Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: http://www.pjms.in/

Original Research Article

Immunohistochemical expression of K-ras protein and p53 in chronic pancreatitis and precursor lesions of pancreatic carcinoma- 5 year study in a tertiary care centre

Prasanth P S[1], Preethamol S[2,*]

[1]*Dept. of Radiology, SUT Academy of Medical Sciences, Trivandrum, Kerala, India*
[2]*Dept. of Pathology, Government Medical College, Thiruvananthapuram, Kerala, India*

ARTICLE INFO

Article history:
Received 24-04-2021
Accepted 18-08-2021
Available online 30-04-2022

Keywords:
Chronic pancreatitis
Pancreatic adenocarcinoma
IPMN
p53
Kras

ABSTRACT

Introduction: K-ras mutation is an early event and p53 mutation is a late event in the tumorigenesis pathway of carcinoma associated with Chronic Pancreatitis.
Aim : To identify the immunohistochemical expression of K-ras protein and p53 in surgical specimens of pancreas with chronic pancreatitis.
Materials and Methods: A 5 year study of chronic pancreatitis with associated pancreatic lesions received in the Histopathology Department, Government Medical College, Trivandrum. All cases of chronic pancreatitis, (n=60) were histopathologically examined along with the adjacent pancreatic tissue which showed precursor lesions. Immunohistochemical expression of K-ras protein and p53 in all cases were studied.
Results: In a total 60 cases of chronic pancreatitis, adjacent pancreatic tissue showed ductal adenocarcinoma in 20 cases (30%). Pancreatic Intraepithelial neoplasm (PanIN) was present in 6 cases accounting for 10%. 1 case was IPMN and one case was associated with serous cystadenoma.
7% of chronic pancreatitis showed K-ras mutations. Immunostaining for mutated p53 protein always was negative. p53 was positive in Pan IN 1A , Pan IN 1B, IPMN and carcinoma cells in 0 of 4(0%), 0 of 2(0%), 0 of 1(0%), and 18 of 20(90%), respectively, and K-ras was positive in 3 of 4 (75%), 2 of 2(100%), 1of 1(100%) and 12 of 20(60%), cases respectively.
Conclusion : Chronic pancreatitis and precursor lesions may progress to the development of pancreatic cancer. Further molecular studies for evaluation of oncogenes may be done in future for targeted therapy in pancreatic cancer.

1. Introduction

Pancreatic malignancy is the second leading cause of digestive cancer related death after colon cancer.[1–3] In 70-90% of cases patients presents with unresectable tumor.[4] Hence understanding the molecular pathogenesis of the neoplasm is of great importance. It has been noted that there is an increased incidence of development of pancreatic malignancy in patients with chronic pancreatitis.[5,6] Among the various genetic alterations suggested for pancreatic malignancy, in our study on chronic pancreatitis and precursor lesions we immunohistochemically determined the expression of K-ras and p53 to identify whether it can aid in the early detection of pancreatic cancer.

* *Corresponding author*.
E-mail address: drpreetha801@gmail.com (Preethamol S).

https://doi.org/10.18231/j.pjms.2022.015

2. Aims of the Study

To identify the immunohistochemical expression of K-ras protein and p53 in cases of chronic pancreatitis and precursor lesions of pancreatic carcinoma.

3. Materials and Methods

This retrospective descriptive study of five years was conducted in the Department of Pathology and materials were obtained from Surgical Departments, Govt. Medical College Hospital, Thiruvananthapuram after obtaining Institutional Ethical clearance.

The specimens we received included trucut biopsies, distal pancreatectomy and whipples resection. These tissues were fixed in 10% formalin, samples from representative areas were processed, stained with Hematoxylin and Eosin and studied under light microscope.

The study population included 60 patients with chronic pancreatitis of which 20 cases having pancreatic ductal adenocarcinoma in the adjacent pancreas.

The gross and microscopic features of chronic pancreatitis and lesions associated with it were studied and analysed. Immunohistochemical staining with K-ras and p53 was done in all cases. The expression of immunohistochemical staining with K-ras and p53 was also analysed in chronic pancreatitis and associated lesions in adjacent pancreas incuding pancreatic ductal adenocarcinoma.

