Detection of Ki-ras oncogene mutations in pancreatic diseases : helpful or irrelevant ?

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Ki-ras mutations are found in a large number of human cancers. In pancreatic ductal adenocarcinoma, the mutations are commonly restricted to the codon 12 and are observed in 85% to 100% of these tumours. These findings come from surgical specimens in the beginning of the nineties, using techniques based on Polymerase Chain Reaction (PCR) (1-4). In the era of molecular biology, such techniques were planned to be routinely used in order to improve the diagnostic approach of ductal adenocarcinoma by detecting codon 12 mutations in pancreatic juice or Wirsung brushings collected during ERCP, in material obtained by fine needle puncture, or in the stool and in the blood (5-10). An increasing number of reports have thereby enthusiastically claimed that Ki-ras mutations are specific and early markers of pancreatic carcinoma development (7,10). However, by increasing the number of patients studied and by improving the sensitivity of PCR techniques, some concerns have recently appeared as to the specificity and the relevance of this marker in patients presenting with a pancreatic disease.

In this review, we will aim to define the usefulness but also the limits of this diagnostic genetic approach of pancreatic cancer and its differentiation from chronic pancreatitis. The "Ki-ras profile" of other pancreatic neoplasms will be also reviewed.

Ki-ras mutations in ERCP samples in the diagnosis of pancreatic ductal adenocarcinoma

ERCP is highly accurate for diagnosing and differentiating malignant biliary and pancreatic duct strictures with a sensitivity and a specificity of approximately 80% (11-12). However, in the majority of the cases, a cytological confirmation is mandatory before the final management decision. The reported yield of brush cytology for detecting malignant strictures remains low ranging from 40% (bile duct) to 70% (pancreatic duct), while its specificity is high (more than 90%) (13-16). However, some authors have reported better sensitivity and accuracy ((80%) using endobiliary forceps biopsy or needle aspiration which require considerable technical skill and which are not routinely applicable to the pancreatic duct (17-18).

We and others have previously reported in preliminary studies that Ki-ras analysis from specimens obtained during ERCP may help the clinician to differentiate between malignant and benign strictures and is more accurate than conventional cytology (6,8,19). The overall reported sensitivity was approximatively 80 to 85% and the specificity near 100% (6,7,8,19).

These data relied however on a limited number of patients with pancreatic cancer and controls represented by normal patients or patients suffering from chronic pancreatitis.

In a further prospective study, we have studied 125 patients undergoing ERCP in our department for biliopancreatic strictures (49 with pancreatic adenocarcinomas and 76 with chronic pancreatitis). Based on this larger series, we were able to confirm the better sensitivity of the Ki-ras analysis compared to conventional cytology. Sensitivity of Ki-ras analysis from both pancreatic duct or biliary duct (when the pancreatic duct was not accessible for brushing) was superior to that of cytology (80% and 71% versus 51 and 35% respectively). In contrast, Ki-ras mutations were detected in 19/76 patients (25%) with chronic pancreatitis ; these mutations were similar to those observed in pancreatic adenocarcinoma (20). None of these patients were found to have a tumour and therefore the calculation of the specificity of the Ki-ras analysis shows a decreased value (72%), lower than that recorded for cytology which was 100%. The overall accuracy of both methods was however similar (70 vs 74%) and the combination of the two analyses was able to improve only marginally the diagnostic approach by the brushing technique (20). This study underlines that results of Ki-ras analysis from ductal brushings should be carefully interpreted when chronic pancreatitis coexists and that finding a mutant ras should not prompt the clinicians to propose immediate surgical resection in such patients.

Ki-ras mutations in chronic pancreatitis : early marker or irrelevant of neoplastic change ?

Mucinous cell hyperplasia are frequently seen in pancreatic tissue of patients suffering from chronic pancreatitis. Such lesions have further been subdivided into simple hyperplasia, papillary hyperplasia and

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atypical hyperplasia. All these lesions can be encountered in the vicinity of cancers and are therefore considered by many authors as premalignant lesions (21-22). Ki-ras mutations were found in mucinous cell hyperplasia lesions present around the carcinoma or from pancreatic tissue of chronic pancreatitis obtained after microdissection with a high frequency (more than 60%) and were identical to those observed in carcinomas (10,23). Such results indicated a clonal origin of cells comprising the mucinous cell hyperplasia and suggesting a neoplastic and/or pre-cancerous nature (10,23-24).

Ki-ras mutations were also detected in two patients with chronic pancreatitis who developed pancreatic carcinoma 18 and 40 months later supporting the fact that they can represent an early marker of malignant development (7).

