Published in final edited form as:

Am J Surg Pathol. 2008 December ; 32(12): 1905–1909. doi:10.1097/PAS.0b013e31818371cd.

Undifferentiated Carcinoma with Osteoclastic Giant Cells (UCOCGC) of the pancreas associated with the Familial Atypical Multiple Mole Melanoma syndrome (FAMMM)

Jan-Bart M Koorstra^{1,3}, Anirban Maitra, MBBS³, Folkert HM Morsink, BA¹, Paul Drillenburger, MD², Fiebo JW ten Kate, MD¹, Ralph H Hruban, MD³, and G Johan A Offerhaus, MD PhD¹

¹Department of Pathology University Medical Center Utrecht ²Department of Onze Lieve Vrouwe Gasthuis Amsterdam, The Netherlands ³Department of The Sol Goldman Pancreatic Cancer Research Center, the Johns Hopkins Medical Institutions, Baltimore MD, USA

Abstract

The Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome is caused by a germline mutation of *p16*. More than 90% of the sporadic pancreatic carcinomas contain genetic alterations that inactivate *p16*. Patients with the FAMMM syndrome have an increased risk of developing pancreatic cancer. Ductal adenocarcinoma is the most common cancer of the pancreas and the one encountered in patients with FAMMM syndrome. Undifferentiated carcinoma with osteoclastic giant cells, also referred to as UCOCGC of the pancreas, is a rare variant of pancreatic cancer. An UCOCGC of the pancreas associated with FAMMM syndrome is described in this report. Molecular analysis confirmed a germline *p16*- Leiden deletion in the UCOCGC, accompanied by somatic loss of heterozygosity (LOH) of the second *p16* allele, and absence of p16 protein expression in the neoplastic cells. It is the first case reported and it provides additional evidence that UCOCGC can be considered as a variant of conventional ductal adenocarcinoma of the pancreas.

Keywords

Undifferentiated carcinoma with osteoclastic giant cells; pancreas; Familial Atypical Multiple Mole Melanoma syndrome; *P16* Leiden deletion; *KRAS2* codon 12 mutation

The *p16* gene on chromosome 9p is a tumor suppressor that inhibits the cell cycle by binding Cdk4 and Cdk6 and interfering with their ability to phosphorylate RB1.^{6,7} More than 90% of the sporadic pancreatic adenocarcinomas show complete loss of function of p16 due to a combination of deletion, inactivating mutation and/or hypermethylation of the promoter region.^{9,13} The Familial Atypical Multiple Mole Melanoma (FAMMM) syndrome can be caused by germline mutations of *p16*.³ Families with the FAMMM syndrome are not only at risk for melanoma but also for the development of pancreatic cancer.⁴ FAMMM patients have a 20- to 34-fold higher risk of pancreatic cancer compared to the general population, although germline *p16* mutations *per se* contribute to significantly less than 5% of all familial pancreatic cancer cases. To the best of our knowledge, there are no significant differences in the age of onset, histology, and prognosis of FAMMM-associated ductal adenocarcinomas compared to

those occurring in the non-syndromic setting, although the paucity of cases renders a definitive conclusion as unwarranted.

A variety of different types of pancreatic cancer exists, but the most common type is pancreatic ductal adenocarcinoma and this is also the entity that typically is encountered in patients with the FAMMM syndrome.² An unusual variant of pancreatic cancer is the anaplastic carcinoma that is accompanied by non-neoplastic osteoclastic like multinucleated giant cells, originally described by Rosai.⁸ The giant cells in this neoplasm resemble cells observed in giant cell tumors of bone. A growing body of evidence suggests that UCOCGC of the pancreas is best regarded as a variant of ductal adenocarcinoma with an associated non-neoplastic giant cell reaction.¹

In this report a case of UCOCGC is described associated with the FAMMM syndrome due to a *p16-Leiden* deletion.² It is the first reported case of UCOCGC associated with FAMMM. The case provides indirect support for the notion that UCOCGC can be considered as a variant of ductal adenocarcinoma.

Case Report

A 39 year-old male was referred to us from another hospital. The patient had presented with abdominal discomfort, pain, jaundice and weight loss and had a history of a previous hospitalization for pancreatitis due to alcohol abuse. Computed tomography (CT) and magnetic resonance imaging (MRI) showed heterogeneity and calcification in the pancreas consistent with chronic pancreatitis and no evidence for malignancy although a tumor in the head of the pancreas could not be ruled out with certainty. Endoscopic retrograde cholangiopancreatography (ERCP) revealed an ampullary lesion (Figure 1A), and the biopsy was initially interpreted as reactive, since epithelial markers were negative on immunohistochemistry.

The patient's family history was remarkable for the occurrence of multiple melanomas and occasionally pancreatic cancer due to the FAMMM syndrome caused by a *p16-Leiden* deletion.

