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KRAS mutant allele-specific imbalance is associated with worse prognosis in pancreatic cancer and progression to undifferentiated carcinoma of the pancreas

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Abstract

KRAS codon 12 mutations are present in about 90% of ductal adenocarcinomas and in undifferentiated carcinomas of the pancreas. The role of *KRAS* copy number changes and resulting *KRAS* mutant allele-specific imbalance (MASI) in ductal adenocarcinoma (*n*=94), and its progression into undifferentiated carcinoma of the pancreas (*n*=25) was studied by direct sequencing and *KRAS* fluorescence *in situ* hybridization (FISH). Semi-quantitative evaluation of sequencing electropherograms showed *KRAS* MASI (ie, mutant allele peak higher than or equal to the wild-type allele peak) in 22 (18.4%) cases. *KRAS* FISH (performed on 45 cases) revealed a trend for more frequent *KRAS* amplification among cases with *KRAS* MASI (7/20, 35% *vs* 3/25, 12%, *P*=0.08). *KRAS* amplification by FISH was seen only in undifferentiated carcinomas (10/24, 42% *vs* 0/21 pancreatic ductal adenocarcinoma, 0%, *P*=0.0007). In 6 of 11 cases with both undifferentiated and well-differentiated components, transition to undifferentiated carcinoma was associated with an increase in *KRAS* copy number, due to amplification and/or chromosome 12 hyperploidy. Pancreatic carcinomas with *KRAS* MASI (compared to those without MASI) were predominantly undifferentiated (16/22, 73% *vs* 9/97, 9%, *P*<0.001), more likely to present at clinical stage IV (5/22, 23% *vs* 7/97, 7%, *P*=0.009), and were associated with shorter overall survival (9 months, 95% confidence interval, 5–13, *vs* 22 months, 95% confidence interval, 17–27; *P*=0.015) and shorter disease-free survival (5 months, 95% confidence interval, 2–8 *vs* 13 months, 95% confidence interval, 10–16; *P*=0.02). Our findings suggest that in a subset of ductal adenocarcinomas, *KRAS* MASI correlates with the progression to undifferentiated carcinoma of the pancreas.

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Keywords

fluorescence *in situ* hybridization; *KRAS*; mutant allele-specific imbalance; pancreatic cancer; undifferentiated carcinoma

Activating *KRAS* codon 12 mutations are present in the majority (about 90%) of pancreatic ductal adenocarcinomas.[1–3] Undifferentiated carcinomas of the pancreas, many of which contain pleomorphic large cells or osteoclast-like giant cells, are considered to be variants of pancreatic ductal adenocarcinoma and tend to have a worse prognosis than pancreatic ductal adenocarcinoma. Undifferentiated carcinomas of the pancreas also harbor *KRAS* mutations,[4] and when accompanied by ductal adenocarcinoma, both components harbor the same *KRAS* mutation.[5–7]

Mutations in the *KRAS* oncogene are typically heterozygous and the ratio of the mutant allele to wild-type allele may be balanced or unbalanced.[1,8,9] In rare instances, the mutant allele becomes dominant, either through deletion of the wild-type allele and/or through copy number gain of the mutant allele. This phenomenon is termed ‘mutant allele-specific imbalance’ (MASI) and reflects increased ‘dosage’ of the mutant allele.[8,10,11] Cancers that exhibit *KRAS* MASI appear to behave aggressively, as has been reported in both lung[12] and colon[13] adenocarcinomas.

The mechanism and significance of *KRAS* MASI in human pancreatic ductal adenocarcinoma and undifferentiated carcinoma is unclear. In a mouse model of pancreatic ductal adenocarcinoma, loss of the *KRAS* wild-type allele was shown to be associated with a higher incidence of metastasis.[14] Compared with preneoplastic changes in the pancreas, a ‘second hit’ in the *KRAS* gene (loss of the wild-type allele or gene amplification) may be associated with the invasive process.[15] The whole-genome loss of heterozygosity study of human cell lines derived from primary and metastatic pancreatic tumors confirmed that loss of heterozygosity at *KRAS* locus is indeed an event associated with metastases.[14] Other studies have identified amplification of the *KRAS* mutant allele without the loss of the wild-type allele in cell lines,[16,17] mouse models[9] and human pancreatic ductal adenocarcinoma,[17] a feature believed to contribute to pancreatic ductal adenocarcinoma progression.[18] In fact, amplification of the mutated *KRAS* allele in a case of pancreatic ductal adenocarcinoma metastatic to a lymph node was shown about a quarter of century ago.[19] Finally, both *KRAS* loss and gain were reported in an array comparative genomic hybridization study of 13 pancreatic ductal adenocarcinomas. [20]

Our aim was to describe the role of *KRAS* MASI in pancreatic tumorigenesis. Specifically, we have employed combined interpretation of *KRAS* sequencing and *KRAS* fluorescence *in situ* hybridization (FISH) to characterize *KRAS* copy number changes that are associated with the transition of pancreatic ductal adenocarcinoma into undifferentiated carcinomas. This is the first study to focus on *KRAS* allelic imbalance in pancreatic ductal adenocarcinoma and to directly compare conventional ductal adenocarcinoma to undifferentiated carcinoma of the pancreas.

