ORIGINAL ARTICLE

Molecular characterisation of pancreatic ductal adenocarcinoma in patients under 40

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Background: Pancreatic ductal adenocarcinoma (PDAC) rarely affects people under 40.

Objectives: To determine whether the clinical, pathomorphological and genetic features of PDAC occurring in young patients (≤ 40 years) differ from those in elderly patients.

Methods: Clinical and pathomorphological data were obtained from seven patients presenting with PDAC, with age ranging from 35 to 40 years of age (mean 38 years). All tumours were characterised by using immunohistochemistry and molecular genetics.

Results: All seven patients were women and lacked an association to cancer-predisposing genetic syndromes. Four patients were smokers and one had non-hereditary chronic pancreatitis. Pathomorphologically, tumours in three patients displayed moderate differentiation and four showed poor differentiation including one adenosquamous carcinoma. All tumours showed overexpression of transforming growth factor $\beta 1$ and loss or significant reduction of Smad4. Accumulation of p53 and overexpression of epidermal growth factor receptor (EGFR) were seen in five and four patients, respectively. No expression of p16, oestrogen hormone receptor or progesterone receptor was found. Mismatch repair gene products (MutL homologue 1 (MLH1), MSH2 and MSH6) were expressed in all tumours. Mutational analyses showed K-ras mutations in only three of the seven tumours.

Conclusion: A large clinical, pathomorphological and genetic overlap of PDAC in young patients aged under 40 is seen with that in elderly patients. The existence of yet undefined initiating events of pancreatic carcinogenesis is suggested by the low rate of K-ras mutations, in at least a subgroup of young patients.

Pancreatic ductal adenocarcinoma (PDAC) typically affects people in their late adult life with 80% of PDAC arising between the ages of 60 and 80. Whereas other pancreatic neoplasms, such as solid pseudopapillary neoplasms or endocrine tumours, commonly occur in younger people, the incidence of ductal adenocarcinoma is exceptionally rare in people under the age of 40 years.^{1,2} Epidemiological studies suggest a possible association of PDAC with cigarette smoking, predisposing diseases (eg, chronic pancreatitis) and a number of genetic syndromes, including hereditary pancreatitis, familial adenomatous polyposis, familial atypical multiple mole melanoma syndrome, Peutz-Jeghers syndrome, hereditary non-polyposis colon cancer, Fanconi anaemia, and familial breast cancer.^{3–5} Furthermore, a very limited number of families are affected by familial pancreatic cancer syndrome.⁶

To date, however, it remains unclear whether PDAC of young patients can be specifically linked to certain predisposing factors, and whether PDAC in young and elderly patients differs on the molecular level. In this study, we characterised PDAC in patients under the age of 40 years and compared these findings with data reported on the common type of PDAC.

MATERIAL AND METHODS

From the surgical pathological archives of the Institute of Pathology at the University of Heidelberg, Germany, formalin-fixed paraffin wax-embedded tissue samples were obtained from seven patients under 40 years of age, who had undergone pancreatic resections for ductal adenocarcinoma between 1990 and 2004. Clinical data were collected from the files of the Department of General Surgery.

For histological evaluation, sections were stained with haematoxylin and eosin (H&E). Histological typing, grading and staging was carried out independently by two pathologists trained in pancreatic histology, according to the criteria recommended by the World Health Organization.⁵

Immunohistochemistry

Immunohistochemical analyses were carried out with primary antibodies directed against p16 (1:200; clone G175-405; BD PharMingen, San Diego, California, USA), p53 (1:100; clone DO7; Dako, Carpinteria, California, USA), Smad4 (1:50; rabbit polyclonal; Santa Cruz Biotechnology, Santa Cruz, California, USA), transforming growth factor $\beta 1$ (1:20; rabbit polyclonal; Santa Cruz Biotechnology), β -catenin (1:200; clone 14; BD Transduction Laboratories, Lexington, KY, USA), epidermal growth factor receptor (1:50; clone 31G7; Zymed Laboratories, San Francisco, California, USA), oestrogen hormone receptor (1:50; clone 1D5; Dako), progesterone hormone receptor (1:50; clone PGR636; Dako), HER2/neu (polyclonal rabbit, 1:500; A0485; Dako) and the mismatch repair gene products MLH1 (1:100; clone G168-15; BD PharMingen), MSH2 (1:100; AB2; Oncogen Research, Cambridge, Massachusetts, USA) and MSH6 (1:200; clone 44; BD Transduction Laboratories), using the avidin-biotin-complex method. If necessary, antigen retrieval was achieved by microwave pretreatment in citrate buffer (p16, p53, Smad4, β -catenin, oestrogen and progesterone hormone receptors, HER2/neu and MSH6), by microwave pretreatment in EDTA (MLH1 and MSH2) or by pronase digestion (epidermal growth factor receptor) of the slides.