Deeper sections of the ampullary biopsy (Figure 1B) showed osteoclast like giant cells which expressed CD68 on immunohistochemistry (Figure 1D), whereas immunolabeling for CAM 5.2, pancytokeratin, and CK19 was negative. Immunolabeling for S-100 protein, Melan-A, and HMB-45, performed to rule out melanoma, were also negative. A diagnosis of UCOCGC was considered and the decision was made to do a mutational analysis of *KRAS2* codon 12, since a previous study by our group had demonstrated that the neoplastic cells of UCOCGC often harbour *KRAS2* gene mutations and that this mutant DNA can be phagocytosed by, and detected in, the non-neoplastic giant cells.¹¹ A *KRAS2* gene mutation was found (Figure 1C) and, in combination with the family history, it was interpreted as sufficient evidence to justify surgery. A pylorus preserving partial pancreaticoduodenectomy was performed and the surgical specimen grossly revealed a soft hemorrhagic neoplasm in the pancreatic head. The tumor measured 3cm in diameter with involvement of the ampulla (Figure 2A), adjacent to the distal common bile duct. On cut section the tumor was partially cystic and had necrotic areas (Figure 2B). The resection margins were free. Microscopically, the neoplasm had the typical features of an UCOCGC with anaplastic pleomorphic mononucleated cells and scattered osteoclast like multinucleated giant cells without atypia (Figure 2C). Focal osteochondroid differentiation was present. PanIN lesions of various degrees were seen, ranging from PanIN 1A-3. Mutation analysis of *p16* performed on the neoplastic cells confirmed the germ line *p16-Leiden* deletion and demonstrated loss (LOH) of the remaining wild-type allele (Figure 2D). Immunolabeling for the p16 protein revealed that the neoplastic anaplastic cells were negative, while the non-neoplastic osteoclast-like giant cells were positive (Figure 2C, inset).

The patient had an uneventful recovery and was discharged from the hospital after 10 days.

DNA analysis

Genomic DNA was isolated from formalin fixed, paraffin embedded tissue of the initial biopsy, the resected tumor and normal tissue respectively.

The DNA extracted from the biopsy was subjected to PCR amplification of the *KRAS2* gene and subsequent sequence analysis for codon 12 mutations was performed as described elsewhere.⁵

The DNA extracted from the surgically resected tumor and normal tissue was PCR amplified for mutational analysis for the *p16-Leiden* deletion, as previously described.² This deletion extends 19 bp and removes nucleotides at positions 225-243 of exon 2.² Loss of heterozygosity (LOH) was defined as a decrease of at least 40% in the intensity of one allele in the tumor sample compared to the matched wild type allele in the normal tissue. The quotient of the peak height ratios of normal and tumor tissue was considered as indicative for LOH if <0.6 and >1.6 corresponding with 40% reduction of one allele.¹⁰ It was estimated that from the micro-dissected tumor tissue for LOH analysis 50-70% of the cells were tumor cells.

Discussion

The FAMMM syndrome is characterized by the familial segregation of multiple melanomas and atypical precursor nevi and can be caused by germline mutations of the *p16* tumor suppressor gene.²⁻⁴ Carriers of this germline mutation are not only at risk for melanomas and its precursors, but they also have a significantly increased risk of pancreatic carcinoma.^{3,4} The 20- to 34-fold higher risk of pancreatic cancer in FAMMM patients translates into a 10- 20% risk of developing pancreatic cancer by the age of 70 years. This is not surprising, since the large majority of sporadic (non-syndromic) pancreatic adenocarcinomas harbor acquired *p16* gene mutations, leading to loss of function of this tumor suppressor.^{6,7,9,13} FAMMM family members with a specific 19 bp deletion of *p16*, the so called *p16-Leiden* deletion, have an estimated risk of almost 20% of developing pancreatic cancer by the age of 75 years.² Pancreatic cancers that are encountered in association with the FAMMM syndrome are typically conventional ductal adenocarcinomas, although unusual cases of adenosquamous and neuroendocrine carcinoma have been reported.^{2,12} The UCOCGC of the pancreas is considered a rare variant of ductal adenocarcinomas, characterized by the presence of reactive multinucleated osteoclast like giant cells. Accumulating evidence suggests that UCOCGCs are undifferentiated ductal carcinomas arising from precursor ductal lesions.¹ In addition to morphologic observations, this hypothesis is supported by immunolabeling for the p53 protein which reveals abnormal accumulation of the protein only in the undifferentiated cells. The osteoclast-like giant cells do not accumulate the p53 protein, suggesting that they are *TP53* gene wild-type. Interestingly, we have previously shown that the non-neoplastic multinucleated osteoclast like giant cells can phagocytose degraded neoplastic cells, and, as a result, some of these non-neoplastic cells contain phagocytosed mutant *KRAS2* genes. *KRAS2* gene analysis was used in the current case to arrive at the proper diagnosis.¹¹ It is at the same time yet another example of the great value of molecular DNA analysis as an adjunct to conventional microscopy.