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Materials and methods

Patients

The slides and reports of all possible undifferentiated carcinomas of the pancreas in the surgical pathology files of the authors' institutions were reviewed. All cases that fulfilled the criteria for undifferentiated carcinoma (with or without osteoclast-like giant cells) and had tissue blocks available for study were included (cases were collected from 2001 through 2011). A consecutive cohort of pancreatic ductal adenocarcinoma cases resected between 2007 and 2009 at UPMC with tissue blocks available for analysis were also studied. This study was approved by the University of Pittsburgh Institutional Review Board (# PRO11070413). Only *KRAS*-mutated cases were included in this study; *KRAS* wild-type cases were excluded (two undifferentiated carcinomas and 12 ductal adenocarcinomas were *KRAS* wild-type). Basic clinicopathologic data are summarized in Table 1. Clinical staging was based on 7th edition of the American Joint Committee on Cancer manual.[21]

KRAS Exon 2 Codons 12 and 13 Mutational Analysis

Exon 2 *KRAS* codon 12 and 13 mutations were assessed by direct sequencing in both directions (forward and reverse) as previously described.[22] Tumor areas with >50% tumor cells (based on review of hematoxylin and eosin-stained slides) were microdissected from the 4-μm unstained formalin-fixed paraffin embedded sections.

Review of Sequencing Electropherograms and Determination of the *KRAS* Mutant to Wild-type Allelic Peak Height Ratio

Based on the variation of the *KRAS* mutant and wild-type allelic peak heights on the sequencing electropherograms, all cases were divided into two groups: (1) Tumors with mutant allele peak lower than the wild-type allele peak (Figure 1a) were considered negative for MASI; (2) Cases with mutant allele peak higher than or equal to the wild-type allele peak (Figures 1b, c, and d) were considered positive for *KRAS* MASI.

KRAS FISH

Dual-color *KRAS* FISH analysis was successfully performed on 45 cases using a Spectrum Green labeled chromosome enumeration probe 12 (CEP12) (Abbott Molecular, Des Plaines, IL, USA) and a Spectrum Orange labeled *KRAS* locus specific (RP11-295I5, CHORI, Oakland, CA, USA) probe, as previously described.[12,13,23,24] Amplification was defined as a *KRAS*/CEP12 ratio of >2 (Figure 2a). Chromosome 12 hyperploidy was defined as >40% of cells with >2 CEP12 signals (Figure 2b). Chromosome 12 monosomy was defined as >40% of cells with one CEP signal (Figure 2c). At least 60 cells were analyzed in each tested case. Nuclei were counterstained with DAPI, 4′,6-diamidino-2-phenylindole. The basic clinicopathologic features of cases selected for FISH were similar to the cases for which FISH analysis was not performed.

Statistical Analysis

Overall survival was assessed from the time of biopsy-confirmed diagnosis or resection to death from any cause based on review of medical record and social security death index.

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Living patients were censored at the time of last clinical follow-up. Disease-free survival was assessed from the time of biopsy-confirmed diagnosis to disease recurrence (eg, local recurrence, distant metastasis) or death of any cause. Follow-up for one case was not available. A *t*-test was used to characterize the relationship between quantitative variables, and the Fisher's exact test was used to characterize the relationship between categorical variables. Median survival intervals with 95% confidence intervals were estimated using the Kaplan–Meier method, with statistical significance of differences between groups estimated by log-rank test. A *P*-value of <0.05 was defined as statistically significant. Statistical analysis was performed using SPSS 20 (Somers, NY, USA).

Results

Clinicopathologic Comparison of Ductal Adenocarcinoma with Undifferentiated Carcinoma of the Pancreas

Ninety-four conventional pancreatic ductal adenocarcinomas and 25 pancreatic undifferentiated carcinomas harboring *KRAS* mutations were included in this study. The 'undifferentiated carcinoma' variant of pancreatic carcinoma includes a spectrum of morphologies. In our study, the undifferentiated carcinomas could be divided into three subtypes (Figure 3): ten (40%) tumors were composed of two cell populations: pleomorphic round or polygonal neoplastic mononuclear cells admixed with osteoclast-like giant cells (six of these cases also contained anaplastic giant cells), 10 (40%) tumors were composed of a population of medium-to-large anaplastic epithelioid cells (anaplastic carcinoma) with variable cohesiveness, nuclear-to-cytoplasmic ratios and spindle cell components (in four of these cases, large cells contained abundant pink cytoplasm) and five (20%) tumors were composed of mixed small monotonous and anaplastic cells forming cohesive nests in a sometimes fibrous stroma.

