Dysregulation of KRAS signaling in pancreatic cancer is not associated with KRAS mutations and outcome

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is a tumor with a poor prognosis, and no targeted therapy is currently available. The aim of the present study was to investigate the prognostic significance of the expression of V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), downstream signaling pathway genes and the association with clinical characteristics in PDAC patients undergoing radical surgery. Tumors and adjacent non-neoplastic pancreatic tissues were examined in 45 patients with histologically verified PDAC. KRAS and B-Raf proto-oncogene, serine/threonine kinase (BRAF) gene mutation analysis was performed using the KRAS/BRAF/phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α array. The transcript profile of 52 KRAS downstream signaling pathway genes was assessed using quantitative-polymerase chain reaction. KRAS mutation was detected in 80% of cases. The genes of four signaling pathways downstream of KRAS, including the phosphoinositide 3-kinase/3-phosphoinositide-dependent protein kinase 1/V-akt murine thymoma viral oncogene homolog 1, RAL guanine nucleotide exchange factor, Ras and Rab interactor 1/ABL proto-oncogene-1, non-receptor tyrosine kinase, and RAF proto-oncogene serine/threonine-protein kinase/mitogen-activated protein kinase pathways, exhibited differential expression in PDAC compared with that in the adjacent normal tissues. However, no significant differences in expression were evident between patients with KRAS-mutated and wild-type tumors. The expression of KRAS downstream signaling pathways genes did not correlate with angioinvasion, perineural invasion, grade or presence of lymph node metastasis. Additionally, the presence of KRAS mutations was not associated with overall survival. Among the KRAS downstream effective signaling pathways molecules investigated, only v-raf-1 murine leukemia viral oncogene homolog 1 expression was predictive of prognosis. Overall, KRAS mutation is present in the majority of cases of PDAC, but is not associated with changes in the expression of KRAS downstream signaling pathways and the clinical outcome. This may partly explain the failure of KRAS-targeted therapies in PDAC.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC; Online Mendelian Inheritance in Man no. 260350) ranks fourth in the leading causes of cancer-associated mortality in Western countries (1). Despite diagnostic and therapeutic advances, the prognosis of PDAC remains poor. Only 20% of patients present with potentially resectable disease at the time of diagnosis, while due to the high propensity for tumor recurrence, the 5-year overall survival (OS) rate in patients undergoing surgery with radical intent is usually <25%. Although a number of prospective clinical trials have demonstrated that adjuvant systemic therapy improves the patient outcome following surgery, adjuvant chemotherapy appears to be effective only in a minority of patients, and the majority of the patients ultimately succumb to the disease. The prognosis of metastatic patients is extremely poor, with a median OS time of <1 year (2). Consequently, novel regimens of adjuvant treatment are being investigated and there is currently no definitive standard of adjuvant therapy.

In PDAC, mutations in the V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) gene occur in 75-90% of cases, representing the most frequent, as well as the earliest, genetic alteration. KRAS mutations, specifically in codons 12 and 13, lead to constitutive activation of downstream signaling pathways that are important for tumor initiation, development and spread (3,4). KRAS signaling is highly complex and dynamic, with various downstream effector pathways interconnected at different levels by cross-signaling and feedback loops (5). The four major KRAS downstream pathways reported in PDAC are RAF proto-oncogene serine/threonine-protein kinase (RAF)/mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/3-phosphoinositide-dependent protein kinase 1 (PDK1)/ABL proto-oncogene-1, non-receptor tyrosine kinase (ABL), RAL guanine nucleotide exchange factor, and Ras and Rab interactor 1 (RIN1)/ABL pathways (Fig. 1) (6-10). This multiplicity of downstream pathways may partly explain the failure of existing efforts to target epidermal growth factor receptor, KRAS or serine/threonine-protein kinase B-raf (BRAF) using specific inhibitors, underlining the complexity of genetic changes and the resistance of the cancer cells.

The aim of the present study was to assess the association between gene expression from the four major KRAS-effective pathways in PDAC and the clinical features of the patients, and to evaluate the potential predictive and prognostic significance.

