Mutational Profiling of Kinases in Human Tumours of Pancreatic Origin Identifies Candidate Cancer Genes in Ductal and Ampulla of Vater Carcinomas

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Abstract

Background: Protein kinases are key regulators of cellular processes (such as proliferation, apoptosis and invasion) that are often deregulated in human cancers. Accordingly, kinase genes have been the first to be systematically analyzed in human tumors leading to the discovery that many oncogenes correspond to mutated kinases. In most cases the genetic alterations translate in constitutively active kinase proteins, which are amenable of therapeutic targeting. Tumours of the pancreas are aggressive neoplasms for which no effective therapeutic strategy is currently available.

Methodology/Principal Findings: We conducted a DNA-sequence analysis of a selected set of 35 kinase genes in a panel of 52 pancreatic exocrine neoplasms, including 36 pancreatic ductal adenocarcinoma, and 16 ampulla of Vater cancer. Among other changes we found somatic mutations in ATM, EGFR, EPHA3, EPBB2, and KIT, none of which was previously described in cancers.

Conclusions/Significance: Although the alterations identified require further experimental evaluation, the localization within defined protein domains indicates functional relevance for most of them. Some of the mutated genes, including the tyrosine kinases EPHA3 and EPBB2, are clearly amenable to pharmacological intervention and could represent novel therapeutic targets for these incurable cancers.

Introduction

Protein kinases are key regulators of different and complex cellular processes such as cell cycle progression, differentiation, apoptosis and invasion [1–4]. The protein kinase complement (defined as “kinome”) represents a significant fraction of the human genome, and recently Manning et al. organized it into a dendogram containing nine broad groups of genes [5]. Alterations in a kinase gene can lead to an aberrant protein activity, which may have a role in cancer initiation and progression [6,7]. These alterations, including point mutations and deletions in conserved domains, often result in constitutively activated kinases, which are potential therapeutic targets for cancer treatment. Indeed, there are several small molecule inhibitors currently in use or being evaluated in clinical trials [8–10]. Mutations in a kinase gene may also result in its inactivation, as for genes involved in the maintenance of genome stability [11,12] or controlling cell-cell communication [13]. In recent years, extensive sequence analysis of tumour genomes and particularly of kinase gene family has been conducted in different epithelial tumours leading to the identification of different somatic mutations [14–22]. These works point to a subset of kinase genes with known or potential relationship with solid tumour development, as they display a relatively high frequency of mutations.

To determine the presence of mutations potentially relevant as therapeutic targets, we conducted a DNA-sequence analysis of a selected set of kinase genes in a panel of two different pancreatic neoplasms, including pancreatic ductal adenocarcinoma (PDAC), and ampulla of Vater cancer (AVC). For these tumour types no effective therapeutic agents are currently available [23,24]. For example, PDAC is highly aggressive and resistant to conventional and targeted therapeutic agents, resulting in a dismal 5-year survival rate of 3% to 5% [24]. Here we present the mutational profile of 35 genes belonging to the kinase gene families in PDAC and AVC. Specifically we found non-synonymous mutations in the following genes: ATM, BRAF, EGFR, EPHA3, EPBB2, ERBB2, FGFR2, KIT, and PIK3CA. Among these changes, there were both well-characterized mutations and mutations affecting aminoacid not previously found to be mutated in human cancers.
Materials and Methods

Ethics Statement

All research involving human participants was approved by the University and Hospital Trust’s Institutional Board. Informed consent was obtained in writing from living patients or relatives for all tissues used in this study.

Samples

The panel of 52 pancreatic cancer samples was obtained from the tumour bank maintained by the Department of Pathology, Section of Anatomic Pathology, University of Verona (Verona, Italy), except for six of pancreatic adenocarcinoma samples provided by Dr. Daniela Campani (University of Pisa). Samples were collected according to the ethical requirements and regulations of the review boards of both the University of Verona (Verona, Italy) and the University of Pisa (Pisa, Italy). This panel included 36 PDAC and 16 AVC (see supplementary Table S1 for detailed clinical information of cancer samples). The 23 PDAC tumors were passaged in vitro as cell lines or in nude mice as xenografts to remove contaminating non-neoplastic cells [25]. Cell lines were harvested after a maximum of six in vitro passages for nucleic acid preparation. Thirteen PDAC samples were previously described human pancreatic tumour cell lines [26] (see supplementary Table S2 for details). Tissue samples estimated to contain more than 80% of tumour cells were used. Genomic DNA was isolated using DNeasy Blood and Tissue kit (Qiagen, Milan, Italy). Matched normal DNA was used to determine whether the mutations identified were somatic or germline. No matched normal sample was available for the 13 established cell lines included in the study. Genomic DNA was further isolated after cryostat enrichment from frozen tissues of primary PDAC to confirm the mutations eventually identified in the corresponding xenografted tumors.