Sixteen (64%) of the undifferentiated carcinomas were associated with ductal adenocarcinomas; eight tumors had separate foci of pancreatic ductal adenocarcinoma comprising from 30–60% of the entire tumor mass (two of these cases also had intraductal papillary mucinous neoplasms and one had a mucinous cystic neoplasm) and eight tumors had microscopic foci of pancreatic ductal adenocarcinoma (two of these also had mucinous cystic neoplasms and one had an intraductal papillary mucinous neoplasm). Hence, there were three cases of undifferentiated carcinoma that were associated with mucinous cystic neoplasms and three that were associated with intraductal papillary mucinous neoplasms.

As a group, undifferentiated carcinomas were larger than pancreatic ductal adenocarcinomas (4.2cm *vs* 3.3 cm, *P*=0.04). Patients with undifferentiated carcinoma tended to present at younger age than patients with pancreatic ductal adenocarcinoma (average age of 63 years *vs* 67 years, *P*=0.1). Otherwise, the distribution of patients' gender, tumor site (head/neck *vs* body/tail), pT, pN, clinical stage, and type of *KRAS* mutation were similar between these two histologically defined groups.

Overall average follow-up was 20 months. The median overall survival tended to be shorter for patients with undifferentiated carcinomas when compared with overall survival of patients with pancreatic ductal adenocarcinomas (10 months, 95% confidence interval, 6–14

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vs 21 months, 95% confidence interval, 15–27, *P*=0.12). Similarly, disease-free survival tended to be shorter for patients with undifferentiated carcinoma (8 months, 95% confidence interval, 5–11 *vs* 12 months, 95% confidence interval, 9–15, *P*=0.4).

While histologic type (pancreatic ductal adenocarcinoma *vs* undifferentiated carcinoma), patients' age, gender, and type of *KRAS* mutation did not correlate with overall survival or disease-free survival, clinical stage was predictive of disease-free survival and overall survival (Figure 4a). For instance, estimated median overall survival for Stage II patients was 21 months (95% confidence interval, 16–26), while it was 2 months (95% confidence interval, 0.8–3) for Stage IV patients.

Allelic Peak Height on Sequencing Electropherograms and *KRAS* Amplification by FISH

While reviewing *KRAS* sequencing electropherograms of all 119 pancreatic carcinomas, we noticed that 22 cases showed mutant allele peak higher or equal to the wild-type allele peak (Figure 1). In an attempt to identify the mechanism of the allelic imbalance suggested by sequencing electropherograms, *KRAS* FISH was performed on 45 cases, including 20 cases with mutant allele peak higher or equal to the wild-type peak and 25 cases with mutant allele peak lower than the wild-type peak (Table 1).

Chromosome 12 hyperploidy or monosomy appeared to be independent of allelic peak height on sequencing electropherograms. An equal number of cancers with chromosome 12 hyperploidy or monosomy had mutant allele peak higher/equal to the wild-type allele peak and mutant allele peak lower than the wild-type allele peak. These findings suggest that hyperploidy and monosomy may not preferentially target the chromosome harboring the *KRAS* mutant allele.

KRAS amplification was seen more frequently among cases with MASI compared with cases with no MASI (7/20, 35% *vs* 3/25, 12%, *P*=0.08; Table 1). Among cases with *KRAS* amplification, the average *KRAS*/CEP12 ratio was 3.6 (2.02–9.75). In six cases, *KRAS* amplification was associated with monosomy or hyperploidy. In four of six such cases, mutant allele peak was higher or equal to the wild-type peak.

Finally, in two undifferentiated carcinomas (one with osteoclast-like giant cells and one with anaplastic cells) sequencing electropherograms revealed mutant allele only (Figure 1d). Only one of these cancers has successful FISH testing and no FISH abnormalities were identified, suggesting that the likely mechanism of MASI in this cancer is homozygous mutation or uniparental disomy. As uniparental disomy and homozygous mutations are affecting very short DNA segments, these two mechanisms cannot be detected by FISH.

KRAS FISH: Pancreatic Ductal Adenocarcinoma *vs* Undifferentiated Carcinoma

KRAS FISH was performed on 21 cases of pancreatic ductal adenocarcinoma and 24 cases of undifferentiated carcinoma (Table 2). Fifteen of 21 (71%) tested pancreatic ductal adenocarcinomas showed no chromosome 12 abnormalities or *KRAS* copy number alteration. However, only 8 of 24 (33%) undifferentiated carcinomas showed no *KRAS* FISH abnormalities. Therefore, abnormal *KRAS* FISH result (ie, chromosome 12 hyperploidy, monosomy, or *KRAS*/CEP12 >2) was more common in undifferentiated carcinomas

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(*P*=0.0017) (Table 2). Most intriguingly, *KRAS* amplification was seen only in undifferentiated carcinomas. Ten of 24 (42%) undifferentiated carcinomas showed *KRAS* amplification, compared to none of the pancreatic ductal adenocarcinomas (*P*=0.0007) (Table 2).