Materials and methods

Patients. A cohort of 45 consecutive patients with PDAC who underwent surgery with curative intent was recruited from two oncology centers in the Czech Republic (Institute of Clinical and Experimental Medicine, Prague; and University Hospital, Masaryk University, Brno, Czech Republic) between August 2008 and January 2012. Inclusion criteria were: i) Adult operable patients with suspected pancreatic carcinoma based on clinical imaging methods; ii) patients who provided informed consent; and iii) pancreatic carcinoma diagnosis was verified by collaborating pathologist. None of the patients had received prior chemotherapy. Characteristics of the patient cohort are summarized in Table I. The tissue specimen collection and processing, and the data retrieval were as described previously (11).

All patients signed an informed consent form, in accordance with the requirements for ethical approval, which was provided by the Institutional Review Boards of the Institute of Clinical and Experimental Medicine and University Hospital, Masaryk University, Brno.

Isolation of nucleic acids and cDNA synthesis. Tissue samples were homogenized and total RNA and DNA was isolated as previously described (12,13). cDNA was synthesized using 0.5 µg total RNA and characterized as previously described (14). cDNA was then pre-amplified by TaqMan® PreAmp Master mix to enrich the specific targets for gene expression analysis using TaqMan Gene Expression assays (Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA) (Table I). The cDNA pre-amplification was performed with 5 µl cDNA using 14 pre-amplification cycles (10 min at 95°C and 14 cycles of 15 sec at 95°C), and the pre-amplification uniformity of cDNA was checked according to the procedure recommended by the manufacturer (Thermo Fisher Scientific, Inc.).

Quantitative polymerase chain reaction (qPCR). qPCR was performed using the Viia7 Real-Time PCR system using TaqMan Gene Expression assays (Table I), with optimized primer and probe sets and TaqMan Gene Expression Master mix (Thermo Fisher Scientific, Inc.). Processing of precursor 4, S. cerevisiae, homolog of, mitochondrial ribosomal protein L19, E74-like factor 1 and eukaryotic translation initiation factor 2B subunit 1 were used as reference genes for studies of gene expression in human pancreatic carcinoma based on our previously published data (15). Determination of transcript levels was performed exactly as previously described (10) and the qPCR study adhered to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines (16).

KRAS and BRAF mutation status. KRAS and BRAF gene mutation analysis was performed using the KRAS/BRAF/phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) (KBP) Array (EV3799A/B; Randox Laboratories Ltd., Crumlin, Northern Ireland) according to the manufacturer’s instructions. The assay is based on a combination of multiplex PCR and biochip array hybridization for high discrimination between multiple wild-type and mutant DNA regions in the KRAS (mutations in codons 12, 13 and 61), BRAF (V600E mutation) and PIK3CA (mutations in codons 542, 545 and 1,047) genes. Providing there are enough copies of DNA present, ~1% of mutants can readily be detected in a background of wild-type genomic DNA. A unique primer set is designed for each mutation target (and control), which will hybridize to a complementary discrete test region (DTR) on the biochip array. Each DTR corresponds to a particular mutation target. One of the target-specific primers in each pair contains a biotin label, which on addition of streptavidin-horseradish peroxidase conjugate permits chemiluminescence detection of hybridized products on the biochip array. Dedicated software processes produced automatic results.
Statistical analysis. Differences in gene expression profiles between tumor and paired non-neoplastic control tissues and between wild-type and KRAS-mutated samples were evaluated using the RT² Profiler PCR Assay Data Analysis v3.5 program (Qiagen GmbH, Hilden, Germany). This gene expression analysis suite performs fold-change calculations from raw quantification cycle values for reference and target genes based on the $\Delta\Delta$Cq method described by Livak and Schmittgen (17), and enables hierarchical clustering of gene expression profiles between the compared groups of patients and data. Differences in intratumoral gene expression levels between patients stratified by clinical data were evaluated by the Kruskal-Wallis test.

OS was defined as the time between the date of surgery and all-cause mortality. Surviving patients were censored at the last follow-up in December 2015. Patients were divided into two groups by the median intratumoral gene expression levels of individual genes and the survival functions were computed by the Kaplan-Meier method, with statistical significance evaluated by the Breslow test using SPSS v16.0 (SPSS, Inc., Chicago, IL, USA).

P<0.05 was considered to indicate a statistically significant difference. All P-values are departures from two-sided tests. The correction for multiple testing was applied according to the Bonferroni and the false discovery rate (FDR) methods.