3.1. Immunohistochemical staining

The 5 μm serial sections of each block were adhered to poly-L-lysine covered slides, and heated at 50°C for 1 hour. After paraffin elimination with xylene and a staged ethyl alcohol dehydration, sections were placed in methanol (300 ml) and hydrogen peroxide (10 mL) for 20 minutes in order to saturate endogenous peroxidase. After washing in water, sections were placed in citrate buffer solution and heated for 20 minutes. After cooling, samples were processed with TRIS buffer solution for 10 minutes. Sections were then incubated for 12 hours with p21 monoclonal Ab (Kras) AN434-5N-BIOGENEX diluted at a 1:50 ratio. After washing with TRIS buffer solution, sections were stained using the Universal LSAB peroxidase II kit SUPER SENSITVE POLYMER HRP/DAB and developed by diaminobenzidine.

A 1:150 dilution ratio of p53 antibody AN23 9-BIOGENEX was used for p53 immunohistochemical staining. The remaining procedure was identical to the K ras immunohistochemical staining process.

3.2. Immunohistochemical staining results

Positivity for p53 immunohistochemical staining was microscopically determined by identification of dark brown-stained nuclei and cytoplasmic positivity for Kras under 40x power. The sample was subjectively standardized as: few foci of positive staining was considered as focally positive (f+), fewer than 10% positive staining was 1+ fewer than 25% but greater than 10% positive staining was 2+, fewer than 50% but greater than 25% positive staining was 3+ and diffuse or greater than 50% positive staining was 4+. Nuclear staining was considered positive for p53 immunostaining and a score of 1+ or greater was considered positive. Kras immunostaining was cytoplasmic and a score of 1+ or greater was considered positive.

4. Results

We studied 60 cases of chronic pancreatitis with associated pancreatic lesions for their histopathological and immunohistochemical correlation. The results observed were as follows. Surgical specimens received were Trucut biopsy (28%), distal pancreatectomy (40%) or Whipples resection (32%). Age group varied from 14-78 years. Most common age group was between 41-60 yrs. Pancreatic ductal adenocarcinoma was noted in patients over 45yrs of age. Single case of serous cystic neoplasm was noted in 64yr female. Precursor malignant lesions were seen in all age groups .The male to female ratio was 7:5, showing slight male predominance. Most common site of chronic pancreatitis was head of pancreas in 39 cases accounting for 65% followed by tail of pancreas in cases accounting for 12%.

Histopathological study of adjacent pancreatic tissue is summarized in Table 1.

Table 1: Histopathological study of adjacent pancreas

Adjacent pancreas	No: of cases
Normal (excluding trucut biopsies)	43/43 (100%)
Pan IN 1A	4/60 (6 %)
Pan IN 1B	2/60 (3%)
IPMN	1/60 (1.6%)
Ductal adenocarcinoma	20/60 (30%)
Serous cystic neoplasm	1/60 (1.6%)

4.1. Immunohistochemical expression

Immunohistochemical study for Kras and p53 was done in all cases of chronic pancreatitis, Pan IN 1A and Pan IN 1B, IPMN, ductal adenocarcinoma and in adjacent normal pancreatic tissue.

The 60 specimens of chronic pancreatitis demonstrated different types of ductal lesions. Immunohistochemical expression of Kras and p53 in normal pancreatic tissue, chronic pancreatitis and precursor lesions is shown in Table 2.

Table 2: Immunohistochemical staining: summary

	Normal	Chronic pancreatitis	Pan IA	Pan IB	IPMN	PDAC
K-ras	0/43(0%)	4/60(7%)	3/4(75%)	2/2(100%)	1/1(100%)	12/20(60%)
P53	0/43(0%)	0/60(0%)	0/4(0%)	0/2 (%)	0/1(0%)	18/20(90%)

Serous cystic neoplasms show no immunostaining for p53 or Kras.