In our department, we have started a prospective study in patients with chronic pancreatitis but without any evidence of pancreatic tumour as assessed by CT-scan, endoultrasonography and brush or needle aspiration cytology. In the 76 first patients evaluated, Ki-ras mutations were found in pancreatic brushings obtained during ERCP in 19 of them (25%) (20,25). All patients are prospectively followed up and within a mean follow-up period of 6 months, none has shown the development of carcinoma, suggesting that the presence of a mutant ras does not predict *rapid* cancer development. Recently, Furuya *et al.* reported a series of 54 patients with chronic pancreatitis and observed a mutant ras in 20 of them (37%). After a mean follow-up period of 78 months, they did not record any cancer development in those patients (26).

When chronic pancreatitis coexists, interpretation of such analysis should be thus extremely careful since the relevance of mutant ras is presently still unknown and requires a longer follow-up period. In any case, Ki-ras gene probably does not represent the unique link between chronic pancreatitis and pancreatic cancer.

In the future, the study assessment of other genetic markers such as microsatellite instability (MIN) or telomerase activity in pancreatic brushing products or pure juice will be probably able to improve the diagnostic and prognostic approaches based on molecular biology methods (27-28).

Ki-ras mutations in hyperplastic duct cells of the pancreas without pancreatic disease

Mucinous cell hyperplasia can be also found in the pancreas free from pancreatic cancer or chronic pancreatitis, i.e. in the elderly as shown in autopsy studies. Recently, a provocative study from Tada *et al.* reported the presence of different types of Ki-ras codon 12 mutations in hyperplastic foci from autopsy samples. However, the most frequent mutation type among ductal hyperplasia was different from those observed in the case of chronic pancreatitis or cancer (29). Although this type of report may lead to increase the confusion regarding the role and the relevance of ras mutations in the pancreas, this study showed that some types of mutants have a low potential for malignant transformation and that hyperplastic foci with these types might remain or regress more frequently than those with other types of mutations.

Ki-ras mutations in other pancreatic neoplasms

The biological behaviour as well as the potential aggressiveness of intraductal papillary mucinous neoplasms (IPMN) remain poorly understood. Little is known about their genetic profile. Ki-ras mutations are found in a large majority of this type of neoplasms ranging from 31% to 90% comparable to the frequency observed in ductal adenocarcinoma (30-32). However, due to the limited number of recorded cases, the exact significance of mutant ras remains unknown and controversial although mutant ras seems to be more frequent in the case of high grade dysplasia or carcinoma than in the case of absence of any dysplasia in our personal series (32).

Data regarding the presence of Ki-ras mutations in cystic neoplasms such as serous or mucinous cystadenoma or carcinoma are extremely limited although mutant ras are also detected in these types of neoplasms.

Finally, Ki-ras mutations are also found in neuroendocrine tumors and with a high frequency (60 to 100%) in ampullary tumors (33).

Conclusions

Ki-ras analysis in brushing samples collected during ERCP provides a good sensitivity but a disappointing specificity in pancreatic diseases especially when chronic pancreatitis coexists. In contrast, cytology appears to remain a highly specific but poorly sensitive method while the combination of the two methods can moderately improve the diagnostic approach of an equivocal stricture observed at ERCP.

Whether Ki-ras activation represents a potential and early marker of cancer development remains to be carefully assessed by long-term prospective follow-up of patients harbouring a mutant ras. Currently, there is no clinical justification to propose pancreatic resection on the unique basis of finding a mutant ras.

The ras mutations detected in microscopic foci of mucinous hyperplasia do not solve the questions about the precursor lesions of pancreatic carcinomas. Further investigations are required to clarify the molecular profile of hyperplasia and its possible link with cancer development.

References

1. ALMOGUERA C., SHIBATA D., FORRESTER K., MARTIN J., ARNHEIM N., PERUCHO M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell*, 1988, **53** : 549-554.
2. HRUBAN R.H., VAN MANSFELD A.D., OFFERHAUS G.J., VAN WEERING D.H., ALLISON D.C., GOODMAN S.N., KENSLER T.W.,

BOSE K.K., CAMERON J.L., BOS J.L. Ki-ras oncogene activation in adenocarcinoma of the human pancreas : a study of carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and ellele-specific oligonucleotide hybridization. *Am. J. Pathol.*, 1993, **143** : 545-554.