Abbreviation: PDAC, pancreatic ductal adenocarcinoma

Figure 1 Immunohistochemical findings in pancreatic ductal adenocarcinomas of seven young patients under 40 years of age. (A) Nuclear accumulation of p53 (case 6). (B) Loss of expression of Smad4 within the tumour (case 5); non-neoplastic pancreatic tissue of the same patient displays regular Smad4 expression (insert). (C) Cytoplasmic staining of transforming growth factor (TGF β 1; case 6). (D) Expression of epidermal growth factor receptor, shown in the adenosquamous carcinoma (case 4). (E) Staining of the cell membrane with β -catenin (case 3). (F) Nuclear expression of MutL homologue 1 (MLH1; case 2). (G) Nuclear expression of MSH2 (case 7). (H) Nuclear expression of MSH6 (case 1). Original magnification: $\times 100$ (B–E); $\times 200$ (A, F–H).

Mutation analysis

For molecular analyses, 10- μ m sections ($n = 20$) were manually microdissected and genomic DNA was extracted using standard protocols.

For mutational analyses, exons 1 and 2 of *K-ras* and exons 11 and 15 of *BRAF* were amplified by PCR under standard conditions using the following primers: *K-ras* 1F: 5'-GTG TGA CAT GTT CTA ATA TAG TCA-3'; *K-ras* 1R: 5'-GAA TGG TCC TGC ACC AGT AA-3'; *K-ras* 2F: 5'-TCA AGT CCT TTG CCC ATT TT-3'; *K-ras* 2R: 5'-TGC ATG GCA TTA GCA AAG AC-3'; *BRAF* 11F: 5'-TCC CTC TCA GGC ATA AGG TAA-3'; *BRAF* 11R: 5'-CGA ACA GTG AAT ATT TCC TTT GAT-3'; *BRAF* 15F:

5'TCA TAA TGC TTG CTC TGA TAG GA-3'; *BRAF* 15R: 5'-GGC CAA AAA TTT AAT CAG TGG A-3'. After purification, the PCR products were processed for analysis of single strand conformational polymorphism. All samples were bidirectionally sequenced on an ABIPrism 377 DNA sequencer (Applied Biosystems, Foster City, California, USA), using the BigDye Termination kit (Applied Biosystems).

RESULTS

Tables 1 and 2 and in figs 1 and 2 show a summary of the clinical, pathomorphological and molecular findings.

Figure 2 Missense point mutations (arrow) in the *K-ras* gene were detected on codon 12 of (A) case 5, (B) case 6 and (C) case 7. The remaining four patients displayed wild-type copies of *K-ras* (WT).

Clinical data

All seven patients in this study were women, with ages ranging from 35 to 40 years (mean 38 years) at the time of diagnosis. Five patients displayed risk factors for pancreatic cancer: four women were smokers and one had been diagnosed with chronic pancreatitis due to pancreas divisum. None of the patients was known to have hereditary pancreatitis, familial atypical multiple mole melanoma syndrome, Peutz-Jeghers syndrome, hereditary non-polyposis colorectal cancer syndrome, or hereditary breast or ovarian cancer. No reports were found for pancreatic cancer in first-degree relatives; however, other neoplasms occurred in five families: one case of colon cancer, prostate cancer, acute

lymphatic leukaemia, one not further defined tumour of the testis and one not further determinable cancer.

Depending on the location of the tumours (5 head, 1 tail and 1 corpus or tail) the patients underwent pancreaticoduodenectomies (Whipple resections; $n = 5$) or pancreatic left resections ($n = 2$).