Results

Study population. The study was performed on 45 patients with resected (R0 resection in >90% of cases) PDAC who had not received any prior neoadjuvant therapy. Overall, 80% (36/45) of patients harbored KRAS mutations in the DNA of the tumor tissue, while BRAF mutations were not found in any sample (Table II). The majority of patients (76%; n=34) received adjuvant chemotherapy consisting of nucleoside analogs (gemcitabine and/or 5-fluorouracil).

The median OS time was 23.7 months, with 18% of patients (n=8) alive at the time of data cut off (December 2015).
Table II. List of TaqMan gene expression assays used in the study.

| Gene abbreviation | Gene name                                                                 | Assay ID                  |
|------------------|---------------------------------------------------------------------------|---------------------------|
| AKT1             | V-akt murine thymoma viral oncogene homolog 1                             | Hs00178289_m1             |
| AKT2             | V-akt murine thymoma viral oncogene homolog 2                             | Hs01086102_m1             |
| ARAF             | V-raf murine sarcoma viral oncogene homolog 1                             | Hs00176427_m1             |
| BRAF             | V-raf murine sarcoma viral oncogene homolog B1                            | Hs00269944_m1             |
| GRB2             | Growth factor receptor-bound protein 2                                    | Hs00257910_s1             |
| GSK3B            | Glycogen synthase kinase 3-β                                              | Hs00275656_m1             |
| KRAS             | V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog                      | Hs00364284_g1             |
| MAP2K1           | Mitogen-activated protein kinase kinase 1                                 | Hs00983247_g1             |
| MAP2K2           | Mitogen-activated protein kinase kinase 2                                 | Hs04194606_gH             |
| MAP2K7           | Mitogen-activated protein kinase kinase 7                                 | Hs00178198_m1             |
| MAP3K1           | Mitogen-activated protein kinase kinase kinase 1                          | Hs00394890_m1             |
| MAP3K2           | Mitogen-activated protein kinase kinase 2                                 | Hs01109981_m1             |
| MAP3K4           | Mitogen-activated protein kinase kinase kinase 4                          | Hs00245958_m1             |
| MAP3K7           | Mitogen-activated protein kinase kinase kinase 7                          | Hs01105682_m1             |
| MAPK1            | Mitogen-activated protein kinase 1                                        | Hs01046830_m1             |
| MAPK10           | Mitogen-activated protein kinase 10                                       | Hs00373455_m1             |
| MAPK14           | Mitogen-activated protein kinase 14                                       | Hs01051152_m1             |
| MAPK3            | Mitogen-activated protein kinase 3                                        | Hs00946872_m1             |
| MAPK7            | Mitogen-activated protein kinase 7                                        | Hs00611114_g1             |
| MAPK8            | Mitogen-activated protein kinase 8                                        | Hs00177083_m1             |
| MAPK9            | Mitogen-activated protein kinase 9                                        | Hs00177102_m1             |
| MKNK1            | Mitogen-activated protein kinase-interacting serine/threonine kinase 1    | Hs00374376_m1             |
| MKNK2            | Mitogen-activated protein kinase-interacting serine/threonine kinase 2    | Hs01046586_g1             |
| MTOR             | Mechanistic target of rapamycin                                           | Hs00234508_m1             |
| PAK1             | p21 protein-activated kinase 1                                            | Hs0176815_m1              |
| PDPK1            | 3-phosphoinositide-dependent protein kinase 1                             | Hs00176884_m1             |
| PIK3CA           | Phosphatidylinositol 3-kinase, catalytic, α                               | Hs00907966_m1             |
| PIK3CG           | Phosphatidylinositol 3-kinase, catalytic, γ                               | Hs00277911_m1             |
| PLK3             | Polo-like kinase 3                                                        | Hs00177725_m1             |