The number of cases with *KRAS* amplification was too low for a meaningful outcome analysis; however, compared with patients without *KRAS* amplification, patients with carcinomas characterized by *KRAS* amplification showed a trend for worse median overall survival (8 months, 95% confidence interval, 0.7–15, *vs* 17 months, 95% confidence interval, 12–22 months, *P*=0.12). This is best explained by the fact that *KRAS* amplification was seen in undifferentiated carcinomas only.

KRAS Copy Number Alterations in Cases with Paired Ductal Adenocarcinoma and Undifferentiated Components in the Primary Tumor

In 11 cases of undifferentiated carcinoma with adjacent ductal adenocarcinoma, *KRAS* FISH was performed on both components (Table 3). In six of these cases (and in three additional cases without *KRAS* FISH data) *KRAS* sequencing was repeated on the well-differentiated component and in all cases the *KRAS* mutations were identical in both components.

In 6 of 11 cases, transition from the conventional ductal adenocarcinoma component to undifferentiated carcinoma was associated with an increase in *KRAS* copy number, either due to amplification and/or chromosome 12 hyperploidy. In three cases with *KRAS* sequencing performed on both components, FISH findings provided mechanistic explanation for the allelic peak height change seen on sequencing electropherograms (Table 3). For instance, in case #6, while the *KRAS* mutant peak was lower than the wild-type allele peak in the conventional ductal adenocarcinoma component, the height of two peaks was equal when sequencing was performed on the undifferentiated component. This change in peak height was accompanied by *KRAS* amplification with an increase in the *KRAS*/CEP12 ratio from 1.28–9.75.

Morphologic Characterization of Undifferentiated Carcinoma with *KRAS* MASI and *KRAS* FISH Abnormalities

As noted above, only undifferentiated carcinomas had *KRAS* amplification by FISH, and all three of the morphologic subtypes had at least one case showing amplification. Of the 10 undifferentiated carcinomas with osteoclast-like giant cells, seven (70%) had *KRAS* MASI. In five of these cases the likely mechanism of MASI was identified by *KRAS* FISH: *KRAS* amplification in four cases and chromosome 12 hyperploidy in one case. Of the 10 cases with predominantly anaplastic epithelioid cells (anaplastic carcinoma), five (50%) had *KRAS* MASI and the likely mechanism of MASI was amplification in three cases and chromosome 12 hyperploidy in one case. Four (80%) tumors with a more cohesive or nested growth pattern had *KRAS* MASI and the likely mechanism of MASI was amplification in one case and chromosome 12 monosomy in one case.

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Clinicopathologic Correlates of *KRAS* MASI in Pancreatic Carcinomas

Pancreatic carcinomas with *KRAS* MASI (compared to those without MASI) were predominantly of undifferentiated type (16/22, 73% *vs* 9/97, 9%, $P<0.001$), more likely to present at clinical stage IV (5/22, 23% *vs* 7/97, 7%, $P=0.009$), and were associated with shorter overall survival ($P=0.015$) and disease-free survival ($P=0.02$) (Table 1 and Figure 4b). In subgroup analysis, clinical stage maintained its prognostic significance in both MASI and no MASI cases (for overall survival). Interestingly, clinical stage did not correlate with disease-free survival in the MASI group while it was significantly associated with disease-free survival in the no MASI group. The latter result allows speculating that in some cohorts the prognostic value of *KRAS* MASI may be independent of clinical stage.

Discussion

Undifferentiated carcinomas of the pancreas are rare and a head-to-head comparison of *KRAS*-mutated undifferentiated carcinomas to *KRAS*-mutated pancreatic ductal adenocarcinomas has not been previously reported. As *KRAS* mutations are so common in pancreatic cancer, we wanted to study a homogeneous group of tumors and hence only included *KRAS*-mutated cases. In prior studies, *KRAS* mutational status was employed to confirm the ductal cell origin of undifferentiated carcinomas. [4–7] Specifically, identical *KRAS* mutations were shown both in undifferentiated and ductal adenocarcinoma components in about 20 cases reported in the literature.[5–7] Along with the cytokeratin profile, the knowledge of *KRAS* status was important to suggest that undifferentiated carcinomas represent variants of pancreatic ductal adenocarcinoma. The aim of our study was to characterize the role of *KRAS* MASI in pancreatic ductal adenocarcinoma and its progression to undifferentiated carcinoma.

The potential role of *KRAS* MASI in tumor progression is based on observations that *KRAS* MASI is associated with pancreatic ductal adenocarcinoma metastases,[14] as well as aggressive behavior in lung and colon adenocarcinomas.[12,13] *KRAS* MASI may lead to elevated KRAS mRNA levels[24] and increased RAS activity in murine pancreatic adenocarcinomas and other carcinomas.[8,9] In a variety of pancreatic ductal adenocarcinoma models, deletion of the wild-type allele and/or an increased copy number of the mutated allele was associated with more aggressive features. The status of the wild-type allele is also relevant, as increasing evidence indicates that the wild-type *KRAS* allele may act as a tumor suppressor.[25–27]