Genes, PCR and Sequencing

Thirty-five genes belonging to the protein kinase gene family were chosen on the basis of their high frequency of mutations in human cancers other than pancreatic as assessed in previous works [14–22]. These genes were: AKT2, ATM, ATR, AURKC, BRAF, BRD2, DDR1, DYRK2, E2F1, E2F2, E2F3, E2F4, E2F5, ERBB2, ERBB4, FGFR1, FGFR2, FGFR3, FGFR4, FLT1, FLT3, FRAP1, KDR, KIT, MAP2K4, MET, NTRK2, NTRK3, PAK1, PDGFRα, PDGFRβ, PI3KCA, PTPN11, SLK, TGFBR2. Primers for amplification and sequencing of DNA were designed using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and refer to National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) reference sequence files with the Gene and Transcript ID (RefSeq) provided in supplementary Table S3. PCR primers were designed to amplify the selected exons and the flanking intronic sequences, including splicing donor and acceptor regions. PCR products were ~400 bp in length, with multiple overlapping amplifiers for larger exons. PCR conditions, purification, and direct sequencing have been previously described [14].

Data analysis

Sequence differences to the NCBI reference sequence were identified via manual inspection of aligned electropherograms assisted by the Mutation Surveyor software package (SoftGenetics, State College, PA). The genetic alteration identified were cross-referenced to variant information from the NCBI SNP database, the Ensemble Genome Browser (http://www.ensembl.org), The Swiss-Prot (http://ca.expasy.org) and GeneBank databases http://www.ncbi.nlm.nih.gov/Genbank), the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic), and literature. In addition to non-synonymous genetic alterations, we detected numerous silent sequence variations that were analyzed using the ASSP (http://www.es.embnet.org/~mwang/assp.html) sequence analysis tool to exclude that cryptic splices sites may be activated. These silent mutations are not presented and further analyzed here. All new sequence data has been deposited in GenBank.

Results and Discussion

We performed a mutational profiling of 35 kinase genes in a panel of 36 PDAC, and 16 AVC, including primary tumours, xenografts and cell lines. For each gene, all exons in which somatic mutation had been previously identified were analyzed. Exon specific primers were designed to amplify and sequence the coding region, and at least 15 intronic bases at both the 5’ and 3’ ends, including the splicing donor and acceptor sites. A total of 8,321 PCR products, spanning over 3 Mb of tumour genomic DNA, were generated and subjected to direct sequencing. Of the 147 exons extracted, 92% furnished good sequence traces (i.e., more than 90% of bases in the target region had a Phred score [defined as ~10[log10(raw per-base error)]] of at least 20 in at least three-quarters of the samples analyzed), and therefore were analyzed searching for specific mutations. Changes previously described as single nucleotide polymorphisms (SNPs) were excluded from further analysis. To ensure that the eventually observed mutations were not PCR or sequencing artifacts, amplicons were independently re-amplified and re-sequenced in the corresponding tumours. All verified changes were resequenced in parallel with the matched normal DNA, to distinguish between somatic mutations and SNPs not previously described.

This approach led to the identification of a total of 10 different non-synonymous mutations (Table 1). Among these mutations, 9 were missense; one was a small insertion, whilst no mutations were found in the splice sites or UTR regions (Table 1).

