Wild-type K-ras has a tumour suppressor effect on carcinogen-induced murine colorectal adenoma formation


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SUMMARY

K-ras mutations are found in ~40% of human colorectal adenomas and carcinomas and contribute to colorectal tumour formation at an early stage. Wild-type K-ras has been reported to be deleted in some tumours, but the consequences of changes in wild-type K-ras copy number for experimental colorectal carcinogenesis have not been investigated. To characterize the effects of K-ras copy number changes on formation of carcinogen-induced colorectal neoplasms in mice, wild-type (K-ras<sup>+/+</sup>) and heterozygous K-ras exon 1 knockout (K-ras<sup>+/−</sup>/C0) mice were given 10 weekly treatments of 1, 2-dimethylhydrazine (DMH) to induce colorectal tumours. Colorectal expression levels of K-ras<sub>4A</sub> and 4B transcripts in K-ras<sup>+/−</sup>/C0 mice were ~50% decreased compared with K-ras<sup>+/+</sup> mice. One year after DMH treatment, survival of K-ras<sup>+/−</sup>/C0 mice decreased from 88 to 82% compared with wild-type mice. Colorectal adenomas significantly increased from 0.52 ± 0.15 in K-ras<sup>+/+</sup> mice to 0.87 ± 0.14 in K-ras<sup>+/−</sup>/C0 mice (mean ± SEM per mouse, <i>P</i> < 0.01); total tumour volume increased 2.13-fold (<i>P</i> < 0.05). Comparing K-ras<sup>+/+</sup> with K-ras<sup>+/−</sup>/C0 murine adenomas, Ki-67-positive proliferating tumour cells significantly increased from 7.77 ± 0.64% to 9.15 ± 0.92% and cleaved caspase-3-positive apoptotic tumour cells decreased from 1.40 ± 0.37% to 0.80 ± 0.22% (mean ± SEM, <i>P</i> < 0.05 for both). No K-ras or B-raf mutations were detected in the adenomas. Immunohistochemical studies showed no significant changes in extracellular signal regulating kinase/mitogen-activated protein kinase (Erk/MapK) or PI3K/Akt pathway activation in the adenomas. In conclusion, the data collectively show that a 50% reduction in K-ras gene dosage and RNA expression promoted experimental colorectal tumourigenesis, consistent with wild-type K-ras having a tumour suppressor effect on carcinogen-induced murine colorectal adenoma formation.

Keywords

1, 2-dimethylhydrazine, adenoma, carcinogen, colorectal, K-ras, mouse, wild-type

K-Ras protein is critical in the regulation of cellular proliferation, apoptosis and differentiation, and is mutated in ~30% of all human tumours and ~40% colorectal adenomas and carcinomas (Malumbres & Barbacid 2003; Arends et al. 1993, 1994; Luo et al. 2011). Once activated by ligand-mediated extracellular stimuli, normal K-Ras protein activates multiple downstream pathways, including the Map-Kinase pathway (Raf, Mek, Erk), the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, the Ral GDP dissociation stimulator (RalGDS) pathway and others (Olson & Marais 2000; Markman et al. 2010; Campbell et al. 1998). K-ras activating mutations render K-Ras an oncoprotein that is constitutively active (locked in the GTP-bound form), leading to deregulated activation of Ras protein functions (Malumbres...
& Barbacid 2003). The oncogenic alleles of Ras genes have been referred to as dominant alleles, because they demonstrate transforming ability in the presence of normal allele expression (Malumbres & Barbacid 2003; Marshall 1991), but this dogma has been challenged (Diaz et al. 2005).