Pathomorphological characteristics

Pathological examination of the pancreatic tumours showed three moderately differentiated and four poorly differentiated ductal adenocarcinomas. One poorly differentiated carcinoma represented an adenosquamous variant (case 4). The coexistence of any other pancreatic neoplasms was excluded by extensive sampling of the operation specimen.

All patients presented in locally advanced stages of disease (pT3). Regional lymph node metastases were detected in six patients. At the time of diagnosis, one patient displayed a mass in the right lung, which was later diagnosed as metastasis of the pancreatic carcinoma (patient 2).

Immunohistochemical findings

Overexpression of transforming growth factor $\beta 1$ was found in all seven tumours. Six tumours showed complete loss in Smad4 expression and one tumour displayed a significant reduction in Smad4. Accumulation of p53 was seen in five cases, with proportions of positively staining tumour cells of 20% (case 7), 30% (case 4), 50% (case 6), and 70 to 80% (cases 1 and 2). Expression of epidermal growth factor receptor was detected in four cases, in which, the proportions of positively staining tumour cells were 30% (cases 3 and 7), 50% (case 2) and 70% (case 4). None of the tumours showed expression of p16, oestrogen receptor or progesterone receptor. No nuclear or cytoplasmic accumulation of β -catenin was found.

Mismatch repair gene products (MLH1, MSH2 and MSH6) were expressed in all tumours (MLH1 analyses failed in two patients).

Mutation analysis

Mutational analyses showed *K-ras* mutations in three cases, all affecting codon 12, whereas four cases presented wild-type copies of *K-ras* (exons 1 and 2). No mutations were detected in exons 11 and 15 of BRAF (exon 11 mutational analysis failed in case 4).

DISCUSSION

In this study, we present clinical, pathomorphological and molecular findings in seven patients (≤ 40 years) with PDAC. PDAC is among the most frequent malignancies of adult life,

Table 1 Clinical and pathological findings

Patient no.	Sex/age (years)	Diagnosis	Stage	Location	Treatment	Risk factors	Neoplastic history
1	F/39	Moderately differentiated PDAC	pT3 pN0 cM0	Head	Whipple	None	Brother: testicular tumour
2	F/38	Poorly differentiated PDAC	pT3 pN1 pM1	Head	Whipple	Smoking	Son: ALL
3	F/36	Poorly differentiated PDAC	pT3 pN1 cM0	Head	Whipple	Chronic pancreatitis	Mother: died of cancer
4	F/35	Poorly differentiated PDAC; adenosquamous variant	pT3 pN0 cM0	Tail	Extended left resection	Smoking	Father: prostate cancer
5	F/40	Moderately differentiated PDAC	pT3 pN1 cM0	Head	Whipple	Smoking	Both parents died of colon cancer
6	F/40	Moderately differentiated PDAC	pT3 pN1 cM0	Corpus or tail	Subtotal left resection	None	Unknown
7	F/38	Poorly differentiated PDAC	pT3 pN1 cM0	Head	Whipple	Smoking	Benign breast tumour
							Osteochondroma
							Sister died of cancer
							Grandmother: colon and breast tumours

ALL, acute lymphatic leukaemia; F, female; PDAC, pancreatic ductal adenocarcinoma.

Table 2 Immunohistochemical and molecular findings

Patient no.	K-ras	BRAF	p16	p53	Smad4	EGFR	TGFβ1	β-catenin	ER	PR	MLH1	MSH2	MSH6
1	WT	WT	—	+	—	—	+	memb	—	—	+	+	+
2	WT	WT	—	+	—	+	+	memb	—	—	+	+	+
3	WT	WT	—	—	—	+	+	memb	—	—	+	+	+
4	WT	WT*	—	+	—	+	+	memb	—	—	+	+	+
5	G12R	WT	—	—	—	—	+	memb	—	—	ni	+	+
6	G12D	WT	—	+	(—)	—	+	—	—	—	ni	+	+
7	G12V	WT	—	+	—	+	+	memb	—	—	+	+	+

EGFR, epidermal growth factor receptor; ER, oestrogen receptor; memb, immunohistochemical staining of cell membrane; ni, not informative; PR, progesterone receptor; TGFβ1, transforming growth factor β1; WT, wild type.

+, positive immunohistochemical staining; —, negative immunohistochemical staining; (—), reduced immunohistochemical staining.