With regard to PDAC we analyzed a total of 36 samples, 13 of which were established cell lines. A total of 7 different missense mutations affecting the following genes were found: BRAF, EGF, EPHA3, EPID2, FGFR2, FGFR3, PIK3CA (Table 1). Of these mutations FGFR2P582L and PIK3CAE545R were identified in adenocarcinoma cell lines (PT45 and GER, respectively) for which no matched normal sample was available. Therefore, the somatic status of these mutations could not be ascertained. The PIK3CAE545K mutation has been previously related to cancer and extensively characterized [27–30]. Although mutations of FGFR2 have been previously found in human cancers [16,31,32], no mutations of this gene have been reported to be associated with pancreatic cancers to date. Our characterization of established pancreatic tumour cell lines with the respect to the genetic alterations in this potential cancer target family finally provides suitable cell systems for data interpretation, target validation, as well as preclinical models for the development of novel targeted cancer drugs. BRAFV600E has been previously related to cancer and characterized [33]. The finding of a BRAF mutation in PDAC is somehow expected since its related pathway is altered in almost all the pancreatic adenocarcinomas [34], although individual BRAF mutations are quite infrequent in this type of cancer and generally occur in tumours that lack KRAS mutations [35,36]. Interestingly, the mutation we found was in homozygous state (Figure 1B), which is not expected for a protein that acts in a dominant fashion. The same missense somatic mutations of EPID2 (D283H) were found in two different PDAC samples (Figure 1A), one of which also displayed a missense mutation in the PIK3CA gene (Figure 1C).
The mutations identified in BRAF and EPHB2 were re-analyzed in the corresponding primary tumours (Supplementary Figure S1) to further confirm the presence of mutations and thus exclude the possibility that the genetic alterations could be the consequence of the graft in nude mice. In particular, the sequencing analysis of the primary cancer corresponding to the xenograft 377 (Table 1) also confirmed the homozygous state of the BRAF gene. The Eph receptors represent the largest subfamily of trans-membrane tyrosine kinase receptors and are primary involved in the process of cell adhesion and migration during development, homeostasis and disease [13,37]. In this study, we report the mutational profile of four members of this family (EPHA3, EPHA7, EPHB2, and EPHB6), which have been found frequently mutated or silenced in previous study on human cancers [13]. For example, mutations of EPHB2 that presumably lead to a loss of activity have been found in colorectal and prostate cancer [38,39], whilst mutations in EPHA3 have been described in melanoma, lung and colorectal cancer [14–16,19]. Thus far, no mutations of EPHA3 have been reported to be associated with pancreatic cancer. Otherwise, a very recent work that deeply analyzed the protein-coding genes in pancreatic adenocarcinomas reported the occurrence of an intronic point mutation of EPHA3 [34]. The mutations of EPHB2 and EPHA3 we found in this study localized in the evolutionary conserved Cysteine extracellular domain that is thought to be involved in determining the binding affinity to their ligands as well as the tetramerization of activated receptors [40]. Indeed, these aminoacid changes (D283H and K207N) may affect the binding of the receptors to their ligands and therefore possibly disrupt the normal signalling cascade. Furthermore, increasing evidence suggest a role of ephrin receptor/ephrin system in invasiveness in cancer as well as its potential relevance for therapeutic targeting [41]. Mutations of EGFR (L815F) affecting the kinase domain was harboured in only one of the PDAC samples according to the low incidence of EGFR somatic alterations found in pancreatic cancer by others [42,43]. Finally, we report for the first time a somatic mutation in the kinase domain of KIT (R740K) in PDAC cancer. KIT, also designated as CD117, is frequently affected by activating mutations in gastro-intestinal stromal tumours [44,45], thus representing a logical therapeutic target for this malignancy [46]. Although several studies suggest an involvement of KIT in pancreatic carcinogenesis, no somatic mutations have been previously found [47–49].

Concerning AVC we analyzed a total of 16 samples, including 15 primary tumours and one cell line. We found a total of four different somatic mutations, of which three were missense and one was a small insertion, affecting the following genes (Table 1): ATM, EGFR, EPHA3, and ERBB2. The somatic mutation ERBB2V777L has been previously found in human cancers [50,51]. Furthermore, a careful analysis of ERBB2 sequence electropherogram showed that the peak corresponding to the mutation was minor as compared to its wild type counterpart (Figure 1C). This suggest the occurrence of this variant only in a subpopulation of tumour cells of the samples. One of the most interesting alterations we found was the insertion occurring in the exon 22 of EGFR that leads to a premature stop codon at amino acid 896.

