancreatic cancers, but rare in chronic pancreatitis<sup>[6-8]</sup>. Attempt at detection of such genetic change have been made in plasm<sup>[9-12]</sup>, pancreatic juice samples<sup>[13-16]</sup>, fine needle tumour aspirates<sup>[17-19]</sup>, and stool samples<sup>[20-22]</sup>. However, at present there exists completely different viewpoints about these preliminary results. In this article we review previous studies of prospective follow-up of patients with chronic pancreatitis positive for K-ras gene at codon 12 and evaluated its significance mutation in detecting early pancreatic cancer and in differentiating pancreatic cancer from chronic pancreatitis.

## K-RAS GENE MUTATIONS

K-ras gene is the locus for the c-k-ras protooncogene, lying on chromosome 12p12, and is about 45 000 bp in lenth. It encodes for a 2.0 kb transcript which is highly conserved across species, and is translated into the p21-ras protein. These proteins are located in the plasma membrane and could transduce growth and differentiation signals from activated receptors to protein kinases within the cell<sup>[23]</sup>. p21-ras protein are in a weak GTP-bound, active state, thereby altering transduction into the cell<sup>[23]</sup>. The majority of mutations have been found at K-ras codons 12 and 13, and to a lesser extent, at codon 61<sup>[24,25]</sup>. These mutations are somatic rather than in the germ-line, and consist of single base-pair substitutions which lead to the change of one amino acid in the protein. The wild-type K-ras gene encodes for glycine (GGT) at codon 12, and the most common amino acid substitution is aspartic acid for glycine (46%), followed by valine (32%), arginine (13%), cystein (5%), serine (1-2%), and alanine (<1%). These mutations presumably result in the K-ras protein product (p21-ras) remaining in the GTP-bound, activated state, which may promote cell proliferation. The reason for the specificity of these mutations to condon 12 is not entirely clear. This location appears to confer higher change in the p21 ras protein's 3 dimensional structure and ras-GAP binding characteristics.

K-ras mutations were thought to be an early event in pancreatic tumorigenesis<sup>[24]</sup>. Is it true? In animal models, weekly exposed to doses of nitrosamines and serially sacrificed at 8,12, 14, 16, or 24 weeks, K-ras mutations were found in 26% of hyperplastic lesions,46% of papillary hyperplastic lesions,76% of carcinomas *in situ*, and 80% of invasive pancreatic carcinomas<sup>[25]</sup>. In human pancreas, K-ras point mutation at codon 12 is found hyperplasia without dysplasia<sup>[26,27]</sup>, severe dysplasia<sup>[26]</sup> or carcinoma *in situ*<sup>[26]</sup> even within multifocal hyperplastic foci of ductal epithelium in histologically normal pancreas<sup>[26]</sup>. K-ras point mutations rate seems to increase regularly from normal duct cells to flat or papillary hyperplasia observed in chronic pancreatitis tissue<sup>[26]</sup>. Such epithelial lesions mainly associated with chronic pancreatitis are thought to be potentially premalignant ductal lesions. However, there existed completely different objections about the significance of presence of the presence of K-ras mutations<sup>[28,29]</sup>.

## CLINICAL SIGNIFICANCES OF K-RAS MUTATIONS

### *K-ras mutation and pancreatic cancer screening test*

Chronic pancreatitis (CP) was considered to be a risk factor

for the development of pancreatic carcinoma (PC)<sup>[30,31]</sup>. The detection of K-ras mutations in the duodenal or pancreatic juice has been held to be a tool for pancreatic cancer early diagnosis. One application of K-ras mutation testing for pancreatic cancer is the screening of pancreatic juice samples. In order to evaluate the significance of K-ras mutation, a prospective follow-up of study of patients with CP in the detection of early pancreatic cancer and K-ras mutations at codon 12 has been carried out. In Berthelemy's series<sup>[32]</sup>, two patients free of pancreatic mass had no evidence of pancreatic cancer, when K-ras was first studied, but developed tumors 18 and 40 months, respectively, after identification of K-ras mutations. Boadas *et al*<sup>[33]</sup> collected 50 patients' pancreatic juice samples, including 49 patients with CP and one patient proceeding from a PC family screening. K-ras mutation was detected by PCR-RFLP (restriction fragment length polymorphism). As a result, K-ras mutation was detected in 8/49 patients (16%) with CP, one of whom developed PC during the follow-up, allowing surgical resection of early cancer.