*not informative for exon 11.

typically occurring at ages between 60 and 80 years; this tumour is exceedingly rare in young people (≤ 40 years). According to—and including—a recent study,¹ only 30 patients under 40 years have been reported from 1818 to date, the youngest patient being a 7-year-old boy.⁷ Although all seven patients in the present series were women, in general, there seems to be a slight predilection for male sex, as 23 of 37 reported patients were men (62%; female:male ratio 1:1.6).

None of the patients of the present study displayed an association with a genetic syndrome. Nevertheless, various benign and malignant tumours had occurred in first-degree relatives of five patients. Previously reported PDAC in young patients included four patients with familial pancreatic cancer syndrome,^{6–8,9} two patients with Peutz-Jeghers syndrome,^{10,11} one association with hereditary non-polyposis colorectal cancer,¹² and one patient with juvenile polyposis.¹³ Furthermore, four tumours had occurred after radiotherapy or immunosuppressive treatment of neoplastic haematological diseases.^{14–17}

In the current study, five women had established risk factors for pancreatic cancer. Four women were cigarette smokers, with smoking showing the strongest positive association with risk of PDAC,³ second only to lung cancer in men and women (relative risk 1.3 and 2.5).¹⁸ Furthermore, one patient was diagnosed with non-hereditary chronic pancreatitis, which is associated with an increased risk for PDAC after a history of several years.³

The tumours in the present study did not show evident pathomorphological characteristics deviant from the common type of ductal adenocarcinoma. This is in good accordance with the results obtained in the only pre-existing larger series, containing 10 PDAC cases in young patients.¹ In this recent study, Lüttges *et al*¹ did not find any marked pathomorphological differences between PDAC in young and elderly patients. They, however, observed a greater rate of variants, including mucinous non-cystic tumours and adenosquamous carcinomas, representing 40% of the collective instead of an expected rate of 10%. This could not be reproduced in our collective, which contained only one PDAC variant, an adenosquamous carcinoma.

On the molecular level, PDAC and its precursor lesions, pancreatic intraepithelial neoplasias, were found to show a sequential acquisition of mutations, similar to the adenoma-carcinoma sequence in colorectal carcinomas.^{19–20} The most commonly affected genes include the proto-oncogene K-ras (90–95%) as well as the tumour suppressors p16 (90%), p53 (50–70%) and DPC4/Smad4 (55%).^{21–25} Both our findings and the previously reported results¹ indicate that PDAC in young patients harbour mutations in p16 and p53 to a degree similar to that found in PDAC in older patients. In further accordance with previously reported data, mismatch repair gene products were detected in all tumours in the present series, indicating microsatellite-stable genotypes.

In contrast with earlier findings, where inactivation of Smad4 occurred in about 55% of PDAC,^{24,25} all tumours in the present series displayed a loss of or significant reduction in Smad4. Inactivation of Smad4 was associated with a biologically aggressive form of PDAC.^{26,27} The fact that six of the seven tumours in the present series showed regional or distant spread at the time of diagnosis may well be compatible with an aggressive phenotype of these neoplasms. Nevertheless, appropriate follow-up information, which at this time is not available for the patients in the present series, will have to be awaited before drawing any conclusions.

Whereas inactivation of Smad4 has been shown to represent a late event in the neoplastic progression of PDAC,²⁴ mutations in K-ras were assumed early if not in the initial steps in the tumorigenesis of PDAC.^{21,28} It is all the more remarkable that K-ras mutations were found in only three of the current seven patients (42%), indicating that at least a subgroup of PDAC in young patients may be genetically distinguished from the common type of PDAC. In the common type of PDAC, K-ras mutations occur in 90–95% of cases.²⁹ In addition, K-ras mutations were detected in pancreatic intraepithelial neoplasias, where their frequency increases in correlation to the grade of the lesions.²⁸ The assumption that K-ras mutations play a crucial part in the early steps if not in the initiation of the tumorigenesis of PDAC was supported by examinations on genetically changed mice²¹: directing endogenous expression of K-ras to progenitor cells of the mouse pancreas resulted in the induction of ductal lesions, equivalent to pancreatic intraepithelial neoplasias. Some of these lesions spontaneously progressed to invasive and metastatic adenocarcinoma, establishing pancreatic intraepithelial neoplasias as precursor lesions of PDAC.