In conclusion, in this study we identified 10 different mutations affecting 9 kinase genes in pancreatic ductal adenocarcinoma and ampulla of Vater cancers. No definite pattern of somatic mutations was identified for each tumour types and only one alteration (EPHA3K207N) showed overlap between the tumor types analyzed. In agreement with the results from previous studies we observed a low frequency of specific somatic mutations in kinase genes [15,16,18–22,34]. Except for the FGFR2 and PIK3CA mutations that affected human tumour cell lines, the remaining 8 mutations were found in samples derived from primary tumours and were somatic in origin as assessed by the sequencing of the matched

**Table 1. Mutations Identified in Protein Kinase Genes.**

| Gene | Nucleotide Changea | Amino acid changeb | Mutation Type | Zygosityc | Tumor Type | Sample |
|------|--------------------|--------------------|---------------|-----------|------------|--------|
| ATM  | c.2679 C>T         | p.R823C            | Missense      | Heterozygous | AVC        | 107p   |
| BRAF | c.1452 G>T         | p.G464V            | Missense      | Homozygous | PDAC       | 377    |
| EGFR | c.2689 C>T         | p.L815F            | Missense      | Heterozygous | PDAC       | 369    |
| EGFR | g.173783-173784insA | 892insA            | Insertion     | Heterozygous | AVC       | 160p   |
| EPHA3| c.846 G>T          | p.K207N            | Missense      | PDAC       | 549      |
| EPHA3| c.846 G>T          | p.K207N            | Missense      | PDAC       | 135p     |
| EPHB2| c.865 G>C          | p.D283H            | Missense      | PDAC       | 549      |
| EPHB2| c.865 G>C          | p.D283H            | Missense      | PDAC       | 370      |
| ERBB2| c.2759 G>T         | p.V777L            | Missense      | AVC        | 119p     |
| FGFR2| c.2337 C>T         | p.S582L            | Missense      | n.d.       | PDAC      | PT45   |
| KIT  | c.2340 G>A         | p.R740K            | Missense      | PDAC       | PP161    |
| PIK3CA| c.3297 A>G        | p.H1047R           | Missense      | n.d.       | PDAC      | GER    |

NOTE: The mutations are listed by gene alongside the samples in which they were found. The nucleotide numbering uses the A of the ATG translation initiation start site as nucotide + 1, based on reference sequences provided in Supplementary Table S3. 
ac., cDNA sequence; gc., genomic sequence; ins., insertion; p., protein sequence; fs, frameshift mutation.

aThe genetic alteration identified were cross-referenced with variant information from databases and literature (see Materials and Methods section for details).
bThe insertion of the nucleotide A causes a frameshift and leads to a premature stop codon at amino acid 896.
cn.d., not determined.

**Figure 1C**
normal DNA (data not shown). With regard to PDAC recently Jones et al performed a comprehensive genetic analysis leading to the identification of a defined set of partially overlapping signalling pathways that were altered, despite the fact that the alterations affecting the individual component varied widely between individual tumours [34]. Indeed, none of the somatic mutations described by Jones et al were found in our analyses and viceversa. Our and previous results thus suggest that a deep genetic analysis for each gene could be performed in a large series of pancreatic ductal adenocarcinoma samples to unravel the contribution of a specific gene to pancreatic tumorigenesis.

Finally, none of the alterations we found in primary tumours were previously described in cancers. These alterations require further experimental evaluation to determine their functional relevance and in some cases may turn out to represent passenger rather than driver mutations. On the other hand, the localization within defined protein domains indicates functional relevance of most of the genetic alterations identified. Moreover, the mutations affect genes that are potentially relevant as target of pharmacologic intervention for these types of cancers. It is the case of the most promising alterations we found affecting the EPHA3 and EPHB2 genes in pancreatic adenocarcinoma and AVC samples, considering their emerging role as attractive therapeutic target in cancer [41].

Supporting Information

Figure S1  Examples of somatic mutations identified in primary PDAC samples. The chromatograms refer to the sequence of tumor samples. A, homozygous mutation in BRAF (g.143148 G>T, p.G464V). B, heterozygous mutation in EPHB2 (g.152108 G>C, p.D283H). Arrows indicate the location of missense mutation. Numbers above the sequence traces are part of the software output. The nucleotide numbering uses the A of the ATG translation initiation start site as nucleotide +1, based on reference sequences provided in Supplementary Table S3. A, mutation in PDAC; B, mutation in PDAC; C, mutation in AVC. Abbreviations: g., genomic sequence; p., protein sequence.

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Table S1 Clinical information on the pancreatic tumours included in the study.
Found at: doi:10.1371/journal.pone.0012653.s001 (1.35 MB DOC)

Table S2 Pancreatic tumor cell lines included in this study.
Found at: doi:10.1371/journal.pone.0012653.s002 (0.06 MB DOC)

Table S3 Protein kinase genes and primers used for PCR amplification and sequencing.
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Author Contributions

Conceived and designed the experiments: VC AS. Performed the experiments: VC RR. Analyzed the data: SB DC AB. Contributed reagents/materials/analysis tools: NF DC AB. Wrote the paper: VC AS. Collected the samples: RR. Critical comments: SB AB. Approved the final version of the manuscript: AS.