Another interesting clinical application of K-ras mutation testing for pancreatic cancer is the screening of stool samples. Caldas and colleagues<sup>[20]</sup> tested stool samples from patients with pancreatic adenocarcinoma, cholangiocarcinoma, and chronic pancreatitis for K-ras mutations. Stool samples were frozen, then approximately 1 g was resuspended in buffer, extracted in phenol-chloroform, and precipitated. PCR products resulting from this template were subcloned and plaque hybridizations using specific oligonucleotide probes for different K-ras mutations. Positive stool samples were found in 6 of 11 patients with carcinoma of pancreas (all of which had mutations in paraffin-embedded tumor sections), in none of 3 chronic pancreatitis specimens (all negative on paraffin sections) and in 2 of 3 cholangiocarcinomas (one positive and one negative on paraffin sections, the one negative stool sample was positive for K-ras mutation on paraffin section). When mutations were detected in stool specimens, 5 of 6 cases had the same amino acid substitution as seen in the paraffin-embedded sections. Negative controls using *E. coli* DNA were performed, and no mutation was detected. The frequency of K-ras mutations approximates only 40-50% in pancreatic cancer, it was presumed that these mutations were derived from exfoliated cells from pancreatic cancer. The sensitivity and specificity of K-ras mutation as a screening tool were not high, and therefore its clinical application is limited at present.

The significance of codon 12 K-ras mutation in CP is still debated. Queneau *et al*<sup>[34]</sup> examined the pure pancreatic juice from the diagnosed 36 patients with chronic pancreatitis based on the criteria of Cambridge and Marseilles classifications. Ten patients were positive for K-ras point mutation at codon 12, pancreatic cancer was discovered at an invasive stage in two patients, respectively in 7 and 17 months after disclosure of a K-ras mutation. Unfortunately the disclosure of pancreatic cancer after follow-up of patients with chronic pancreatitis positive for a K-ras mutation was not associated with an early stage at diagnosis and an improved prognosis. Lohr *et al*<sup>[35]</sup> reported that K-ras gene mutation was found in 6 of 66 patients with chronic pancreatitis, pancreatic neoplasm occurred in none of the mutation in patients over a mean follow-up period of 26 (4-54) months, no pancreatic cancer developed during follow-up. Analysis of K-ras gene mutation seemed to have little use for detection pancreatic neoplasm in patients with chronic pancreatitis<sup>[36,37]</sup>.

Conflicting reports have raised the question whether K-ras gene mutations in chronic pancreatitis are related to the development of pancreatic neoplasm. According to epidemiological data: about 4%<sup>[30]</sup> patients with chronic pancreatitis will develop pancreatic cancer during the course

of the disease. Interestingly, the risk of cancer seemed predominant in the first few years of chronic pancreatitis<sup>[31]</sup>. No clinical, morphological, or histocytological information could help differentiate patients with cancer from the others during the follow-up of chronic pancreatitis. Moreover, K-ras mutations were found in 63-71% of the microdissected specimens of benign mucous cell hyperplasia of the pancreatic ductal epithelium with chronic inflammation<sup>[26]</sup>, only about 1% of patients harboring such precursor lesions were believed to develop pancreatic adenocarcinoma. Disclosure of a K-ras point mutation in chronic pancreatitis seemed not sufficiently predictive of malignant transformation, and even in some cases of chronic pancreatitis early occurrence of p53 gene but not K-ras mutations has been reported<sup>[38]</sup>. Therefore, detection of K-ras mutation cannot be recommended at this time for screening pancreatic cancer.

