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ACCELERATED PUBLICATION

Human RASSF7 regulates the microtubule cytoskeleton and is required for spindle formation, Aurora B activation and chromosomal congression during mitosis

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RASSF7, a member of the N-terminal Ras association domain family, has increased expression in various cancers and, on the basis of our previous work in *Xenopus* embryos, may be a regulator of mitosis. In the present study, we address, for the first time, the role of human RASSF7 in mitosis. We demonstrate that RASSF7 is expressed in a broad range of different cell types and that this expression could be enhanced following exposure to hypoxia. Knocking down *RASSF7* in human cell lines inhibited cell growth and induced defects in mitosis, including aberrant spindle formation and a failure in chromosomal congression. In order to understand the molecular basis of the defects in more detail, we analysed the activity of mitotic signalling proteins and found that activation of Aurora B did not occur in cells in which RASSF7 was knocked down. We also show that endogenous RASSF7 protein localizes to the centrosome and demonstrate using microtubule-regrowth assays that RASSF7 is an important regulator of microtubule dynamics. On the basis of these observations, we propose that, owing to its key role in regulating the microtubule cytoskeleton, RASSF7 is required for mitosis in human cells.

Key words: Aurora kinase, centrosome, hypoxia, microtubule, mitosis, Ras association domain family protein (RASSF protein).

INTRODUCTION

RASSF7 (Ras association domain family 7), previously known as HRC1 (HRAS1 cluster 1) [1] and C11orf13, is a member of the N-terminal RASSF family [2], a group of four proteins (RASSF7–RASSF10) which are structurally distinct from the classical RASSF proteins (RASSF1–RASSF6) [3,4]. There is emerging evidence to suggest that at least two members of the N-terminal RASSF family may be tumour suppressors. *RASSF8* has reduced expression in lung cancers and knocking down its expression causes an increase in growth [5–7], and *RASSF10* expression is epigenetically inactivated in leukaemias and thyroid cancers [8,9]. In contrast, microarray studies have demonstrated that *RASSF7* expression is up-regulated in a range of cancers [10–14], but little is known about its function. Our previous work showed that knocking down the expression of *Xenopus rassf7* in embryos caused a failure in spindle formation, nuclear fragmentation and apoptosis [15]. This suggests that RASSF7 is required for mitosis, a complex process involving microtubule nucleation from the spindle pole and the stable attachment of microtubules to the kinetochore [16–18]. However, RASSF7 function has not been studied in species other than *Xenopus* and the role that it plays in mitosis remains unknown. In the present study, we investigated the function of human RASSF7 and established why it is required for mitotic progression. Our data show that knocking down *RASSF7* causes a reduction in cell growth and multiple defects in mitosis, including a failure in spindle formation, Aurora B activation and chromosomal congression. In addition, we have shown that RASSF7 localizes to the centrosome and that it plays a crucial role in regulating microtubule growth. These results indicate that RASSF function is crucially important for mitosis because it regulates microtubule growth from the centrosome.

EXPERIMENTAL

Cell culture

Cell culture medium and reagents were purchased from Sigma. H1792 and HeLa cells were maintained at 37 °C under 5 % CO_2 in DMEM (Dulbecco's modified Eagle's medium), with 10 % fetal bovine serum, 1 % penicillin/streptomycin and 1 % glutamine. Mitotic cell enrichment and the microtubule-depolymerization and -regrowth assays were carried out as described in [19,20]. To expose cells to hypoxic conditions, cultures were placed in a humidified 37 °C multigas incubator with 5 % CO_2/95 % nitrogen for 3 h. Residual O_2 levels were monitored regularly and were found to be consistently less than 0.1 %. Control cultures, maintained under nomoxic conditions (21 % O_2), were processed in parallel.

Cloning, transfection and gene silencing

The coding sequence of human *RASSF7* (IMAGE clone: 40032773) was cloned into the expression vector pcDNA3-DEST53 (Invitrogen) using TOPO® and Gateway® cloning (Invitrogen) to generate a GFP (green fluorescent protein)-fusion

Abbreviations used: CENP-A, centromere protein A; GFP, green fluorescent protein; H a E, haematoxylin and eosin; INCENP, inner centromere protein; PLK1, Polo-like kinase 1; RASSF, Ras association domain family; shRNA, short hairpin RNA; siRNA, small interfering RNA.