| PRKACA           | Protein kinase, camp-dependent, catalytic, α                              | Hs00427274_m1             |
| PRKCA            | Protein kinase c, α                                                       | Hs00925195_m1             |
| PTK1             | Phosphatase and tensin homolog                                            | Hs02621230_s1             |
| PTK2             | Protein-tyrosine kinase, cytoplasmic                                       | Hs01056457_m1             |
| PTK2B            | Protein-tyrosine kinase 2, β                                              | Hs01559708_m1             |
| RAC1             | Ras-related C3 botulinum toxin substrate 1                               | Hs01025984_m1             |
| RAF1             | V-raf-1 murine leukemia viral oncogene homolog 1                          | Hs00234119_m1             |
| RAL1             | V-ral simian leukemia viral oncogene homolog A                            | Hs01564991_g1             |
| RALBP1           | RalA-binding protein 1                                                    | Hs0134988_g1              |
| RALGDS           | Ral guanine nucleotide dissociation stimulator                            | Hs00325141_m1             |
| RAP1A            | Ras-related protein 1A                                                    | Hs01092205_g1             |
| RASA1            | Ras p21 protein activator 1                                               | Hs00963555_m1             |
| RASA2            | Ras p21 protein activator 2                                               | Hs01003325_m1             |
| RHOA             | Ras homolog gene family, member A                                         | Hs00357608_m1             |
| RIN1             | Ras and rab interactor 1                                                  | Hs00182870_m1             |
| RPS6KA2          | Ribosomal protein S6 kinase, 90-kd, 2                                     | Hs00179731_m1             |
| RPS6KA4          | Ribosomal protein S6 kinase, 90-kd, 4                                     | Hs00177670_m1             |
| RPS6KA5          | Ribosomal protein S6 kinase, 90-kd, 5                                     | Hs01046594_m1             |
| SHC1             | SHC transforming protein                                                  | Hs01050699_g1             |
| SOS1             | Son of sevenless, Drosophila, homolog 1                                   | Hs00362316_m1             |
| SOS2             | Son of sevenless, Drosophila, homolog 2                                   | Hs00412876_g1             |
| SRC              | V-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene                    | Hs01082238_g1             |
| STAT3            | Signal transducer and activator of transcription 3                        | Hs01047580_m1             |
Transcript levels of KRAS signaling pathways genes in PDAC. Considering the pivotal role of KRAS oncogene in the integration and transduction of mitogenic and metabolic signals, the transcript levels of 52 genes covering four major pathways downstream of KRAS were measured (Table I). TheKRAS pathway was significantly dysregulated in tumors compared with that in adjacent non-malignant pancreatic tissues (Fig. 2; Table III). Significant overexpression of genes of the PI3K/PDK1/AKT, RAL guanine nucleotide exchange factor, and RIN1/ABL [phosphatidylinositol 3-kinase, catalytic, α/γ (PIK3CA/G), p21 protein-activated kinase 1, V-ral simian leukemia viral oncogene homolog A, RalA-binding protein 1, Ras-related C3 botulinum toxin substrate 1, RIN1, protein-tyrosine kinase, cytoplasmic, and V-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene] pathways were observed, leading to cytoskeletal remodeling, endocytosis, cell spreading and migration (Table III; Fig. 1). By contrast, genes of the RAF/MAPK pathway exhibited significantly lower expression in tumors compared with that in the paired adjacent non-malignant pancreatic tissues (particularly in genes ARAF, BRAF, V-RAF-1 murine leukemia viral oncogene homolog 1 (RAFI), mitogen-activated protein kinase kinase, mitogen-activated protein kinase 1, mitogen-activated protein kinase-interacting serine/threonine kinase 1/2 (MKNK1/2) and ribosomal protein S6 kinase, 90-kd, 2. All these results remained significant after FDR adjustment for multiple testing and the majority remained significant after Bonferroni correction (Table III; Fig. 1).