#### *Utility of K-ras mutation in differentiating pancreatic cancer from chronic pancreatitis*

**Fine needle aspiration (FNA) of pancreatic cancer and K-ras gene** The knowledge that pancreatic cancer frequently harbor K-ras mutations has been applied to the histopathologic diagnosis of pancreatic mass. Because cytologic diagnosis from FNA depends upon accurate sampling, a negative test does not rule out carcinomas. However, in equivocal cases, detection of K-ras mutation might help to conform the diagnosis of pancreatic cancer. Urgell<sup>[19]</sup> prospectively examined a total of 84 consecutive having a pancreatic mass who were clinically suspected of pancreatic cancer for the confirmation and follow-up of their chronic pancreatitis. Fine needle aspiration specimens were taken for both cytology and the presence of K-ras mutations. By cytology and/ or the patient's clinical course (death within a year), the authors concluded that the final diagnoses were 60 pancreatic cancers, 2 mucinous cystic tumours, 4 endocrine tumours and 6 other malignancies, 10 chronic pancreatitis, 1 acute pancreatitis and 1 tuberculosis. Cytology offered a conclusive diagnosis in 63 of 84 (75%) cases, inconclusive report in 21 cases (25%) (9 cases with suspicious cells and 12 cases with insufficient material). The presence of malignant cells in the FNA samples from patients with pancreatic cancer was 65%. No false-positives were detected in the remaining 24 conclusive FNAs. K-ras mutations were detected in 46 of 60 FNA samples (77%) from cancers and no mutations were detected in the remaining 24 FNA samples. The combined molecular and cytological approach offered an 88% sensitivity with a 100% specificity. A similar study was performed by Urban and colleagues<sup>[17]</sup>, who examined 20 consecutive patients undergoing FNA for pancreatic lesions. Sixteen samples were successfully amplified by PCR, 10 (of 11) pancreatic cancers and 1 (of 1) cystadenocarcinoma had K-ras mutations, while no mutations were found in 3 patients with chronic pancreatitis and 1 patient with an islet cell tumor. Two patients having a benign cytologic diagnosis had K-ras mutations and were ultimately proven to have pancreatic cancers. Pathologic diagnosis alone was established in 13 of 16 cases, but when pathology and K-ras mutations were combined, all the 16 patients were correctly diagnosed. When adequate samples were obtained, the sensitivity rate was 92% and specificity was 100% in this study. K-ras mutation in combination with cytologic analysis might be a helpful tool for the diagnosis of pancreatic carcinomas.

**K-ras point mutation at codon 12 in pancreatic juice** Differentiating pancreatic adenocarcinoma from chronic pancreatitis can be quite difficult particularly when both diseases coexist. Cytological distinction between chronic pancreatitis and pancreatic cancer is occasionally difficult because chronic pancreatitis can induce morphological changes

similar to those seen in well differentiated adenocarcinomas. In addition, pancreatic juice can induce the cell injury and degradation duo to disadvantages of digestive utility induced by various proteases. The main advantages of the gene technique are its objectivity and the lack of dependence on cell integrity and number. Van Laethem *et al*<sup>[39]</sup> analysed prospectively the presence of these mutations in brushing samples collected during ERCP in 45 patients (26 males, 19 females) showing a dominant stricture of the main pancreatic duct at pancreatography. Twenty-four were pancreatic adenocarcinoma, sixteen were chronic pancreatitis, and five intraductal mucin hypersecreting neoplasms. Twenty of 45 patients presented equivocal ERCP findings that did not permit a definite diagnosis. K-ras mutations at codon 12 were detected. Result were compared with those provided by routine brush cytology. A definitive diagnose was established for each patient. Mutations were detected in 20 of 24 patients with pancreatic adenocarcinoma, but in none of the chronic pancreatitis patients and intraductal mucin hypersecreting neoplasms, irrespective of their locations. By contrast, only 13 (Carcinoma of the pancreas head) of 24 pancreatic adenocarcinoma were detected by cytological examination, which yielded four false negative and seven (Carcinoma of the body or tail of pancreas) non-contributive results. sensitivity (duo to the neoplastic site lying in the body or tail of pancreas), specificity, and accuracy of molecular biological and cytological methods were 83-76%, 100-83%, and 90-58%, respectively. Notably the mutations could be detected in six patients with small tumours. Watanabe<sup>[40]</sup> found K-ras mutations from the pancreatic juice in 11 of 20 (55%) pancreatic cancer patients, and in none of 18 patients with chronic pancreatitis. Fifty percent of tumours of the pancreatic head (4 of 8), 67% of the body (6 of 9), and 33% of the tail (1 of 3) had K-ras mutation. When tumours were examined by size, 50% of T1 (1 of 2), 43% of T2 (3 of 7), 50% of T3 (3 of 6), and 80% of T4 (4 of 5) had K-ras mutations. Tada<sup>[41]</sup> found K-ras mutations in the pancreatic juice from 6 of 6 patients with pancreatic cancer, and 1 with intraductal papillary neoplasm. In contrast, 3 patients with chronic pancreatitis were negative for mutations, and this method was sensitive enough to detect 3-30 mutant copies of K-ras in the presence of 3000 000 normal copies of the gene (which would be the equivalent to 0.01 ng of mutant DNA in 1 mg of total DNA).