References

1. Blume-Jensen P, Hunter T (2001) Oncogenic kinase signalling. Nature 411: 355–365.
2. Nigg EA (2001) Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev Mol Cell Biol 2: 21–32.
3. Hunter T (2000) Signaling 2000 and beyond. Cell 100: 113–127.
4. Schlessinger J (2000) Cell signaling by receptor tyrosine kinases. Cell 103: 211–225.
5. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase genes and the pathways they control. Nat Rev Mol Cell Biol 3: 95–113.
6. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57–70.
7. Vogtstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10: 789–799.
8. Fabbrini D, Ruez S, Buchdinger E, Cowan, Jacob SW, Fendrich G, et al. (2002) Protein kinases as targets for anticancer agents: from inhibitors to useful drugs. Pharmacol Ther 93: 79–98.
9. Leary A, Johnston SR (2007) Small molecule signal transduction inhibitors for the treatment of solid tumors. Cancer Invest 25: 347–365.
10. Steeghs N, Norier JW, Gelderblom H (2007) Small molecule tyrosine kinase inhibitors in the treatment of solid tumours: an update of recent developments. Ann Surg Oncol 14: 942–953.
11. Boulton J (2003) Ataxia telangiectasia gene mutations in leukaemia and lymphoma. J Clin Pathol 56: 512–516.
12. Menoyo A, Alazzouzi H, Epsn E, Armengol M, Yamamoto H, et al. (2001) Somatic mutations in the DNA damage-response genes ATR and CHK1 in sporadic stomach tumors with microsatellite instability. Cancer Res 61: 7727–7730.
13. Merlos-Suarez A, Batlle E (2008) Eph-ephrin signalling in adult tissues and cancer. Curr Opin Cell Biol 20: 194–200.
14. Balakrishnan A, Bleeker FE, Lamba S, Rodolfo M, Danzani M, et al. (2007) Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma. Cancer Res 67: 3543–3550.
15. Bardelli A, Parsons DW, Siddiqui N, Park J, Szabo S, et al. (2003) Mutational analysis of the tyrosine kinase in colorectal cancers. Science 300: 949.
16. Davies H, Hunter C, Smith R, Stephens P, Greenman C, et al. (2005) Somatic mutations of the protein kinase gene family in human lung cancer. Cancer Res 65: 7591–7595.

17. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, et al. (2004) A census of human cancer genes. Nat Rev Cancer 4: 177–183.

18. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, et al. (2007) Patterns of somatic mutation in human cancer genomes. Nature 446: 153–158.

19. Sjoblom T, Jones S, Wood LD, Parsons DW, Lin J, et al. (2006) The consensus coding sequences of human breast and colorectal cancers. Science 314: 268–274.

20. Stephens P, Edkins S, Davies H, Greenman C, Cox C, et al. (2005) A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. Nat Genet 37: 590–592.

21. Thomas KK, Baker AC, Delisi RM, Winckler W, Laframboise T, et al. (2007) High-throughput oncogene mutation profiling in human cancer. Nat Genet 39: 347–351.

22. Wood LD, Parsons DW, Jones S, Lin J, Sjoblom T, et al. (2007) The genomic landscapes of human breast and colorectal cancers. Science 318: 1108–1113.

23. Furuse J, Takada T, Miyazaki M, Miyakawa S, Tsukada K, et al. (2008) Guidelines for chemotherapy of biliary tract and ampullary carcinomas. J Hepatobiliary Pancreat Surg 15: 55–62.

24. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA (2006) Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 20: 1218–1249.

25. Sorio C, Bonera A, Orlandini S, Moore PS, Capelli P, et al. (2001) Successful xenografting of cryopreserved primary pancreatic cancers. Virchows Arch 438: 154–158.

26. Moore PS, Sipos B, Orlandini S, Sorio C, Real FX, et al. (2001) Genetic profile of 22 pancreatic carcinoma cell lines: Analysis of K-ras, p53, p16 and DPC4/Smad4. Virchows Arch 439: 798–802.

27. Rader AG, Kang S, Vogi PK (2006) Cancer-specific mutations in PIK3CA are oncogenic in vivo. Proc Natl Acad Sci U S A 103: 1473–1479.