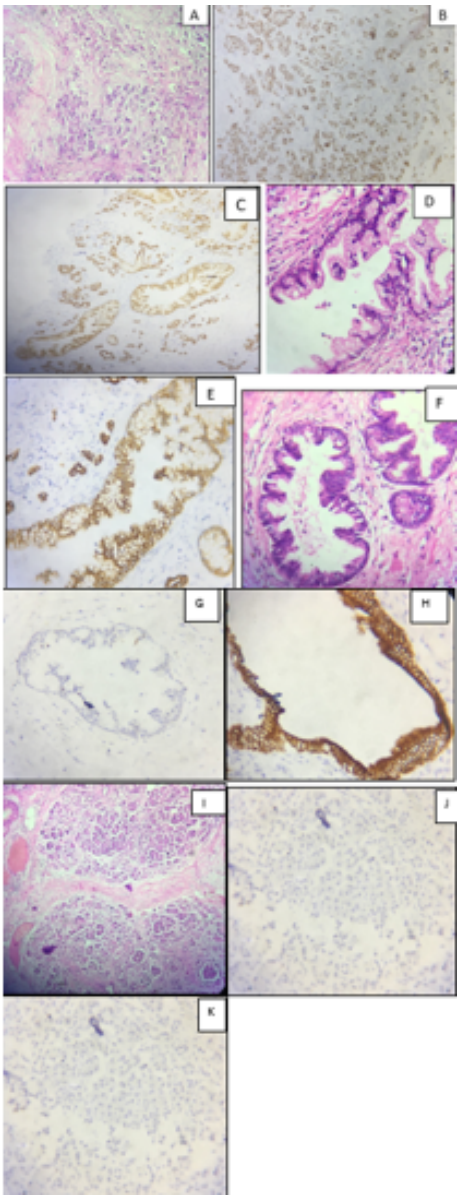

Fig. 1: A: H&E pancreatic cancer x200; **B:** Immunostaining for p53 in pancreatic cancer x200; **C:** Cytoplasmic staining with K-ras in both Pan IN and adjacent invasive adenocarcinoma K-ras ×200; **D:** H&E IPMN (intraductal papillary mucinous neoplasm x 200; **E:** Cytoplasmic staining with K-ras in IPMN ×200; **F:** H&E staining in PanIN 3; **G:** Negative staining for p53 in Pan IN3; **H:** Cytoplasmic staining with K-rasin Pan IN3 ×400; **I:** H & E and; J: p53 negative immunostaining in chronic pancreatitis x 200; **K:** K-ras immunostaining in chronic pancreatitis x 200

5. Discussion

From the molecular analysis of human cancers it is clear that cells acquire mutations in oncogenes and tumor suppressor genes while progressing to malignancies. For a cancer to develop it is said that around three to seven genetic mutations are required.[7] Similar to multistep carcinogenesis in colorectal cancer[8] in which colonic epithelial cells accumulate genetic defects when they progress from normal to adenomatous polyps to invasive carcinoma, pancreatic malignancies also seems to be associated with multiple mutational steps and specific genetic alterations.[9]

On the basis of morphological studies on pancreatic carcinoma a dysplasia - carcinoma sequence has been suggested. So genetic alterations similar to pancreatic carcinoma has been expected to be present in precursor lesions and chronic pancreatitis which has got an increased risk of developing carcinoma pancreas.[10,11]

In our study all cases of pancreatitis there are features of cellular dysfunction, glandular destruction and fibrosis, presumably increase cell turnover which is supposed to be an important increased risk factor for pancreatic cancer as in many other organs.[12]

IPMN are Intraductal mucin producing cystic neoplasms of pancreas with clear malignant potential. Pancreatic malignancy was associated in 60% of cases main duct IPMN.[13]

PanIN is the non invasive precursor of invasive carcinoma.[14,15] Pan IN constitutes a rare but surgically curable, localized disease with a good prognosis following radical resection.[16]

In patients with long-standing chronic pancreatitis, harboring pan IN with alterations in tumor suppressor genes like p16, may develop pancreatic ductal adenocarcinoma.[17]

Andea et al[18] found the rates of pan IN associated with normal pancreas to chronic pancreatitis and to ductal adenocarcinoma were 16%, 60% and 82% respectively and those of grade 3 panIN were 0%, 4%, and 40%, respectively. Pancreatic ductal intraepithelial neoplasia (PanIN) associated with chronic pancreatitis is a risk factor for the development of pancreatic adenocarcinoma.[14–16,19]

The progression of atypical ductal hyperplasia or carcinoma in situ to an invasive adenocarcinoma is well described in the literature, but the time of invasive transformation of the so-called Pan IN lesions is still unclear.

Ductal adenocarcinoma and its variants are the most common neoplasms in the pancreas, representing 85-90% of all pancreatic neoplasms.[20–22] Serous tumors represents 2-5% of all exocrine pancreatic tumours.[20]

The KRAS oncogene (chromosome 12p) is inactivated by point mutation in approximately 90% of pancreatic cancers, these mutations involve codons 12,13 and 61. The ras protein produced by wild type KRAS binds to GTPase activating protein (GAP) and regulates cell cycle progression via the mitogen activated protein kinase (MAPK) and AKT cascades.Activating mutations impair the intrinsic GTPase activity of the KRAS gene product, resulting in a protein that is constitutively active in intracellular signal transduction. Mutations in the KRAS gene are also one of the earliest genetic abnormalities observed in the development of pancreatic neoplasia.[23] In our study Kras was strongly and frequently expressed in pancreatic carcinoma and precursor lesions than in normal tissue similar to earlier study by Sakorafas et al.[24]