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protein. This was transfected into HeLa cells using Lipofectamine™ (Invitrogen). H1792 cells were transiently transfected with siRNA (small interfering RNA) oligonucleotides targeting *RASSF7*, RASSF7 KD1 (Ambion oligonucleotide ID 13242) and RASSF7 KD2 (Ambion oligonucleotide ID 13338) or a control oligonucleotide targeting luciferase (MWG) using Oligofectamine™ (Invitrogen). The soft agar colony-forming assay was conducted as described in [7]. shRNA (short hairpin RNA) silencing of gene expression was achieved using *RASSF7* (code KHO9867N) and scrambled control shRNA. pGeneClip/Neo vectors from SuperArray Bioscience. Approx. 2×10^5 HeLa cells were seeded 1 day before transfection. Transfected cells were selected for 14 days in 1.2 mg/ml G418 (Sigma) and subsequently counted and analysed. The experiment was repeated as described above, except that the seeding of 2×10^5 HeLa cells was carried out after selection.

Immunoblotting

Immunoblotting was carried out and quantified using an Optichem detector with associated software (Ultra Violet Products) as described in [21]. After transfer, the membranes (Whatman) were blocked in 5 % (w/v) non-fat dried skimmed milk powder in Tris-buffered saline containing Tween 20. Antibodies used were rabbit anti-RASSF7 (Aviva Systems Biology ARP34390_T100), mouse anti-β-tubulin (Sigma T4026), mouse anti-(phospho-histone H3) (Abcam 14955). The anti-RASSF7 antibody produced a band at 34 kDa as predicted.

Immunofluorescence

Cells were grown on coverslips, fixed and permeabilized at -20 °C in methanol for 10 min and incubated in blocking solution (PBS, 1 % BSA and 3 % normal goat serum). Primary antibodies were: rabbit anti-pericentrin (Covance PRB-4325), mouse anti-γ-tubulin (Sigma T6557) and mouse anti-RASSF1A (eBioscience 14-6888). The rabbit anti-RASSF7 antibody required antigen retrieval to unmask the epitope, which involved EDTA treatment (1 mM, pH 8.0) for 1.5 h at 37 °C before blocking. For mouse anti-α-tubulin (Sigma T9026), mouse anti-(Aurora A) (BD Biosciences 610938), mouse anti-(Aurora B) (BD Biosciences 611082), rabbit anti-phospho-Aurora (Cell Signaling Technology 2914), rabbit anti-phospho-PLK1 (Polo-like kinase 1) (Biolegend 618601), mouse anti-INCENP (inner centromere protein) (Upstate Biotechnology 05-940), rabbit anti-phospho-CENP-A (centromere protein A) (Millipore 04-792) and rabbit anti-(active caspase 3) (Abcam ab13847) cells were fixed in 4 % (w/v) paraformaldehyde and permeabilized in 0.1 % Triton X-100 at room temperature (22 °C). Incubation with relevant secondary antibodies and DAPI (4′,6-diamidino-2-phenylindole) (1 μg/ml; Sigma) followed. Cells were then mounted in Mowiol (Sigma), visualized using a Zeiss LSM 510 confocal microscope, and images were captured and processed using LSM image browser software.

Promoter methylation analysis

COBRA (combined bisulfite restriction analysis) [8] was used to determine the methylation status of the 5′ CpG island associated with the *RASSF7* gene. Primers used for methylation analysis were 5′-TTTAGGAGYGGGGTTAGATATTTATTT-3′ and 5′-TTAACTACCTCTATCACRCCCCTCCC-3′.

Mouse *in situ* hybridization

In situ hybridization was carried out as described previously [7]. The mouse *Rassf7* probes were generated using a pCMV.sport6 vector (IMAGE clone 4206694) and an SP6 (sense) or T7 polymerase (antisense) following digestion with StuI or AhdI enzymes respectively. H a E (haematoxylin and eosin) staining was performed on paraffin sections using standard methods.

Statistical analysis

Means and S.D. values were calculated and plotted using Microsoft Excel. Each experiment was repeated at least three times. $P < 0.05$ was considered significant. Statistical analysis was carried out using unpaired Student's *t* tests.