Spandidos and colleagues observed that the normal H-Ras protein could have some suppressive role over the mutated H-Ras protein (Spandidos et al. 1990). Our previous studies showed that expression of wild-type K-ras or mutant K-ras induce some overlapping and some different changes to the gene expression profiles in embryonic stem cells, including changes in expression of proliferation-, apoptosis- and differentiation-related genes (Luo et al. 2007a,b). We also demonstrated that murine wild-type K-ras exhibits tumour suppressor activity, as its absence promotes tumorigenesis in murine teratomas (James et al. 2003). Further, Zhang and colleagues showed that wild-type K-ras has tumour suppressor activity and is frequently lost during lung tumour progression (Zhang et al. 2001). Wild-type K-ras has been reported to be deleted in some human cancers, but so far, the role of reductions in copy number of wild-type K-ras in colorectal carcinogenesis has not been adequately investigated; hence, we studied this using a mouse model of carcinogen-induced colorectal tumour formation.

The colon-selective carcinogen 1, 2-dimethylhydrazine (DMH) has been widely used to study chemically induced colorectal tumours. DMH alkylates DNA, producing O6-methylguanine (O6-MeG), which induces GC→AT transitions that may be found in various genes linked to colorectal cancer (Jackson et al. 1997). DMH treatment results in high frequency mutation of K-ras in colorectal adenomas in the rat model (Jacoby et al. 1991), but for DMH-treated mice, K-ras mutations in adenomas are much less common or rare, although DMH is known to induce adenoma formation by either activating Beta-Catenin mutations or inactivating Apc mutations (Jackson et al. 1997; Deschner & Long 1977; Deschner et al. 1979; Diwan et al. 1977). DMH tends to induce more tumours in the distal colon and rectum with similar histopathological appearances to those observed in human sporadic colorectal tumours. DMH is metabolized to aoxymethane (AOM), which has also been used to similar effect in rodent models. Hence, treatment of rodents with these carcinogens provides a useful bioassay to assess colorectal tumour susceptibility in different murine strains, including those with gene knockouts that may influence rates of proliferation or apoptosis.

Mice lacking both K-ras alleles are not viable and die between 12 and 14 days of gestation due to foetal liver defects and anaemia (Johnson et al. 1997); therefore, they could not be tested. Here, we evaluated the effects of 2 or 1 alleles of wild-type K-ras on carcinogen-induced adenoma formation in the large intestine using K-ras+/+ mice and K-ras−/− mice. K-ras−/− mice exhibit normal life expectancy and an overall similar spontaneous tumour incidence to wild-type mice (Johnson et al. 1997), indicating that K-ras−/− mice provide an excellent experimental model to explore the gene dosage effects of wild-type K-ras on colorectal tumorigenesis.

Materials and methods

Mice

The 129/Sv-K-ras−/− mice were previously acquired (Johnson et al. 1997; Luo et al. 2010; Patek et al. 2008). The heterozygous K-ras strain of mice (K-ras+/−) has a deleted exon 1 of the K-ras gene, and this strain was crossed with C57BL/6J mice for >7 generations of crossing, to more than 99% C57BL/6J background. The K-ras genotype was determined by PCR assay as previously described (Johnson et al. 1997; Luo et al. 2010). K-ras+/− and K-ras−/− siblings from the same matings were used for carcinogen treatments.

Ethical approval

The animal model work was approved by the local ethical review process and was carried out under Home Office licence.

Carcinogen treatments

DMH 2HCl (2.0 mg/ml) was dissolved in 0.90% saline containing 1 mM EDTA and 10 mM sodium citrate and the pH adjusted to 6.5 using 0.5 M NaOH. Previously validated DMH treatments consisted of 10 weekly injections of 20.0 mg/kg, starting at age 7–8 weeks (cumulative dose = 200.0 mg/kg DMH-2HCl or 145.0 mg/kg DMH) (Deschner et al. 1979; Luo et al. 2010).

Analysis of intestinal tumours

Mice were inspected daily for signs suggestive of colorectal tumour development at which time they were killed; otherwise they were observed for one year following carcinogen treatment and then killed. The small and large intestines were removed, opened and examined. The numbers of intestinal polyps were counted and measured under a dissecting microscope at ×15 magnification, always by the same investigator (FL). Intestinal tumours were divided and ~30–40% processed for DNA and RNA extraction by standard methods, whereas ~60–70% was formalin-fixed and paraffin-processed for histological examination and immunohistochemistry, as previously described using antibodies to Ki-67, cleaved caspase-3, phospho-Erk and phospho-Akt (Luo et al. 2009, 2010, 2011).