However, no association between KRAS downstream signaling pathway gene expression and tumor characteristics, including tumor size, grade, angioinvasion, lymph node metastasis or perineural invasion, passed the significance threshold of the Bonferroni test.

Impact of KRAS mutation status on transcript levels of target genes. From the 80% of tumor samples with KRAS mutations, the most common mutation, KRASG12D, was present in 33% (n=15) of the tumors. Only 1 tumor was found with a mutation in codon 13, and 2 cases with a mutation in codon 61 (Table II).

Patients divided by the KRAS mutation status significantly differed in terms of the gene expression of 5 of
Table III. Dysregulation of KRAS pathway genes in pancreatic ductal adenocarcinoma tumors in comparison to paired adjacent non-malignant tissues.

| Gene   | Fold-change (tumor vs. non-malignant tissue) | 95% confidence interval | P-value |
|--------|---------------------------------------------|-------------------------|---------|
| AKT1   | 0.73                                        | (0.63-0.83)             | <0.001  |
| ARAF   | 0.72                                        | (0.63-0.81)             | <0.001  |
| BRAF   | 0.84                                        | (0.74-0.93)             | 0.001   |
| GRB2   | 1.37                                        | (1.04-1.69)             | <0.001  |
| KRAS   | 2.04                                        | (1.67-2.41)             | <0.001  |
| MAP2K2 | 0.64                                        | (0.46-0.82)             | <0.001  |
| MAP2K7 | 0.52                                        | (0.39-0.65)             | <0.001  |
| MAP3K1 | 0.85                                        | (0.75-0.95)             | 0.010   |
| MAP3K2 | 1.24                                        | (1.13-1.36)             | <0.001  |
| MAP3K7 | 1.28                                        | (1.14-1.41)             | <0.001  |
| MAPK1  | 0.77                                        | (0.59-0.94)             | <0.001  |
| MAPK14 | 1.27                                        | (1.14-1.40)             | <0.001  |
| MAPK3  | 1.71                                        | (1.26-2.15)             | <0.001  |
| MAPK7  | 1.20                                        | (1.01-1.38)             | 0.006   |
| MAPK8  | 0.81                                        | (0.74-0.88)             | <0.001  |
| MAPK9  | 0.47                                        | (0.38-0.55)             | <0.001  |
| MKNK1  | 0.31                                        | (0.25-0.38)             | <0.001  |
| MKNK2  | 0.35                                        | (0.26-0.44)             | <0.001  |
| PAK1   | 1.27                                        | (1.08-1.45)             | 0.001   |
| PDK1   | 0.73                                        | (0.64-0.81)             | <0.001  |
| PIK3CA | 1.46                                        | (1.25-1.68)             | <0.001  |
| PIK3CG | 2.22                                        | (1.61-2.82)             | <0.001  |
| PLK3   | 1.56                                        | (1.23-1.88)             | <0.001  |
| PTEN   | 1.29                                        | (1.05-1.53)             | 0.006   |
| PTK2B  | 1.68                                        | (1.44-1.91)             | <0.001  |
| RAC1   | 1.65                                        | (1.34-1.96)             | <0.001  |
| RAF1   | 0.62                                        | (0.54-0.69)             | <0.001  |
| RALA   | 1.43                                        | (1.27-1.59)             | <0.001  |
| RALBP1 | 1.60                                        | (1.39-1.81)             | <0.001  |
| RAP1A  | 1.18                                        | (1.07-1.29)             | <0.001  |
| RASAF  | 1.28                                        | (1.12-1.43)             | <0.001  |
| RASA2  | 1.87                                        | (1.51-2.23)             | <0.001  |
| RHOA   | 1.23                                        | (1.13-1.34)             | <0.001  |
| RIN1   | 1.39                                        | (1.10-1.67)             | 0.002   |
| RPS6KA2| 0.65                                        | (0.49-0.81)             | 0.001   |
| RPS6KA4| 1.76                                        | (1.45-2.08)             | <0.001  |
| SHC1   | 1.24                                        | (1.09-1.38)             | 0.001   |
| SOS1   | 1.32                                        | (1.14-1.50)             | <0.001  |
| SOS2   | 0.68                                        | (0.59-0.77)             | <0.001  |
| SRC    | 1.43                                        | (1.16-1.71)             | <0.001  |

Note: aFold-change, 95% confidence interval and P-values were calculated using RT² Profiler PCR Assay Data analysis v3.5 program; bresult that passed Bonferroni's correction for 52 analyzed genes (cut off P=0.001); cupregulated genes. There were 14 additional genes whose expression was not statistically significantly changed and that are therefore not listed in the table: AKT2, GSK3B, MAP2K1, MAP3K4, MAPK10, MTOR, PRKACA, PRKCA, PTEN, PTK2, RALGDS, RAP1A, RPS6KA and STAT3.