28. Benoue T, Kanzaki F, Hikibi Y, Ohata T, Tanaka Y, et al. (2005) Functional analysis of PIK3CA gene mutations in human colorectal cancer. Cancer Res 65: 4562–4567.

29. Samuels Y, Diaz LA, Jr., Schmidt-Kittler O, Cummins JM, Delong L, et al. (2005) Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell 7: 561–573.

30. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptok J, et al. (2004) High frequency of mutations of the PIK3CA gene in human cancers. Science 304: 554.

31. Jung JH, Shin KH, Park JG (2001) Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res 61: 3541–3543.

32. Pollock PM, Gartside MG, Dejeza LC, Powell MA, Mallon MA, et al. (2007) Frequent activating FGFR2 mutations in endometrial carcinomas parallel germline mutations associated with craniostenosis and skeletal dysplasia syndromes. Oncogene 26: 7158–7162.

33. Davies H, Biggell GR, Cox C, Stephens P, Edkins S, et al. (2002) Mutations of the BRAF gene in human cancer. Nature 417: 949–954.

34. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, et al. (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321: 1081–1086.

35. Immervoll H, Hoern D, Kugarah J, Steine SJ, Molven A (2006) Molecular analysis of the EGFR-RAS-RAF pathway in pancreatic ductal adenocarcinoma: lack of mutations in the BRAF and EGFR genes. Virochows Arch 448: 781–786.

36. Ishimaru Y, Yamashita K, Karim Rumi MA, Kadowaki Y, Ishihara S, et al. (2005) BRAF and K-ras gene mutations in human pancreatic cancers. Cancer Lett 199: 169–173.

37. Kullander K, Klein R (2002) Mechanisms and functions of Eph and ephrin signalling. Nat Rev Mol Cell Biol 3: 475–486.

38. Alazouzi H, Davalos V, Kokko A, Domingo E, Woerner SM, et al. (2005) Mechanisms of inactivation of the receptor tyrosine kinase EPHB2 in colorectal cancers. Cancer Res 65: 10170–10173.

39. Hsukko P, Pouciano-Jackson D, Wolf M, Kiefer JA, Azorsa DO, et al. (2004) Nononsense-mediated decay microarray analysis identifies mutations of EPHB2 in human prostate cancer. Nat Genet 36: 979–983.

40. Pasquali LB (2005) Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol 6: 462–473.

41. Campbell TN, Robbins SM (2008) The Eph receptor/ephrin system: an emerging player in the invasion game. Curr Issues Mol Biol 10: 61–66.

42. Lee J, Jang KT, Ki GS, Lim T, Park YS, et al. (2007) Impact of epidermal growth factor receptor [EGFR] kinase mutations, EGFR gene amplifications, and KRAS mutations on survival of pancreatic adenocarcinoma. Cancer 109: 1561–1569.

43. Tzeng GW, Frolov A, Frolova N, Jhala NC, Howard JH, et al. (2007) Epidermal growth factor receptor [EGFR] is highly conserved in pancreatic cancer. Surgery 141: 464–469.

44. Hirota S, Kozaki K, Moriyama Y, Hashimoto K, Nishida T, et al. (1998) Gain-of-function mutations of c-kit in human gastrointestinal stromal tumours. Science 279: 577–580.

45. Zamo A, Bertolaso A, Franceschetti I, Weirich G, Capelli P, et al. (2007) Microfluidic deletion/insertion analysis for rapid screening of KIT and PDGFRα mutations in CD117-positive gastrointestinal stromal tumors: diagnostic applications and report of a new KIT mutation. J Mol Diagn 9: 151–157.

46. Demneti GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumours. N Engl J Med 347: 472–480.

47. Bateman AC, Judd M, Radenkovic D, Johnson CD (2008) CD117/KIT expression in pancreatic adenocarcinoma. Pancreas 36: 76–79.

48. Yasuda A, Sawai H, Takahashi H, Ochi N, Matsuo Y, et al. (2006) The stem cell factor/c-kit receptor pathway enhances proliferation and invasion of pancreatic cancer cells. Mol Cancer 5: 46.

49. Li J, Krief J, Guo J, Fischer L, Giese N, et al. (2003) Effects of ST571 [gleevec] on pancreatic cancer cell growth. Mol Cancer 2: 32.

50. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, et al. (2006) Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. Clin Cancer Res 12: 57–61.

51. Butitta F, Barasi F, Frou F, Friconi L, Ghella A, et al. (2006) Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioalveolar features. Int J Cancer 119: 2506–2519.