Analysis of K-ras and other transcript expression levels

Total RNA (100 ng) from large intestinal tumours or normal tissues from 10 wild-type and 10 K-ras−/− mice were reverse transcribed and amplified in 25 µl volume using the iTaq SYBR Green RT-PCR kit run on an iCycler RT-qPCR machine (Bio-Rad, Hemel Hempstead, UK) as described previously (Patek et al. 2008; Luo et al. 2009). All real-time reverse transcription quantitative polymerase chain reactions (RT-qPCR) were amplified starting with denaturation at 95 °C for 3 min then 45 cycles of 95 °C for 15 s and 60 °C

International Journal of Experimental Pathology, 2014, 95, 8–15
for 1 min using exon-spanning PCR primers for mouse K-ras 4A & K-ras 4B, (as previously described; Patek et al. 2008; Luo et al. 2009), as well as for pRB, p107/RBL1, p130/RBL2, cyclin D1, c-myc and β-actin using the following primers: p107/RBL1 sense primer: 5′ GGA GTG CTA AGA GGA GAC TGT TTG G 3′, p107/RBL1 anti-sense primer: 5′ ATA GGA ACC AGT GTG ATT TCT CCA G 3′; p130/RBL2 sense primer: 5′ GAC TAG CTC CTT AGC GCT CTT TCT T 3′, p130/RBL2 anti-sense primer: 5′ AAG TTC GGT GCA CTG GAT TAT AGA G 3′; cyclin D1 sense primer: 5′ TTT CTT TCC AGA GTC ATC TCT CTC ATC TTC TTG C 3′; c-myc sense primer: 5′ AAA TCC TGT ACC TCG TCC GAT TC 3′, c-mycin anti-sense primer: 5′ ATC AAT TTC TTC CTC ATC TTC TTG C 3′; pRB sense primer: 5′ TTT TCT AGT TCA CCC TTA CGG ATT C 3′, pRB anti-sense primer: 5′ ATT TTC TGG AAC TTT TCA GAT GTC C 3′. RT-qPCRs were performed in triplicate, and the average relative expression levels normalized against β-actin levels were calculated as described previously (Patek et al. 2008; Luo et al. 2009).

Bioinformatic analysis

The expression microarray data of the Sanger Cell Line Project (SCLP) (n = 732, the cancer cell lines with mutation data of the common tumour suppressor genes and oncogenes) were downloaded from the Broad Institute server (www.broadinstitute.org), and a Pearson’s correlation coefficient analysis was performed to correlate mRNA levels of KRAS with those of p107/RBL1 and p130/RBL2 genes in cancer cell lines without (n = 648) or with (n = 84) KRAS point mutations. The P-values were computed using an asymptotic confidence interval based on Fisher’s Z transform, and the samples were clustered using the Euclidean distance metric and the complete linkage algorithm. Mean tumour numbers, incidence and tumour volumes for the different animal groups were compared using the unpaired two-tailed Student’s t-test.

Results

Reduction from two alleles to one K-ras allele decreases expression levels of K-ras transcripts in the large intestine

To explore the effects of K-ras hemizygosity on the expression levels of K-ras transcripts in the normal colons of the K-ras+/- mice, real-time reverse transcription quantitative PCR (real-time RT-qPCR) was used to analyse relative expression levels of both K-ras 4A and 4B isoform transcripts normalized against β-actin RNA levels. The relative expression levels of K-ras 4A transcripts decreased from 15.2 ± 1.9 (mean ± SEM) in colons of wild-type mice to 7.1 ± 1.3 in colons from K-ras+/- mice (P < 0.01) (Figure 1). The relative expression levels of K-ras 4B transcripts decreased from 17.6 ± 2.0 (mean ± SEM) in colons of wild-type mice to 8.7 ± 1.6 in colons from K-ras+/- mice (P < 0.01) (Figure 1). K-ras 4A and 4B transcript expression levels were found to be reduced to approximately half in colons of K-ras+/- mice, consistent with the expected gene dosage effect.