To our knowledge this is the first case of UCOCGC of the pancreas in a patient from a family with FAMMM syndrome. DNA analysis of the neoplasm and normal tissue in the resection specimen confirmed the expected 19 bp deletion typical for the *p16-Leiden* deletion, accompanied by LOH of the remaining wild-type allele. Accordingly, p16 immunohistochemistry was negative in the neoplastic cells consistent with loss of function of the protein product. By contrast, the osteoclast-like giant cells in this case showed intact

labelling for the p16 protein, further supporting the hypothesis that these are non-neoplastic reactive cells. The loss of the wild-type p16 allele in this case supports the contention that the UCOCGC of the pancreas in this patient was causally related to the underlying FAMMM syndrome and not a coincidence. Indirectly, the case therefore also shows that it appears indeed legitimate to consider UCOCGC of the pancreas as a variant of ductal adenocarcinoma.

Acknowledgments

Supported by the NIH SPORE (Specialized Programs of Research Excellence) in Gastrointestinal Cancer Grant CA62924 and The Dutch Cancer Foundation KWF (Koningin Wilhelmina Fonds).

References

1. Bergmann F, Esposito I, Michalski CW, et al. Early Undifferentiated Pancreatic Carcinoma With Osteoclastlike Giant Cells: Direct Evidence for Ductal Evolution. *Am J Surg Pathol* 2007;31:1919–25. [PubMed: 18043049]
2. de vos tot Nederveen, Cappel WH.; Offerhaus, GJ.; van, PM., et al. Pancreatic carcinoma in carriers of a specific 19 base pair deletion of CDKN2A/p16 (p16-leiden). *Clin Cancer Res* 2003;9:3598–605. [PubMed: 14506146]
3. Goldstein AM, Fraser MC, Struwing JP, et al. Increased risk of pancreatic cancer in melanoma-prone kindreds with p16INK4 mutations. *N Engl J Med* 1995;333:970–4. [PubMed: 7666916]
4. Hille ET, van Duijn E, Gruis NA, et al. Excess cancer mortality in six Dutch pedigrees with the familial atypical multiple mole-melanoma syndrome from 1830 to 1994. *J Invest Dermatol* 1998;110:788–92. [PubMed: 9579547]
5. Hruban RH, Sturm PD, Slebos RJ, et al. Can K-ras codon 12 mutations be used to distinguish benign bile duct proliferations from metastases in the liver? A molecular analysis of 101 liver lesions from 93 patients. *Am J Pathol* 1997;151:943–9. [PubMed: 9327727]
6. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994;264:436–40. [PubMed: 8153634]
7. Koorstra JM, Hustinx SR, Offerhaus GJ, et al. Pancreatic carcinogenesis. *Pancreatology*. In press
8. Rosai J. Carcinoma of Pancreas Simulating Giant Cell Tumor of Bone - Electron-Microscopic Evidence of Its Acinar Cell Origin. *Cancer* 1968;22:333–43. [PubMed: 5660199]
9. Schutte M, Hruban RH, Geradts J, et al. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res* 1997;57:3126–30. [PubMed: 9242437]
10. van Rees BP, Rouse RW, de Wit MJ, et al. Molecular evidence for the same clonal origin of both components of an adenosquamous Barrett carcinoma. *Gastroenterology* 2002;122:784–8. [PubMed: 11875011]
11. Westra WH, Sturm P, Drillenburger P, et al. K-ras oncogene mutations in osteoclast-like giant cell tumors of the pancreas and liver: genetic evidence to support origin from the duct epithelium. *Am J Surg Pathol* 1998;22:1247–54. [PubMed: 9777987]
12. Whelan AJ, Bartsch D, Goodfellow PJ. Brief report: a familial syndrome of pancreatic cancer and melanoma with a mutation in the CDKN2 tumor-suppressor gene. *N Engl J Med* 1995;333:975–7. [PubMed: 7666917]
13. Wilentz RE, Geradts J, Maynard R, et al. Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. *Cancer Res* 1998;58:4740–4. [PubMed: 9788631]

1. **1A**, endoscopic view ampullary lesion (arrow); **1B**, H&E endoscopic biopsy with osteoclast-like multinucleated giant cells (200X); **1C**, sequence analysis KRAS2 codon 12 mutation; **1D**, immunohistochemistry pankeratin and CD68 (inset).

2. .

2A, gross specimen ampulla with stent; **2B**, cross section tumor pancreatic head; **2C**, H&E tumor resection specimen and immunohistochemistry for p16 (tumor cells have no nuclear expression, multinucleated giant cells are positive); **2D**, p16-Leiden deletion (arrow, 19 bp shift) and LOH remaining wild-type allele (quotient ratios peak height 1.69).