On the contrary, Pugliese *et al*<sup>[42]</sup> believed that cytology rather than mutation was useful in the diagnosis of pancreatic cancer. In their one report cytological examination, and detection of K-ras point mutation were performed, sensitivity of cytology was 74%, that of mutations in 87% in cancer and 40% in chronic pancreatitis. The specificity for cytology and mutation was 100% and 60% respectively. Combining cytology with mutation analysis increased the sensitivity to 93% but reduced the positive predictive value. Matsubayashi *et al*<sup>[43]</sup> reported the occurrence of K-ras point mutations at codon 12 and compared it with cytology in pancreatic juice. K-ras point mutations at codon 12 were detected in seven of 14 (50%) pancreatic cancers, in four of 10 (40%) mucin-producing tumors, in four of 13 (31%) chronic pancreatitis, and in two of (10%) pancreas without definite disorders. K-ras point mutations were detected in nine of 18 (50%) pancreatic juice samples containing cancer cells, in eight of 18 (44%) pancreatic juice samples containing atypical cells, but in none of such samples containing only normal cells. It was concluded that K-ras mutations were not detected in pancreatic cancer exclusively, they could be detected in pancreatic cancer, and also other diseases.

Combining K-ras point mutation at codon 12 with cytology could increase its sensitivity and specificity. In Tada's series<sup>[44]</sup>, cytology and semiquantitatively mutant analysis were

performed using EUS-FNA specimens as well as in pancreatic juice in 34 patients with pancreatic masses (26 cancers and 8 chronic pancreatitis). Quantitative analysis of mutant ras gene supplemented with cytology was more effective in differential diagnosis of pancreatic cancer.

Evidence to date suggests that the progression to pancreatic adenocarcinoma is multifactorial, and perhaps undefined, genetic mutations play major roles. Molecular profiling indicated a large number of genes were differentially expressed in pancreatic cancer and normal pancreas, but the differences were not significant between pancreatic cancer and chronic pancreatitis<sup>[45]</sup>. Combining other marker seemed to contribute to increase the sensitivity and specificity of detection of K-ras gene point mutation at codon 12 for differentiation of the pancreatic cancer from chronic pancreatitis. Myung *et al*<sup>[46]</sup> examined the telomerase activity and K-ras gene mutations in pancreatic juice from 31 patients. Of them 12 had pancreatic cancer, 11 had chronic pancreatitis, and 8 were control patients. K-ras gene mutation was positive in 75% (9 of 12) of pancreatic cancers and in 27% (3 of 11) of cases of chronic pancreatitis but in none of the control patients. Telomerase activity was detected in 92% (11 of 12) of pancreatic cancers and in 18% (2 of 11) of cases of chronic pancreatitis. By combining these two methods, the specificity rose to 100%. Alterations of other genes such as p16, have been described in pancreatic cancer, but they have also been found in chronic pancreatitis<sup>[47]</sup>. Positive for the two mutations could therefore be particularly predictive of malignant conditions and helpful to differentiating pancreatic cancer from chronic pancreatitis. An identical approach has been evaluated with K-ras gene and p53 or p16 alterations, and has given promising results<sup>[47, 48]</sup>.

## CONCLUSIONS

Usefulness and status of K-ras gene point mutation at codon 12 are contradictory, the mutation indicates a preneoplastic condition, or cancer at an early stage, and therefore disclosure of K-ras gene point mutation in chronic pancreatitis seems not sufficiently predictive of malignant transformation, and its detection in combination with clinical and morphological follow-up should not be recommended at this time for screening pancreatic cancer, but can be used as an adjunct to cytology for the differentiation of pancreatic cancer from chronic pancreatitis. The finding of K-ras point mutations at codon 12 in cytology or biopsy samples from pancreatic mass can not specifically confirm the diagnosis of pancreatic cancer. Only raises the possibility for early surgical intervention if these patients are at high risk of developing pancreatic cancer in the future. Further studies are needed to define the value of K-ras mutation screening in patients with other evidences suggesting the presence of pancreatic cancer. Chronic pancreatitis may be positive for the mutation in the absence of cancer, and pancreatic cancer does not necessarily have the mutation. The ultimate goal is to detect pancreatic cancer at a early stage and avoid unnecessary pancreaticoduodenectomies for chronic pancreatitis.

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