K-ras hemizygosity promotes DMH-induced colorectal tumourigenesis in mice

To assess the effects of K-ras hemizygosity on large intestinal tumour formation, 25 wild-type (control K-ras+/+) and 28 hemizygous K-ras+/- mice were given 10 weekly intraperitoneal injections of DMH. Survival over one year was monitored with culling of mice that showed signs of intestinal tumour formation, and the remaining mice were killed at 53 weeks after the start of the DMH treatment. All murine small and large intestines were carefully examined for tumours. Most of the intestinal tumours were present in the distal colon and rectum, with very few in the small intestines, so only colorectal tumours were statistically analysed. The tumours were shown histopathologically to be adenomas, all of which displayed low-grade dysplasia with no differences between the two cohorts, with no invasive adenocarcinomas found in either cohort. The average colorectal tumour numbers increased from 0.52 ± 0.15 adenomas/mouse (mean ± SEM) in the control K-ras+/+ mice to 0.87 ± 0.14 adenomas/mouse in the K-ras+/- mice (P < 0.01) (Figure 2a). Proportions of mice in each group that developed colorectal tumours, termed incidence of colorectal tumours, was significantly higher (60%) in K-ras+/- mice compared with that observed in the control group (44%) (Figure 2b). The relative tumour volume significantly increased from 1.0 in control mice by 2.13-fold in K-ras+/- mice (P < 0.05) (Figure 2c). There were no significant differences in the tumour volume between male and female animals (P > 0.05). The pattern of survival differed between the two groups of mice over a period of 53 weeks, with a decrease from 88.0% in controls to 82.1% in K-ras+/- mice,
but as the mice were killed at the 1-year timepoint, statistical testing of natural survival by log-rank analysis was not possible (Figure 2d). Twenty tumours were analysed by DNA sequencing, 10 adenomas from each group of mice, for the presence of mutations in \textit{K-ras} exons 1, 2 and 3, and also in \textit{B-raf} exon 5, but no mutations were found in either gene in any of the tumours tested. The \textit{Beta-Catenin} gene was not examined for mutations. However, \textit{Apc} exon 15 mutations were identified in 4 of 10 adenomas from wild-type mice and in 5 of 10 adenomas from \textit{K-ras} \textit{+/−} mice.

Carcinogen-induced colorectal adenomas in hemizygous \textit{K-ras} mice show increased proliferation and decreased apoptosis

To assess whether \textit{K-ras} hemizygosity affects adenomatous cell proliferation and apoptosis, adenomas were immunohistochemically analysed for Ki-67 expression and positivity for cleaved caspase-3 using age-matched colorectal adenomas from the two groups of mice (Figure 3). The proportion (in 500 cells counted from 5 to 6 mice) of Ki-67 positive cells in adenomas from \textit{K-ras} \textit{+/−} (9.15 ± 0.92%, mean ± SEM) was significantly higher than that in adenomas from wild-type mice (7.77 ± 0.64%, \(P < 0.05\)) (Figure 3a,b). The proportion (in 500 cells counted from 5 to 6 mice) of adenoma apoptotic nuclei stained positively for cleaved caspase-3 was reduced from 1.40 ± 0.37% (mean±SEM) in wild-type mice to 0.80 ± 0.22% in \textit{K-ras} \textit{+/−} mice (\(P < 0.05\)) (Figure 3c,d).