The possibility of the imbalance between the wild-type and mutant *KRAS* alleles both in pancreatic ductal adenocarcinoma and undifferentiated carcinomas was raised previously. For instance, one of the first reports on incidence of *KRAS* codon 12 mutations in human pancreatic ductal adenocarcinoma suggested that some pancreatic ductal adenocarcinoma cases may ‘contain increased ratios of mutant *vs* normal *KRAS* allele’.[1] Similar observations were made later by others.[14–16,20,28] It was also suggested that a subset of undifferentiated pancreatic carcinoma may harbor homozygous *KRAS* mutation.[6] A more detailed discussion and comparison of *KRAS* MASI incidence, likely mechanism, or clinicopathologic correlates with prior studies is difficult. Other studies analyzed 12p region or *KRAS* locus in fewer cases, by different techniques, without clinical follow-up or detailed/explicit histologic

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description of cases. In fact, no other study employed FISH to characterize *KRAS* copy number alteration in pancreatic carcinomas.

Most prior studies reported primarily on *KRAS* gain/loss associated with regional lymph node or distant metastases in pancreatic ductal adenocarcinoma. For the first time, we have attempted to systematically evaluate *KRAS* MASI associated with transition of ductal adenocarcinomas to undifferentiated carcinoma of the pancreas. To achieve our aim, a sizable cohort of *KRAS*-mutated undifferentiated carcinomas of the pancreas and a cohort of pancreatic ductal adenocarcinoma were evaluated for *KRAS* MASI by semi-quantitative assessment of *KRAS* sequencing electropherograms and *KRAS* FISH. The semi-quantitative nature of direct sequencing was previously shown in other carcinomas, [8,13,24,29] in pancreatic ductal adenocarcinoma, [17] and was further demonstrated in this report: mutant allele peak equal to or higher than the wild-type allele peak was associated with a trend to higher incidence of amplification. In a prior report on direct *KRAS* sequencing in pancreatic ductal adenocarcinoma, it was shown that the *KRAS* allelic peak height as seen on direct sequencing was comparable to the proportion of transformed *Escherichia coli* colonies harboring mutant alleles.[17]

In our study, *KRAS* MASI was identified predominantly in undifferentiated carcinomas, among patients who presented at a more advance clinical stage, and correlated with worse overall survival. The difference in survival of 11 months between the *KRAS* MASI and no *KRAS* MASI groups is quite substantial for a cancer that has a median survival after resection of only 14–20 months[30] and an overall (all stages) 5-year survival rate of 6%.[31] We showed that *KRAS* MASI developed by *KRAS* amplification and/or chromosome 12 hyperploidy or monosomy. It appears that targeted amplification of the mutant allele may be accompanied by the loss of chromosome 12 carrying the wild-type *KRAS* allele or hyperploidy of the chromosome 12 harboring the mutated *KRAS* allele (Figure 5). Of all *KRAS* MASI mechanisms, *KRAS* amplification was the only one limited to undifferentiated pancreatic carcinomas. Pancreatic ductal adenocarcinoma develops through a multistep process that is initiated by an early activating *KRAS* mutation. Our findings suggest that subsequent *KRAS* MASI in a subset of cases correlates with the progression to undifferentiated carcinomas (Figure 5).

Overall, *KRAS* MASI appears to represent an adverse prognostic factor. Therapeutically, there are at least two potential considerations in which *KRAS* MASI appears to be relevant, as well. First, it was shown that p53 restoration in a $KRAS^{G12D}$ mouse model of non-small-cell lung carcinomas induces tumor regression only when *KRAS* wild-type allele was lost or the *KRAS* mutant allele was amplified.[32,33] Second, potential targeted therapy should selectively affect the mutated *KRAS* gene product only, as inhibition of the wild-type *KRAS* gene product could mimic effects of the wild-type allele deletion.[14]

This study is limited by its retrospective design and focus on comparison of pancreatic ductal adenocarcinomas to undifferentiated carcinomas. Larger studies including cases of pancreatic intraepithelial neoplasia and regional lymph node and distant pancreatic ductal adenocarcinoma metastases are needed for a more granular understanding of *KRAS* MASI in pancreatic tumorigenesis.

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In conclusion, we directly compared patients with *KRAS*-mutated pancreatic ductal adenocarcinomas and undifferentiated carcinomas of the pancreas and showed that *KRAS* MASI develops predominantly through *KRAS* amplification and/or chromosome 12 hyperploidy, monosomy and correlates with worse survival.

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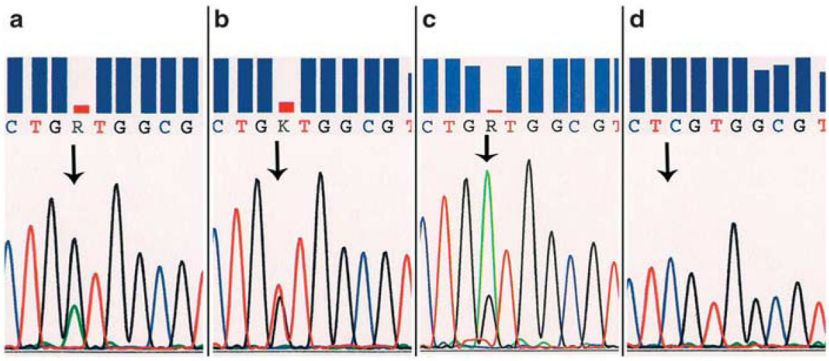

Figure 1.