3. TADA M., OMATA M., OHTO M., Clinical application of ras gene mutation for diagnosis of pancreatic adenocarcinoma. *Gastroenterology*, 1991, **100** : 233-238.
4. LEMOINE N.R., JAIN S., HUGHUES C.M., STADDON S.L., MAILLET B., HALL P.A., KLOPPEL G. K-ras oncogene in preinvasive pancreatic cancer. *Gastroenterology*, 1992, **102** : 230-236.
5. TADA M., OMATA M., KAWAI S., SAISHO H., OHTO M., SAIKI R.K., SNINSKI J.J. Detection of ras gene mutations in pancreatic juice and peripheral blood of patients with pancreatic adenocarcinoma. *Cancer Res.*, 1993, **53** : 2472-2474.
6. VAN LAETHEM J.L., VERTONGEN P., DEVIÈRE J., VAN RAMPELBERGH J., RICKAERT F., CREMER M., ROBBERECHT P. Detection of c-Ki-ras gene codon 12 mutations from pancreatic duct brushings in the diagnosis of pancreatic tumours. *Gut*, 1995, **36** : 781-787.
7. BERTHELEMY P., BOUISSON M., ESCOURROU J., VAYSSE N., RUMEAU J.L., PRADAYROL L. Identification of Ki-ras mutations in pancreatic juice in the early diagnosis of pancreatic cancer. *Ann. Intern. Med.*, 1995, **123** : 188-191.
8. KONDO H., SUGANO K., FUKAYAMA N., KYOGOKU A., NOSE H., SHIMADA K., OHDURA H., FUKUDA H., OHTSU A., YOSHIDA S., SHIMOSATO Y. Detection of point mutations in the Ki-ras oncogene at codon 12 in pure pancreatic juice for diagnosis of pancreatic carcinoma. *Cancer*, 1994, **73** : 1589-1594.
9. SHIBATA D., ALMOGUERA C., FORRESTER K., DURNITZ J., MARTIN S.E., COSGROVE M.M., PERUCHO M., ARNHEURIN N. Detection of c-K-ras mutations in fine-needle aspirates from human pancreatic adenocarcinomas. *Cancer Res.*, 1990, **50** : 1279-1283.
10. CALDAS C., HAHN S.A., HRUBAN R.H., REDSTON M.S., YEO C.J., KERN S.E. Detection of K-ras mutations in the stool of patients with pancreatic adenocarcinoma and pancreatic ductal hyperplasia. *Cancer Res.*, 1994, **54** : 3568-3573.
11. NIEDERAU C., GRENDELL J.H. Diagnosis of pancreatic carcinoma. Imaging techniques and tumor markers. *Pancreas*, 1992, **7** : 66-86.
12. LEE J.G., LEUNG J.W., BAILLIE J., LAYFIELD L.J., COTTON P.B. Benign, dysplastic a malignant-making sense of endoscopic bile duct brush cytology : results in 149 consecutive patients. *Am. J. Gastroenterol.*, 1995, **5** : 722-726.
13. RABINOVITZ M., ZAJKO A.B., HASSANEIN T., SHETTY B., BRAN K.M., SCHADE R.R. Diagnostic evaluation of brush cytology in the diagnosis of bile duct carcinoma : a study in 65 patients with bile duct strictures. *Hepatology*, 1990, **12** : 747-752.
14. FOUTCH D.G., KERR D.M., HARLON J.R., KUMMET T.D. A prospective, controlled analysis of endoscopic cytotechniques for diagnosis of malignant biliary strictures. *Am. J. Gastroenterol.*, 1991, **5** : 577-580.
15. FERRARI A.P., LICHTENSTEIN D.R., SLIVKA A., CHANG C., CARR-LOCKE D.L. Brush cytology during ERCP for the diagnosis of biliary and pancreatic malignancies. *Gastrointest. Endosc.*, 1994, **40** : 140-145.
16. NAKAIZUMI A., TATSUTA M., VEHARA H., YAMAMOTO R., TAKENAKA A. Cytologic examination of pure pancreatic juice in the diagnosis of pancreatic carcinoma. *Cancer*, 1992, **70** : 2610-2614.
17. SUGIYAMA M., ATOMI Y., WADA N., KURODA A., MUTO T. Endoscopic transpapillary bile duct biopsy without sphincterotomy for diagnosing biliary strictures : a prospective comparative study with bile and brush cytology. *Am. J. Gastroenterol.*, 1996, **91** : 465-467.
18. HOWELL D.A., BEVERIDGE R.P., BOSCO J., JONES M. Endoscopic needle aspiration biopsy at ERCP in the diagnosis of biliary strictures. *Gastrointest. Endosc.*, 1992, **38** : 531-535.