Mutations in K-ras activate the RAS-RAF-MEK-ERK-MAP kinase pathway, which mediates cellular responses to growth signals.³⁰ Activation of this pathway by *ras* mutations is also known in various malignancies other than in PDAC, including malignant melanoma, colon cancer and ovarian borderline tumours.^{31–33} In *ras* wild-type tumours of these entities, kinase activation is often achieved by mutations of the BRAF gene, which encodes a downstream protein in the same pathway.³⁴ To date, BRAF mutations (all V599E) in pancreatic cancer were reported in only three cases of K-ras wild-type medullary carcinomas (patient ages not indicated)²⁹ and in two K-ras mutant tubular adenocarcinomas (patient ages 57 and 69 years).³⁵ The present study failed to detect BRAF mutations. Larger series, however, should be analysed to clarify whether BRAF mutations play a part in PDAC in young patients, as this would provide the possibility of applying promising new treatment approaches in at least a subgroup of patients with these fatal tumours. In mice, BRAF inhibitors when given orally, prevented the formation of pulmonary metastases and resulted in rapid regression of established pulmonary metastases from malignant melanoma.³⁶ Currently, BRAF inhibitors are in clinical testing.³⁷

Take home messages

- PDAC typically affects patients between 60 and 80 years of age. It is very rare in patients under 40.
- PDAC of young and elderly patients show a large clinical, pathomorphological and genetic overlap.
- PDAC of young patients seems to be characterised by a low rate of *k-ras* mutations (42% in contrast with expected 90–95%).
- These findings suggest the existence of yet undefined initiating events in at least a subgroup of young PDAC patients, possibly implying access to alternative treatment approaches.

In summary, our analyses of seven sporadic PDAC cases in young patients indicate a large pathomorphological and molecular overlap between these tumours and the common type of PDAC. A distinct subgroup of PDAC, however, may be defined in young patients, which is characterised by wild-type *K-ras* genotypes. Although *BRAF* mutations were not detected in the present study, larger series will have to be analysed to identify other eventual mutations within the RAS-RAF-MEK-ERK-MAP kinase pathway, possibly implying access to alternative treatment approaches for these aggressive tumours.