Sequencing electropherograms, comparing peak heights of the *KRAS* mutant allele and wild-type allele (arrows). (**a**) Mutant allele<wild-type allele. Cases with mutant allele peak lower than the wild-type allele peak are grouped as without mutant allele-specific imbalance (MASI); codon 12 35G>A point mutation (p.G12D). (**b**) Mutant allele=wild-type allele. Peaks are of about equal height; codon 12 35G>T point mutation (p.G12V). (**c**) Mutant allele>wild-type allele. Mutant allele peak higher than the wild-type allele peak; codon 12 35G>A point mutation (p.G12D). (**d**) All mutant allele. The wild-type allele is missing, suggesting complete MASI; codon 12 34 G>C point mutation (p.G12R). This was seen in two cases of undifferentiated carcinoma. Cases illustrated in panel **b**, **c**, and **d** are characterized as cases with MASI, partial (**b**, **c**) or complete (**d**).

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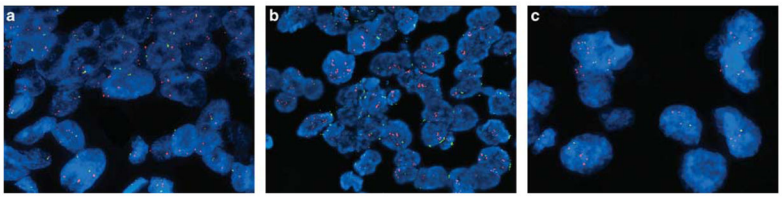

Figure 2.

KRAS fluorescence *in situ* hybridization. (**a**) *KRAS* amplification; numerous *KRAS* (orange) signals and two CEP12 (green) signals per nucleus. (**b**) Example of a case with *KRAS* amplification and chromosome 12 hyperploidy; numerous orange and green signals in each nucleus. (**c**) Example of a case with *KRAS* amplification and chromosome 12 monosomy; numerous *KRAS* (orange) signals and only one CEP12 (green) signal per nucleus.

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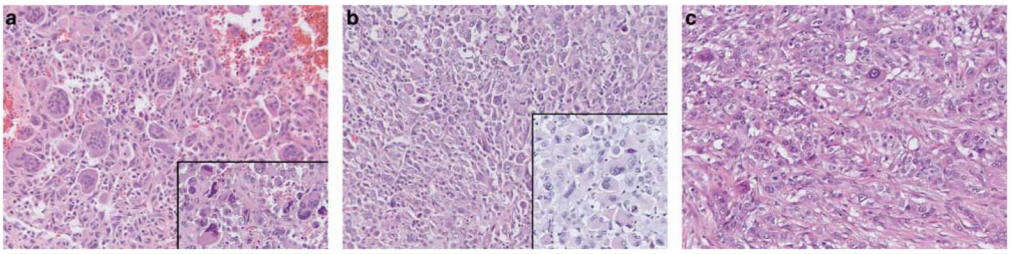

Figure 3.

Representative examples of the undifferentiated carcinomas of the pancreas included in this study (H&E, ×200). (**a**) Undifferentiated carcinoma with osteoclast-like giant cells; some cases also contained anaplastic giant cells (inset) (H&E, ×200); (**b**) Undifferentiated carcinoma containing sheets of medium-to-large anaplastic cells (anaplastic carcinoma); in some cases, the malignant cells contained abundant pink cytoplasm creating a rhabdoid appearance (inset) (H&E, ×200). (**c**) Five cases contained epithelioid cells admixed with anaplastic cells that formed vague nests and trabeculae (H&E, ×200).

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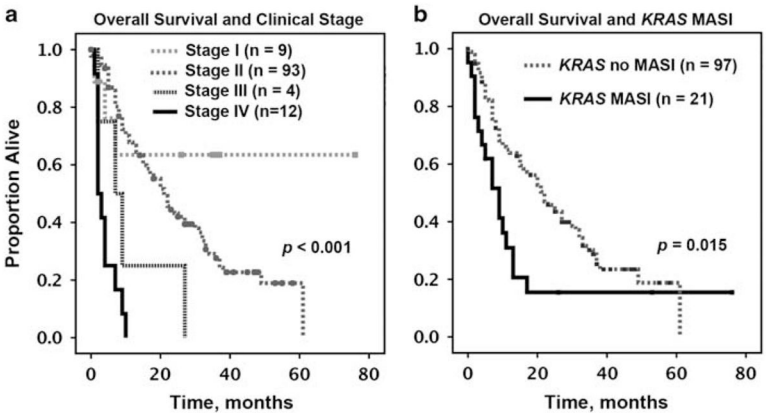

Figure 4.