Investigation of possible mechanisms of how \textit{K-ras} hemizygosity may affect carcinogen-induced colorectal adenoma formation in mice

To investigate possible mechanisms of how \textit{K-ras} may exert a gene dosage effect on colorectal tumourigenesis, evidence of activation of the Erk/MapKinase and PI3K/Akt pathways was sought by immunostaining the adenomas with...
anti-phospho-Erk1/2 and anti-phospho-Akt (both anti-p-Akt-Thr-308 and anti-p-Akt-Ser-473) antibodies. There was no evidence of a detectable change in immunopositivity for either phospho-Erk1/2 or phospho-Akt (at either position p-Akt-Ser-308 or p-Akt-Ser-473) in adenomas from DMH-treated K-ras<sup>+/−</sup> mice compared with adenomas from wild-type mice (data not shown). Real-time RT-qPCR was used to evaluate the transcript expression levels of RB family members <i>pRB</i>, <i>p107/RBL1</i>, <i>p130/RBL2</i>, <i>cyclin D1</i> and <i>c-myc</i> transcripts (mean ± SEM), determined by real-time RT-qPCR. Adenomas from K-ras<sup>+/−</sup> mice showed significantly lower levels of <i>p107/RBL1</i> and <i>p130/RBL2</i>; (P < 0.05 for both) than wild-type mice.

Figure 3 Immunohistochemical staining of the proliferation marker Ki-67 and the apoptosis marker cleaved caspase-3 in colorectal adenomas from age-matched DMH-treated wild-type and K-ras<sup>+/−</sup> mice (a–d, all at magnification ×200): Ki-67 expression in a colorectal adenoma from a DMH-treated wild-type mouse (a) and a K-ras<sup>+/−</sup> mouse (b). Ki-67 positivity in adenomas was significantly higher in the K-ras<sup>+/−</sup> mice than in wild-type mice (P < 0.05). Immunohistochemical staining of cleaved caspase-3 detection in a colorectal adenoma from a DMH-treated wild-type mouse (c) and a K-ras<sup>+/−</sup> mouse (d). Cleaved caspase-3 positive cells in adenomas were significantly lower in frequency in the K-ras<sup>+/−</sup> mice than in wild-type mice (P < 0.05).

Figure 4 Relative RNA expression levels in colorectal adenomas from DMH-treated wild-type K-ras<sup>+/+</sup> and K-ras<sup>+/−</sup> mice, of <i>pRB</i>, <i>p107/RBL1</i>, <i>p130/RBL2</i>, <i>cyclin D1</i> and <i>c-myc</i> transcripts (mean ± SEM), determined by real-time RT-qPCR. Adenomas from K-ras<sup>+/−</sup> mice showed significantly lower levels of <i>p107/RBL1</i> and <i>p130/RBL2</i>; (P < 0.05 for both) than wild-type mice.
with significantly decreased levels of p107/RBL1 and p130/RBL2 transcripts in the colorectal adenomas from K-ras<sup>−/−</sup> mice (93.38 ± 7.92 for p107/RBL1; and 42.90 ± 4.66 for p130/RBL2; mean ± SEM) compared with adenomas from wild-type K-ras<sup>−/−</sup> mice (212.53 ± 31.44 for p107/RBL1; and 109.34 ± 16.77 for p130/RBL2; P < 0.05 for both comparisons), but the transcript expression levels of pRB, cyclin D1 and c-myc showed no differences between K-ras hemizygous and wild-type murine adenomas (Figure 4).

To further investigate the correlation between KRAS and p107/RBL1 or p130/RBL2 transcript levels in human neoplastic samples, a correlation analysis in the largest up-to-date collection of human cancer cell line expression microarray data (n = 732, Sanger Cell Line Project) was performed. There was a weak but statistically significant positive correlation in the expression of both p107/RBL1 and p130/RBL2 with KRAS (Pearson’s correlation, adjusted P-value <0.01 for both) in cell lines without KRAS point mutations (n = 648), indicating that tumour cells with low KRAS expression appear to have low levels of p107/RBL1 and p130/RBL2 expression (Figure S1). When cancer cell lines with mutant KRAS were analysed in the same way, there was no significant correlation between KRAS and either p107/RBL1 or p130/RBL2 expression (data not shown).