RESULTS AND DISCUSSION

We began by establishing which tissues and cell types express RASSF7. *In situ* hybridization was carried out on mouse embryos and adult organs, and *Rassf7* was found to be expressed in all tissues examined; however, the level of expression varied, with particularly high levels seen in the lung and the brain (Figures 1A–1C). Immunoblotting of lysates from mammalian and human cell lines showed a major band which migrated at the predicted size of RASSF7 (34 kDa) (Figure 1D) and this band is specific for RASSF7 because it was reduced by two different knockdown approaches (Figures 2A and 2B). The immunoblotting showed that RASSF7 was expressed in all cell lines tested (Figure 1D). Consistent with the fact that RASSF7 is expressed in a number of different cancerous cell lines, and in contrast with other RASSF proteins [3,4], we found no evidence of *RASSF7* gene silencing by promoter methylation in 57 cancer cell lines (see Supplementary Figure S1A at http://www.BiochemJ.org/bj/430/bj4300207add.htm, and results not shown). *RASSF7* mRNA is up-regulated by hypoxia [22,23], which, given the hypoxic nature of solid tumours, might explain the increase in *RASSF7* expression seen in tumour cells [10–14]. Western blotting demonstrated that RASSF7 protein levels are also increased by hypoxic insult (Figure 1E), showing that RASSF7 protein is likely to be increased by the hypoxic environment found in solid tumours.

Several members of the RASSF family are believed to be tumour suppressors and act to restrain growth [3,4]. Consistent with this idea, knockdown of *RASSF6* or *RASSF8* causes an increase in the anchorage-independent growth of the human lung adenocarcinoma cell line H1792 [7,24]. To establish whether RASSF7 functions in a similar way, we analysed the effect of *RASSF7* knockdown on anchorage-independent growth. *RASSF7* expression was knocked down in cells transfected with RASSF7 KD2 siRNA (Figure 2A). In contrast, RASSF7 KD1 did not efficiently knock down *RASSF7* and this siRNA was used as an additional negative control along with the standard control siRNA (Figure 2A). H1792 cells showed significantly lower colony-forming activity in soft agar after RASSF7 KD2 siRNA treatment as both the size and the number of colonies was reduced compared with the controls (Figure 2A). These findings show that, unlike RASSF6 and RASSF8, which act to restrain growth, RASSF7 is essential for anchorage-independent growth. The fact that RASSF7 is required for growth may explain why its expression is not silenced in the cancer cell lines analysed above.

To address why RASSF7 is required for growth, we carried out shRNA knockdowns in HeLa cells. The alternative knockdown strategy ensures the specificity of the phenotype, and HeLa cells were used as they are easier to analyse than H1792 cells. Consistent with the data in H1792 cells, shRNA knockdown of *RASSF7* in HeLa cells caused a reduction in cell number (Figure 2B). This could be caused by increased apoptosis; however, the percentage of active caspase 3-positive