Overall survival analysis, Kaplan–Meier method, for patients with clinical follow-up. (**a**) Overall survival by clinical stage. (**b**) Overall survival by *KRAS* MASI (as per sequencing electropherogram).

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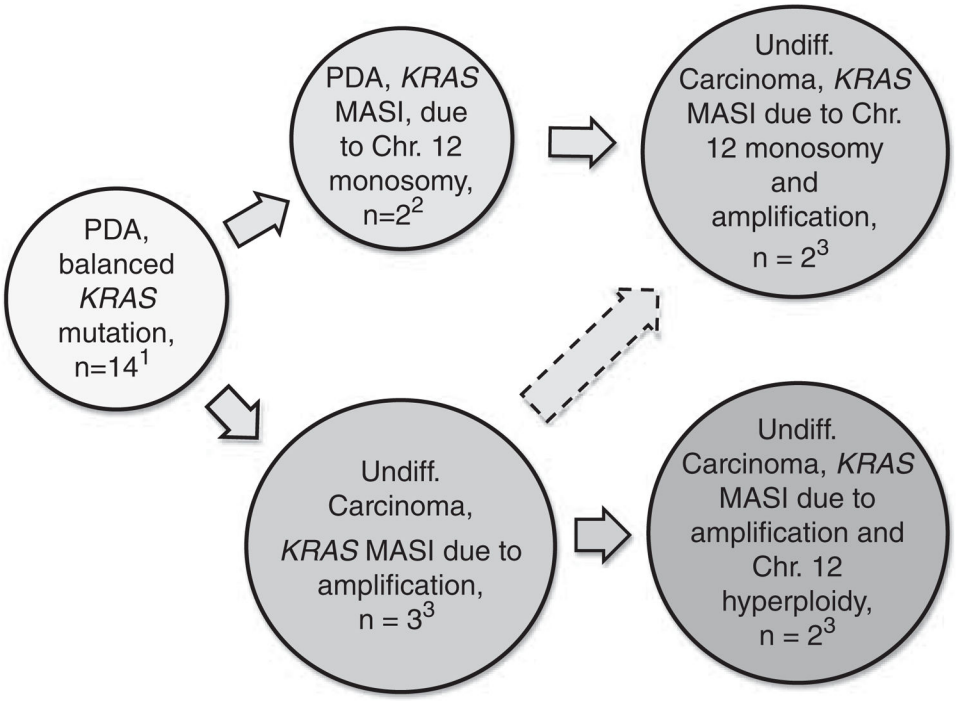

Figure 5.

KRAS copy number alterations and likely *KRAS* allelic imbalance associated with pancreatic ductal adenocarcinoma progression to undifferentiated pancreatic carcinoma. The scheme is based on previously published referenced work and a subset of cases presented in this report. Numbers refer to our cases for which both *KRAS* FISH and sequencing electropherograms were available. Note 1: The largest subset of pancreatic ductal adenocarcinoma (*n*=14) is characterized by normal results of *KRAS* FISH and no *KRAS* MASI. In these cases, the wild-type and mutated *KRAS* alleles are likely to be balanced and the fact that wild-type allele peak is higher than the mutant allele peak on sequencing electropherogram is best explained by the wild-type allele derived from stromal tissue and inflammatory cells. Note 2: Based on sequencing electropherograms, a subset of pancreatic ductal adenocarcinoma is characterized by *KRAS* MASI. *KRAS* FISH was performed on 5 of 6 such cases. Three cases showed normal *KRAS* FISH and *KRAS* MASI may have arisen through chromosome 12 uniparental disomy. In two cases, KRAS FISH revealed chromosome 12 monosomy in 47% and 78% of cells. As wild-type allele was predominant on sequencing electropherogram, the lost copy of chromosome 12 likely harbored the wild-type allele. Note 3: Sixteen of 25 undifferentiated carcinomas showed *KRAS* MASI. *KRAS* FISH was performed on 15 of 16 cases with *KRAS* MASI and amplification alone was identified in three cases. Two cases showed amplification associated with chromosome 12 monosomy, while the other two cases showed amplification associated with chromosome 12 hyperploidy.

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Table 1

Clinicopathologic features of patients with *KRAS*-mutated pancreatic ductal adenocarcinoma and undifferentiated pancreatic carcinoma, overall and by *KRAS* MASI