P53 is the most frequently altered gene in human cancer, which is closely associated with cell cycle regulation.[25] The p53 gene on chromosome 17p is bi-allelically inactivated in approximately 50%-75% of pancreatic cancers, almost always by a combination of intragenic mutation and loss of the second wild–type allele. Alterations of p53 protein function permits cells to bypass DNA damage checkpoints and apoptotic signals; in addition there is emerging evidence to suggest that loss of p53 function may contribute to the genomic instability observed in pancreatic cancers. Immunolabelling for nuclear accumulation of the p53 protein has a modest correlation with the mutation status of p53 gene. By immunohistochemistry, p53 accumulation is usually seen in advanced PanIN-3 lesions, which is consistent with p53 gene mutations being a late event in pancreatic cancer progression.[26–29]

In our study p53 expression was most frequently seen in cancerous lesions, rarely in (with less intensity) precursor lesions and not at all in chronic pancreatitis. Hence over expression of p53 can be used as a useful marker to differentiate carcinomas from dysplasia.

To conclude in this study we tried to analyse the multistep progression of pancreatic carcinoma from a normal pancreatic cell. We studied the histological changes in both malignant and precancerous lesions. These precancerous lesions were more frequently seen in association with pancreatic malignancy. Combining the results of immunohistochemical expression of both the malignant and nonmalignant specimens it is shown that excessive expression of oncogene product was more frequently seen in malignancy than in normal tissue. Ductal lesions in patients with chronic pancreatitis and precursor lesions exhibit K-ras mutations and are negative for p53 protein. So it can be concluded that K-ras mutation is an early event and p53 mutation is a late event in the tumorigenesis pathway of carcinoma associated with Chronic calcific Pancreatitis.[26–29]

Patients with chronic pancreatitis having normal p53 activity, may be good candidates for treatment with EGFR (epidermal growth factor receptor) inhibitors like Erlotinib, which may prevents or delays the development of pancreatic cancer. Further research on this aspect and molecular studies for evaluation of oncogenes may be done in future for targeted therapy in pancreatic cancer.

6. Sources of Funding

No financial support was received for the work within this manuscript.

7. Conflicts of Interest

No conflicts of interest.

References

1. Parkin DM, Bray F, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2002;55(2):74–108. doi:10.3322/canjclin.55.2.74.
2. Statistics, Epidemiology, and end Results Program: Cancer Statistics Review,2000-2004.
3. Oertel JE. The Pancreas :nonneoplastic alterations. *Am J Surg Pathol*. 1989;13(1):50–65.
4. Crist DW, Cameron JL. The current status of the Whipple operation for periampullary carcinoma. *Adv Surg*. 1992;25:21–49.
5. Howes N, Lerch MM, Greenhalf W, Stocken DD, Ellis I, Simon P, et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. *Clin Gastroenterol Hepatol*. 2004;2(3):252–61. doi:10.1016/s1542-3565(04)00013-8.
6. Lowenfels AB, Maisonneuve P, Whitcomb DC. Risk factors for cancer in hereditary pancreatitis. International Hereditary Pancreatitis Study Group. *Med Clin North Am*. 2000;84(3):565–73. doi:10.1016/s0025-7125(05)70240-6.
7. Preston-Martin S, Pike MC, Ross RK, Jones PA, Henderson BE. Increased cell division as a cause of human cancer. *Cancer Res*. 1990;50(23):7415–21.
8. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61(5):759–67. doi:10.1016/0092-8674(90)90186-i.
9. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal tumor development. *N Engl J Med*. 1988;319(9):525–32. doi:10.1056/NEJM198809013190901.
10. Chen J, Baithun SI, Ramsay MA. Histogenesis of pancreatic carcinomas: a study based on 248 cases. *J Pathol*. 1985;146:65–76.
11. Bansal P, Sonnenberg A. Pancreatitis is a risk factor for pancreatic cancer. *Gastroenterology*. 1995;109(1):247–51. doi:10.1016/0016-5085(95)90291-0.
12. Chow HW, Gridley G, Nyren O. Risk of pancreatic cancer following diabetes mellitus: A nation wide cohort study in Sweden. *J Natl Cancer Inst*. 1995;87:930–930.
13. Sohn TA, Yeo CJ, Cameron JL, Hruban RH, Fukushima N, Campbell KA, et al. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. *Ann Surg*. 2004;239(6):788–97. doi:10.1097/01.sla.0000128306.90650.aa.
14. Gyorgy L, Gábor B, Fiore N, Grosfeld JL, Pál O. Pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasms (IPMN). *Magy Seb*. 2006;59(1):12–9.
15. Fernandez C, Chetaille B, Tasei AM, Sastre B, Sahel J, Payan-Defais MJ, et al. From pancreatic intraepithelial neoplasia to cancer: a dramatic progression with K-ras status analysis. *Gastroenterol Clin Biol*. 2005;29(4):465–8. doi:10.1016/s0399-8320(05)80818-8.