**Discussion**

The effects of Ras proteins on regulation of proliferation, apoptosis and tumour formation are complex, as they vary between Ras family members and with their mutational status, expression levels and isoforms (K-ras 4A and 4B) expressed (Malumbres & Barbacid 2003; Luo et al. 2007a, b, 2009, 2011; Johnson et al. 1997; Patek et al. 2008). Here, we show that reduction in gene dosage from two wild-type K-ras alleles to one was associated with a 50% reduction in expression levels of wild-type K-ras transcripts in the K-ras<sup>−/−</sup> colons. This correlated with formation of significantly increased number, incidence and size of colorectal adenomas that do not bear K-ras or B-ras mutations, following treatment with the bowel-selective carcinogen DMH. DMH is known to induce intestinal adenoma formation by either activating Beta-Catenin mutation or inactivating Apc mutation, as found here. These adenomas in K-ras<sup>−/−</sup> mice showed significantly increased proliferation and decreased apoptosis.

The K-ras<sup>−/−</sup> murine colorectal adenomas showed no clear immunohistochemical evidence of increased activation of either the Erk/MapKinase pathway or the PI3K/Akt pathway, as well as no evidence of changes to the expression levels of c-myc or cyclin D1 transcripts by RT-qPCR, compared with those from controls. However, there was a significant reduction in the expression levels of p107/RBL1 and p130/RBL2 transcripts in the K-ras<sup>−/−</sup> colorectal adenomas compared with those from K-ras<sup>−/−</sup> mice, consistent with a previously described relationship between ras and p107/RBL1 and p130/RBL2 expression in other contexts (Diaz et al. 2005; Mason et al. 2004). This finding is of an association with differential gene expression, and this should be confirmed by future functional studies in the relevant cell type.

The RB family of pocket proteins (pRB, p107/RBL1, and p130/RBL2) has been shown to regulate the activity of members of the E2F family of heterodimeric transcription factors by binding directly to E2F family proteins and repressing E2F-mediated transcription (Hurford et al. 1997; Sun et al. 2007; Classon et al. 2000). Others have shown ras signalling either associates with or mediates regulation of the levels of expression of p107/RBL1 and/or p130/RBL2 (Diaz et al. 2005; Luo et al. 2007a,b; Mason et al. 2004). Thus, modulation of E2F family protein activity may link K-ras-mediated transcriptional changes to the control of cell cycle progression, with promotion of DMH-induced adenoma formation. A similar but weak correlation between KRAS and p107/RBL1 and/or p130/RBL2 transcript levels was also seen in a series of 648 human cancer cell lines with wild-type KRAS, but not in cell lines with oncogenic mutations in KRAS, in keeping with the previous observation that the transcriptional effects of KRAS are different depending on its mutation status (Luo et al. 2007a,b), and the proposed mechanism that the tumour suppressor activity of wild-type KRAS appears to involve changes in p107/RBL1 and p130/RBL2 levels. Activating mutations in KRAS have been shown to exert their main oncogenic activity through the canonical MAPK and PI3K/AKT pathways (Malumbres & Barbacid 2003; Luo et al. 2007a,b, 2009, 2010, 2011; Patek et al. 2008), which showed little or no activation in DMH-induced adenomas from mice with either heterozygous deletion or wild-type K-ras, with no mutations in K-ras in the adenomas.

Compared with K-ras hemizygosity promoting methyltransferase (MNU)-induced lung tumour formation (Patek et al. 2008), the present study found that K-ras hemizygosity also significantly promotes DMH-induced colorectal tumorigenesis in vivo, but to a lesser degree. This difference in the degree of tumour promoting effect of K-ras hemizygosity may be due to the single K-ras allele in the K-ras<sup>−/−</sup> mice usually becoming mutated (K-ras c.35G>A (p.Gly12Asp) or G12D mutation) in the MNU-induced lung tumours, but rarely or not becoming mutated in the DMH-induced colorectal tumours on the C57BL/6J background. Here, the tumour promoting effect relates to the gene dosage change being manifested as a ~50% reduction in K-ras transcript expression levels with decreased p107/RBL1 and p130/RBL2 expression, which is hypothesized to act via E2F proteins to modulate proliferation and tumour susceptibility.