The expression profiles of the KRAS signaling pathway as a whole also did not significantly differ between KRAS wild-type and KRAS-mutated tumors (Fig. 3).
Table IV. Downregulation of KRAS pathway genes in PDAC KRAS-mutated tumors compared with cases with wild-type KRAS.

| Gene  | Fold-change\(^a\) (tumor vs. non-tumor) | 95% confidence interval\(^a\) | P-value\(^a\) |
|-------|------------------------------------------|-----------------------------|--------------|
| BRAF  | 0.84                                     | (0.72-0.95)                 | 0.021        |
| MAP3K4| 0.79                                     | (0.67-0.91)                 | 0.035        |
| MAPK8 | 0.84                                     | (0.71-0.97)                 | 0.027        |
| MKNK1 | 0.72                                     | (0.45-0.99)                 | 0.033        |
| SOS2  | 0.77                                     | (0.59-0.94)                 | 0.003        |

\(^a\)Fold-change, 95% confidence interval and P-values were calculated using RT² Profiler PCR Assay Data analysis v3.5 program.

KRAS mutation status had no significant effect on the OS time of the PDAC patients. KRAS wild-type patients experienced a median OS time of 22.3 months, and patients with KRAS mutation experienced a median OS time of 21.0 months (P=0.182).

There was also no association between KRAS mRNA transcript levels and OS time. In contrast to the rest of the pathway, RAF1 showed a significant association with the OS time of the PDAC patients. Patients with RAF1 expression levels lower than the median experienced longer OS times than patients with higher RAF1 expression levels (P=0.030) (Fig. 4). However, this association did not pass Bonferroni correction for multiple testing.

**Discussion**

Mutation analysis of the present cohort of patients with operable PDAC aligns with that of prior studies reporting the presence of KRAS mutation in the majority of PDAC cases (18,19). Additionally, the genes of four KRAS downstream signaling pathways, including the PI3K/PDK1/AKT, RAL guanine nucleotide exchange factor, RIN1/ABL and RAF/MAPK pathways, exhibited differential expression in PDAC compared with that of the adjacent normal tissues, although no significant differences were observed in the expression of these genes between patients with KRAS-mutated and wild-type tumors. The expression profiles of KRAS downstream signaling pathways were not associated with pathological characteristics that reflect tumor biology, including angioinvasion, perineural invasion, grade or presence of lymph node metastasis.

Similar to earlier studies (20-22), the present data indicated that in this cohort of patients (with early-stage disease and following radical surgery) the presence of a KRAS mutation had no effect on the OS time of the patients, although there was limited power to determine associations indicating more minor effects due to the limited size of the patient cohort. Moreover, with the exception of RAF1, no impact was observed of the expression profile of the KRAS downstream major effective signaling pathways on OS. These findings may explain why all previous efforts targeting KRAS failed to improve the patient outcome.
Despite sustained efforts in preclinical and clinical research, PDAC remains a malignancy with an almost uniformly fatal prognosis (23). In contrast to other solid tumors, there has been no major progress in the systemic therapy of PDAC during the last decade. In particular, there is currently no targeted agent with clinically significant activity against this tumor.

Although molecular biomarkers play a crucial role in the management of numerous solid tumors (24), there are currently no useful biomarkers for treatment selection in PDAC. In recent years, a number of negative trials of targeted therapy have been conducted in PDAC (25,26). Consequently, there is an urgent requirement to improve the understanding of PDAC pathogenesis and biology in order to identify novel therapeutic approaches and to define subgroups of patients for tailored therapies. It has been demonstrated that KRAS mutations represent the driver mutations in the majority of PDAC cases. KRAS-targeted agents can be classified into several categories according to the mechanism of action, namely small-molecule RAS-binding ligands, inhibitors of KRAS membrane anchorage, inhibitors that bind to RAS-binding domains of RAS-effector proteins and inhibitors of KRAS expression (27). However, attempts to therapeutically target KRAS or the downstream pathways have all thus far failed in clinical trials (28-32).

In conclusion, as expected, KRAS was mutated in the majority of PDAC cases. The genes of the KRAS downstream signaling pathways, including the PI3K/PDK1/AKT, RAL guanine nucleotide exchange factor, RIN1/ABL and RAF/MAPK pathways, were differentially expressed in PDAC compared with those in adjacent non-neoplastic tissues. However, neither the presence of KRAS mutation nor the extent of KRAS signaling dysregulation was associated with OS time. Among the KRAS downstream signaling pathway genes investigated, only RAF1 expression was predictive of outcome. It is possible that the analysis of post-transcriptional and epigenetic factors associated with KRAS signaling may shed more light onto the molecular biology of PDAC.

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