16. Ledniczky G, Fiore N, Grosfeld JL, Wiebke E. Intraepithelial neoplasms of the pancreas. *Acta Chir Hung*. 1997;36(1-4):195–7.
17. Rosty C, Geradts J, Sato N, Wilentz RE, Roberts H, Sohn T, et al. P16 Inactivation in pancreatic intraepithelial neoplasias (panins) arising in patients with chronic pancreatitis. *Am J Surg Pathol*. 2003;27(12):1495–501. doi:10.1097/00000478-200312000-00001.
18. Andea A, Sarkar F, Adsay VN. Clinicopathological correlates of pancreatic intraepithelial neoplasia: a comparative analysis of 82 cases with and 152 cases without pancreatic ductal adenocarcinoma. *Mod Pathol*. 2003;16(10):996–1006. doi:10.1097/01.MP.0000087422.24733.62.
19. Brockie E, Anand A, Albores-Saavedra J. Progression of atypical ductal hyperplasia/carcinoma in situ of the pancreas to invasive adenocarcinoma. *Ann Diagn Pathol*. 1998;2(5):286–92. doi:10.1016/s1092-9134(98)80020-8.
20. Lowenfels AB, Maisonneuve P, Dimagno EP, Elitsur Y, Gates LK, Perrault JJ, et al. Hereditary pancreatitis and the risk of pancreatic cancer. International Hereditary Pancreatitis Study Group. *J Natl Cancer Inst*. 1997;89(6):442–6. doi:10.1093/jnci/89.6.442.
21. Charpentier KP, Brentnall TA, Bronner MP, Byrd D, Marsh C. A new indication for pancreas transplantation: high grade pancreatic dysplasia. *Clin Transplant*. 2004;18(1):105–7. doi:10.1111/j.1399-0012.2004.00110.x.
22. Langer P, Rothmund M, Bartsch DK. Prophylactic pancreas surgery. *Chirurg*. 2006;77(1):25–32. doi:10.1007/s00104-005-1113-z.
23. Dabbs D. Diagnostic Immunohistochemistry, 2nd Edn. Publisher: Churchill Livingstone; 2006.
24. Sakorafas GH, Lazaris A, Tsiotou AG, Koullias G, Glinatsis MT, Golematis BC, et al. Oncogenes in cancer of the pancreas. *Eur J Surg Oncol*. 1995;21(3):251–3. doi:10.1016/s0748-7983(95)91294-0.
25. Alpert LC, Truong LD, Bossart MI, Spjut HJ. Microcystic adenoma (serous cystadenoma) of the pancreas. A study of14 cases with immunohistochemical and electron-microscopic correlation. *Am J Surg Pathol*. 1988;12(4):251–63. doi:10.1097/00000478-198804000-00001.
26. Mulchay H, Lyautey J, Lederrey C. A prospective study of KRAS mutations in the plasma of pancreatic cancer patients. *Clin Cancer Res*. 1998;4(2):271–5.
27. Haugk B. Pancreatic intraepithelial neoplasia - can we detect early pancreatic cancer ? *Histopathology*. 2010;57(4):503–14. doi:10.1111/j.1365-2559.2010.03610.x.
28. Jeong J, Park YN, Park JS. Clinical Significance of p16 Protein Expression Loss and Aberrant p53 Protein Expression in Pancreatic. *Cancer Yonsei Med J*. 2005;46(4):519–25.
29. Haugk B. Pancreatic intraepithelial neoplasia - can we detect early pancreatic cancer ? *Histopathology*. 2010;57:503–514.

Author biography

Prasanth P S, Assistant Professor

Preethamol S, Associate Professor

Cite this article: Prasanth P S, Preethamol S. Immunohistochemical expression of K-ras protein and p53 in chronic pancreatitis and precursor lesions of pancreatic carcinoma- 5 year study in a tertiary care centre. *Panacea J Med Sci* 2022;12(1):77-81.