This study adds to the accumulating evidence that proto-oncogenic ras can have a tumour suppressor effect. In separate experiments, overexpression of either wild-type K-ras, H-ras or N-ras genes have been reported to partially or completely suppress the transformed phenotype induced by their oncogenic counterparts (Diaz et al. 2005; Spandidos et al. 2007).
et al. 1990; Luo et al. 2007a,b; Spandidos & Wilkie 1988; Singh et al. 2005). K-ras null embryonic stem cells, when stably transfected with wild-type K-ras, induce smaller and more differentiated teratomas than those expressing oncogenic K-ras, indicating the oncosuppressive effect of the wild-type form (James et al. 2003). Loss of wild-type ras or allelic imbalance at ras loci in animal tumours is consistent with the oncosuppressive nature of wild-type ras (Zhang et al. 2001; Guerrero et al. 1985). Cancers of human origin, such as lung adenocarcinomas (Takeuchi et al. 1996), prostate cancer (Kibel et al. 1998), pancreatic cancer (Mahlmaki et al. 1997), breast cancer (Sourvinos et al. 1997) and acute lymphoblastic leukaemia (Baccichet & Sinnett 1997), have been found to show loss of heterozygosity around or near to the K-ras locus on chromosome 12p in some cases, consistent with a tumour-suppressive effect of wild-type K-ras.

In conclusion, following DMH treatment, K-ras hemizygosity promotes formation of increased number, incidence and size of colorectal adenomas that do not bear K-ras mutations. These adenomas showed increased proliferation, decreased apoptosis, along with evidence of an association with decreased transcript expression of p107/RBL1 and p130/RBL2 members of the RB family. Collectively, these data are consistent with expression of wild-type K-ras having a tumour suppressor effect on carcinogen-induced adenoma formation in the murine large bowel.

Acknowledgements

We are grateful to Maggie Green and Ian Purvis (Department of Pathology, University of Cambridge) for technical assistance. GP is a Pfizer Fellow of the Life Sciences Research Foundation. This work was supported by grants from Cancer Research UK and Wellcome Trust.

Author contributions

Feijun Luo, Hongtao Ye, Wenyan Zhang and Gehong Dong performed the experimental work; Rifat Hamoudi and George Poulogiannis carried out the bioinformatics analyses; Feijun Luo and Mark Arends designed the experiments, interpreted the histopathological and expression data and wrote most of the manuscript with some contributions from all of the other authors.

Conflict of interest

There are no disclaimers and no conflicts of interest. The experimental work was supported by a project grant from Cancer Research United Kingdom, and the mouse work was carried out under Home Office Licence.

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Luo F., Brooks D.G., Ye H. et al. (2009) Mutated K-ras<sup>App12</sup> promotes tumourigenesis in Apc<sup>Min</sup> mice more in the large than the small intestines, with synergistic effects between K-ras and Wnt pathways. Int. J. Exp. Pathol. 90, 558–574.

Wild-type K-ras suppresses colorectal tumours


Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Correlation of either p107/RBL1 or p130/RBL2 with KRAS transcript expression in human cancer cell lines with wild-type KRAS. (A) Scatter plots comparing the expression levels of p107/RBL1 (left), or p130/RBL2 (right) with KRAS, both show a significant correlation (P = 5.3 × 10⁻⁷ for p107/RBL1 and P = 0.008 for p130/RBL2, Pearson’s correlation coefficient analysis) with wild-type KRAS expression levels (includes those cell lines with no KRAS point mutation, n = 648, Sanger Cell Line Project). (B) Heatmap of KRAS and p107/RBL1 (top) or p130/RBL2 (bottom) expression levels across the 648 cancer cell lines.