Feature	Overall (*n* = 119)	No MASI (*n* = 97)	MASI (*n* = 22)	*P*-value
Sex				
Men/women	64/55	52/45	12/10	1
Age, average, years	66	67	63.5	0.3
Average tumor size[a], cm	3.5	3.3	4.2	0.08
Tumor site				
Head/neck	92	76	16	0.6
Body/tail	27	21	6	
pT[a]				
1	6	4	2	0.2
2	9	6	3	
3	92	78	14	
4	5	4	1	
pN[a]				
0	40	32	8	0.8
1	70	58	12	
Clinical stage[a]				
I	9	5	4	0.009
II	93	81	12	
III	4	4	0	
IV	12	7	5	
Histologic type				
PDA	94	88	6	<0.001
Undifferentiated	25	9	16	
KRAS AA				
p.G12D	63	52	11	0.9[b]
p.G12V	34	26	8	
p.G12R	16	14	2	
p.G12C	3	3	0	
p.G12A	2	1	1	
KRAS FISH (n = 45)				
Amplification	10	3[c]	7[d]	0.08[e]
Hyperploidy	7	4	3	
Monosomy	5	3	2	
Normal	23	15	8	
Median overall survival, months	20	22 (95 CI, 17–27)	9 (95 CI, 5–13)	0.015
Median DFS, months	15.7	13 (95 CI, 10–16)	5 (95 CI, 2–8)	0.02

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Abbreviations: AA, amino acid; CI, confidence interval; DFS, disease-free survival; FISH, fluorescence *in situ* hybridization; MASI, mutant allele-specific imbalance; PDA, well-differentiated component.

[a]Tumor size not available for 1 case, pT status was not available (biopsy material) for five PDAs and two undifferentiated carcinomas, pN was unknown in nine cases and clinical stage was unknown for one undifferentiated carcinoma.

[b]Based on the distribution of three most common mutations. One case showed a complex insertion/deletion mutation, which was not included in this table.

[c]In one case, amplification was associated with chromosome 12 monosomy (62.3% of cells) and in another case amplification was associated with chromosome 12 hyperploidy (62.9% of cells).

[d]In two cases, amplification was accompanied by monosomy (46.7% and 56.1% of cells) and in two other cases, amplification was associated with hyperploidy (47.7% and 64.5% of cells).

[e]Cases with *KRAS*/CEP12 >2 were compared with cases showing normal *KRAS* FISH.

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Table 2

KRAS FISH results in conventional ductal and undifferentiated pancreatic carcinomas

KRAS FISH (*n* = 45)	Ductal adenocarcinomas (*n* = 21)	Undifferentiated carcinomas (*n* = 24)	*P*-value
Amplification	0	10[a]	0.0007
Hyperploidy	2	5	
Monosomy	4	1	
Normal	15	8	0.0017

Abbreviations: FISH, fluorescence *in situ* hybridization.

[a]In three cases, amplification was associated with chromosome 12 monosomy (46.7–62.3% of cells) and in another three cases, amplification was associated with chromosome 12 hyperploidy (47.7–64.5% of cells).

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Table 3

Cases with both conventional pancreatic ductal adenocarcinoma and undifferentiated carcinoma components: results of *KRAS* FISH and *KRAS* allelic peak height evaluation on sequencing electropherogram

Case no.	Histology	KRAS mutation	Peak height on SE	*KRAS* FISH		
				KRAS/CEP12	% Of cells with hyperploidy	% Of cells with monosomy
1	Undiff.	p.G12D	MA =WT	1.1	41.2%	N
	PDA[a]	p.G12D	MA<WT	1.23	N	N
2	Undiff.	p.G12D	MA =WT	2.02	N	46.7%
	PDA	p.G12D	MA =WT	2.43	N	68.3%
3	Undiff.	p.G12V	MA =WT	2.38	N	N
	PDA	n.t.	n.t.	3.19	N	N
4	Undiff.	p.G12D	MA>WT	2.51	N	N
	PDA	n.t.	n.t.	1.29	50%	N
5	Undiff.	p.G12V	MA>WT	4.36	64.5%	N
	PDA	n.t.	n.t.	1.24	N	47.6%
6	Undiff.	p.G12V	MA =WT	9.75	N	N
	PDA[b]	p.G12V	MA<WT	1.28	N	N
7	Undiff.	p.G12D	MA<WT	1.02	N	N
	PDA[a]	p.G12D	MA<WT	1.03	N	N
8	Undiff.	p.G12D	MA<WT	3.16	62.9%	N
	PDA	p.G12D	MA<WT	1.75	88.5%	N
9	Undiff.	p.G12D	MA<WT	1.1	53.8%	N
	PDA	p.G12D	MA<WT	1.08	11.5%	N
10	Undiff.	p.G12D	MA>WT	1.6	40%	N
	PDA[b]	n.t.	n.t.	1.19	23.3%	N
11	Undiff.	p.G12R	MA<WT	1.16	46.3%	N
	PDA	n.t.	n.t.	1.1	56.3%	N

Abbreviations: FISH, fluorescence *in situ* hybridization; MA, mutant allele; N, normal (<40% of cells with hyperploidy or monosomy); n.t., not tested; PDA, well-differentiated component; SE, sequencing electropherogram; Undiff., undifferentiated component; WT, wild-type allele. The proportion (and size) of the PDA component ranged from 1% (0.3 cm) to 40% (3.6 cm).

[a]The PDA component arose from a mucinous cystadenocarcinoma.

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[b]The microscopic focus of PDA arose from an intraductal papillary mucinous neoplasm.