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REFERENCES

- Lüttges J, Stigge C, Pacena M, et al. Rare ductal adenocarcinoma of the pancreas in patients younger than age 40 years. An analysis of its features and a literature review. *Cancer* 2004;**100**:173–82.
- Solcia E, Capella C, Klöppel G. Tumors of the pancreas. In: Rosai J, Sobin LH, eds. *Atlas of tumor pathology*, 3rd Series, Fascicle 20. Washington, DC: Armed Forces Institute of Pathology, 1997.
- Ghadirian P, Lynch HT, Krewski D. Epidemiology of pancreatic cancer: an overview. *Cancer Detect Prev* 2003;**27**:87–93.
- Van der Heijden MS, Yeo CJ, Hruban RH, et al. Fanconi anemia gene mutations in young-onset pancreatic cancer. *Cancer Res* 2003;**63**:2585–8.
- Klöppel G, Hruban RH, Longnecker DS, et al. Ductal adenocarcinoma of the pancreas. In: Hamilton SR, Aaltonen LA, eds. *World Health Organization classification of tumours. Pathology and genetics of tumours of the digestive system*. Lyon: IARC Press, 2000.
- Tersmette AC, Petersen GM, Offerhaus GJ, et al. Increased risk of incident pancreatic cancer among first-degree relatives of patients with familial pancreatic cancer. *Clin Cancer Res* 2001;**7**:738–44.
- Smith JH III, Baugh C, Reppun T. Obstructive jaundice secondary to pancreatic adenocarcinoma in a seven-year-old male. *J Pediatr Surg* 1985;**20**:184–5.
- Reimer RR, Fraumeni JF Jr, Ozols RF, et al. Pancreatic cancer in father and son. *Lancet* 1977;**1**:911.
- Ehrenthal D, Haeger L, Griffin T, et al. Familial pancreatic adenocarcinoma in three generations. A case report and a review of the literature. *Cancer* 1987;**59**:1661–4.
- Bowlby LS. Pancreatic adenocarcinoma in an adolescent male with Peutz-Jeghers syndrome. *Hum Pathol* 1986;**17**:97–9.
- Thatcher BS, May ES, Taxier MS, et al. Pancreatic adenocarcinoma in a patient with Peutz-Jeghers syndrome—a case report and literature review. *Am J Gastroenterol* 1986;**81**:594–7.
- Karak PK, Mukhopadhyay S, Singh SP, et al. Multiple primary coexisting malignancy in young patients. *Trop Gastroenterol* 1994;**15**:99–103.
- Walpole IR, Cullity G. Juvenile polyposis: a case with early presentation and death attributable to adenocarcinoma of the pancreas. *Am J Med Genet* 1989;**32**:1–8.
- Tavassoli FA, Lynch RG. Occult adenocarcinoma of the pancreas in a 17-year-old patient with immunosuppressed leukaemia. *Gastroenterology* 1974;**66**:1054–7.
- Lack EE, Cassady JR, Levey R, et al. Tumors of the exocrine pancreas in children and adolescents. A clinical and pathologic study of eight cases. *Am J Surg Pathol* 1983;**7**:319–27.
- Deutsch M, Rosenstein MM, Ramanathan RK. Pancreatic cancer in a young adult after treatment for Hodgkin's disease. *Clin Oncol* 1999;**11**:280–2.
- Simchuk E, Welch JP, Orlando R. Antepartum diagnosis of pancreatic carcinoma: a case report. *Conn Med* 1995;**59**:259–62.
- Neuget AL, Habibul A, Robinson E. Pancreas cancer as a second primary malignancy: a population based study. *Cancer* 1995;**16**:589–92.
- Hruban RH, Wilentz RE, Kern SE. Genetic progression in pancreatic ducts. *Am J Pathol* 2000;**156**:1821–5.
- Schneider G, Schmid RM. Genetic alterations in pancreatic carcinoma. *Mol Cancer* 2003;**2**:15.
- Hingorani SR, Petricoin EF, Maitra A, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell* 2003;**4**:437–50.
- Rozenblum E, Schutte M, Goggins M, et al. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res* 1997;**57**:1731–4.
- 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.
- Wilentz RE, Iacobuzio-Donahue CA, Argani P, et al. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res* 2000;**60**:2002–6.
- Lüttges J, Gahleitner H, Broker V, et al. Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. *Am J Pathol* 2001;**158**:1677–83.
- Biankin AV, Morey AL, Lee CS, et al. DPC4/Smad4 expression and outcome in pancreatic ductal adenocarcinoma. *J Clin Oncol* 2002;**20**:4531–42.
- Tascilar M, Skinner HG, Rosty C, et al. The SMAD4 protein and prognosis of pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2001;**7**:4115–21.
- Lüttges J, Neumann S, Jesnowski R, et al. Lack of apoptosis in PanIN-1 and PanIN-2 lesions associated with pancreatic ductal adenocarcinoma is not dependent on K-ras status. *Pancreas* 2003;**27**:e57–62.
- Calhoun ES, Jones JB, Ashfaq R, et al. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: potential therapeutic targets. *Am J Pathol* 2003;**163**:1255–60.
- Peyssonnaud C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. *Biol Cell* 2001;**93**:53–62.
- Caduff RF, Svoboda-Newman SM, Ferguson AW, et al. Comparison of mutations of Ki-RAS and p53 immunoreactivity in borderline and malignant epithelial ovarian tumors. *Am J Surg Pathol* 1999;**23**:323–8.
- Vant Veer LJ, Burgering BM, Versteeg R, et al. N-ras mutations in human cutaneous melanoma from sun-exposed body sites. *Mol Cell Biol* 1989;**9**:3114–6.
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;**319**:525–32.
- Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54.
- Ishimura N, Yamasawa K, Karim Rumi MA, et al. BRAF and K-ras gene mutations in human pancreatic cancers. *Cancer Lett* 2003;**199**:169–73.
- Collisson EA, De A, Suzuki H, et al. Treatment of metastatic melanoma with an orally available inhibitor of the Ras-Raf-MAPK cascade. *Cancer Res* 2003;**63**:5669–73.
- Garnett MJ, Marais R. Guilty as charged: B-Raf is a human oncogene. *Cancer Cell* 2004;**6**:313–9.