19. LEE J.G., LEUNG J.W., COTTON P.B., LAYFIELD U., MANNON P.J. Diagnostic utility of K-ras mutational analysis on bile obtained by endoscopic retrograde cholangiopancreatography. *Gastrointest. Endosc.*, 1995, **42** : 317-320.
20. VAN LAETHEM J.L., BOURGEOIS V., PARMA J., DELHAYE M., COCHAUX P., VELU TH., DEVIÈRE J., CREMER M. Relative contribution of Ki-ras gene analysis and brush cytology during ERCP for the diagnosis of biliary and pancreatic diseases. *Gastrointest. Endosc.*, 1997, in press.
21. CUBILLA A.L., FITZGERALD P.J. Morphological lesions associated with human primary invasive non-endocrine pancreas cancer. *Cancer Res*, 1976, **36** : 2690-2698.
22. KOZUKA S., SASSA R., TAHI T., MASSAMOTO K., NAGASAWA S., SAGA S., HASEGAWA K., TAKUCHI M. Relation of pancreatic duct hyperplasia to carcinoma. *Cancer*, 1979, **43** : 1418-1428.
23. YANAGISAWA A., OHTAHE K., OHASHI K., HORI M., KITAGAWA T., SUGANO H., KATO Y. Frequent c-Ki-ras oncogene activation in mucous cell hyperplasias of pancreas suffering from chronic pancreatitis. *Cancer Res.*, 1993, **53** : 953-956.
24. RIVERA J., ROLL C.J.N., GRAEME-COOK F., FERNANDEZ DEL CASTILLO C., SHU P., LAKEY N. Analysis of Ki-ras oncogene mutations in chronic pancreatitis with ductal hyperplasia. *Surgery*, 1997, **121** : 42-49.
25. VAN LAETHEM J.L., BOURGEOIS V., DELHAYE M., PARMA J., COCHAUX P., VELU TH., DEVIÈRE J., CREMER M. Ki-ras analysis during ERCP in patients with chronic pancreatitis : high prevalence but undetermined relevance. *Gastroenterology*, 1997, **112** : A90.
26. FURUYA N., KAWA S., AKAMATSU T., FURIHATA K. Long-term follow-up of patients with chronic pancreatitis and K-ras gene mutation detected in pancreatic juice. *Gastroenterology*, 1997, **113** : 593-598.
27. BRENTNALL T.A., CHEN R., LEE J.G., KIMMEY M.B., BRONNER M.P., HAGGITT R.C., KOWDLEY K.V., HECKER L.M., BYRD D.R. Micro-satellite instability and K-ras mutations associated with pancreatic adenocarcinoma and pancreatitis. *Cancer Res.*, 1995, **55** : 4264-4267.
28. ABRUZZESE J., ZANG L., LARRY L., CHIAO P., EVANS D. The use of telomerase activity for the early diagnosis of pancreatic cancer. *Pancreas*, 1996, **13** : 427 (Abstract).
29. TADA M., OHASHI M., SHIRATORI Y., OKUDAIRA T., KOMATSU Y., KAWABE T., YOSHIDA H., MACHINAMI R., KISHI K., OMATA M. Analysis of K-ras gene mutation in hyperplastic duct cells of the pancreas without pancreatic disease. *Gastroenterology*, 1996, **110** : 227-231.
30. SESSA F., SALCIA E., CAPELLA C., BANATO M., SCARPA A., ZAMBANI G., PELLAGATA N.S., RANZONI G.N., RICKAERT F., KLOPPEL G. Intraductal papillary-mucinous tumours represent a distinct group of pancreatic neoplasms : an investigation of tumour cell differentiation and K-ras, p53 and c-erb B2 abnormalities in 26 patients. *Virchows Archiv.*, 1994, **425** : 357-367.
31. TADA M., OMATA M., OHOTO M. Ras gene mutations in intraductal papillary neoplasms of the pancreas. *Cancer*, 1991, **67** : 634-637.
32. VAN LAETHEM J.L., DEVIÈRE J., HITTELET A.B., RICKAERT F., DELHAYE M., PARMA J., VELU TH., CREMER M. Identification of K-ras oncogene mutations in intraductal papillary mucinous tumours during ERCP : incidence and clinical interest. *Acta Gastroenterol. Belgica*, 1996, **59** : C47.
33. EBERT M., HOFFMANN J., STOCK-SCHNEIDER R., ROESSNER A., MALFERTHEINER P. K-ras gene mutations in rare pancreatic and ampullary tumours. *Digestion*, 1997, **58** : A-62.