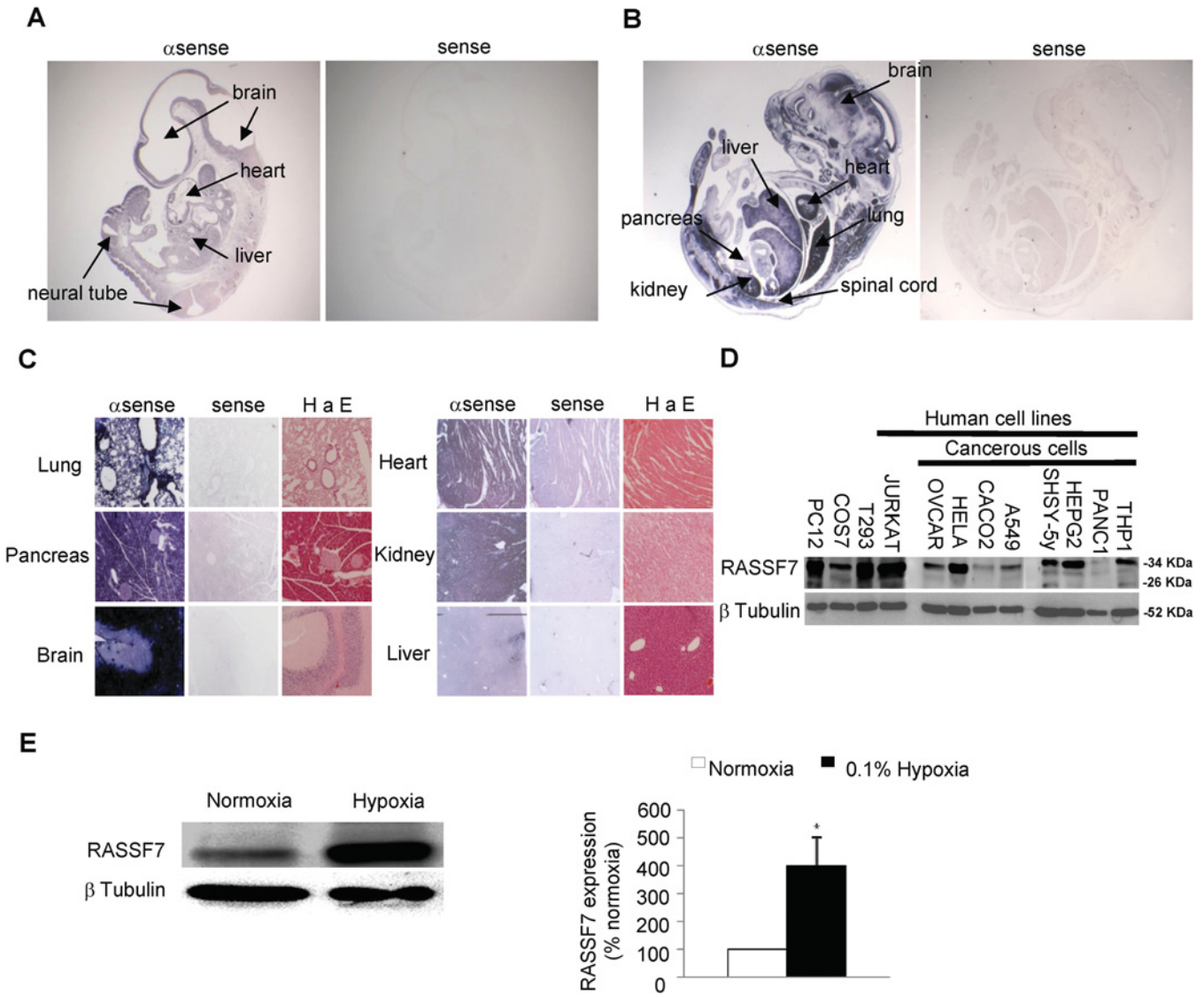

Figure 1 RASSF7 is expressed in a wide range of cell types

(**A**) *In situ* hybridization on E (embryonic day) 10.5 mouse embryos (×22). (**B**) *In situ* hybridization on E15.5 mouse embryos (×8). (**C**) *In situ* hybridization on mouse adult tissues (×50). The H a E staining shows the tissue architecture. (**D**) Western blotting shows expression of RASSF7 protein in different mammalian cell lines. Molecular masses are indicated in kDa. (**E**) RASSF7 protein expression levels increased in HeLa cells exposed to hypoxic conditions. $^*P < 0.05$ compared with corresponding controls. αsense, antisense.

RASSF7 KD cells was not significantly different from controls (see Supplementary Figure S2 at http://www.BiochemJ.org/bj/430/bj4300207add.htm). To establish whether the reduction in cell number was caused by a defect in mitosis, analogous to that seen in *Xenopus*, knockdown cells were examined and mitotic aberrations in metaphase *RASSF7*-knockdown cells were clearly evident. Loss of RASSF7 resulted in a failure in chromosomal congression, demonstrated by an increase in the number of mitotic cells which had failed to align their chromosomes (63 % compared with 18 % in controls, $n = 300$, $P < 0.01$) (Figure 2C), an increase in metaphase cells with lagging chromosomes (21.5 % compared with 6 % in controls, $n = 300$, $P < 0.01$) (Figure 2C, white arrows) and a decrease in metaphase cells with correctly aligned DNA (15.7 % compared with 75.7 % in controls, $n = 300$, $P < 0.001$). Spindle defects were also found in RASSF7-deficient cells, with spindles showing less pronounced polarization towards the DNA and a more radial organization of microtubules (Figure 2C, red arrows). Finally, there was a small increase in the rate of multi-polar spindles (32.6 % compared with 23.7 % in controls, $n = 300$, $P < 0.05$) (Figure 2C, arrowheads).

In order to understand the molecular basis for these mitotic defects, we examined the effect of *RASSF7* knockdown on established regulators of mitosis. Analysis of the activation of PLK1 [25], using an antibody which recognizes the active form, showed similar staining in *RASSF7*-knockdown and control cells (Figure 3A). The localization of RASSF1A, which is required for progression through mitosis [26–28], to the centrosome appeared to be the same in knockdown and control cells (Figure 3B). The localization and activation of Aurora A and Aurora B [29] was also examined (Figures 3C and 3D). An antibody against Aurora A and B and a phospho-specific antibody which recognizes the active form of Aurora A and B enzymes were used. Aurora A was present at the centrosome in RASSF7 (Figure 3C, arrows) and Aurora B was present at the kinetochores

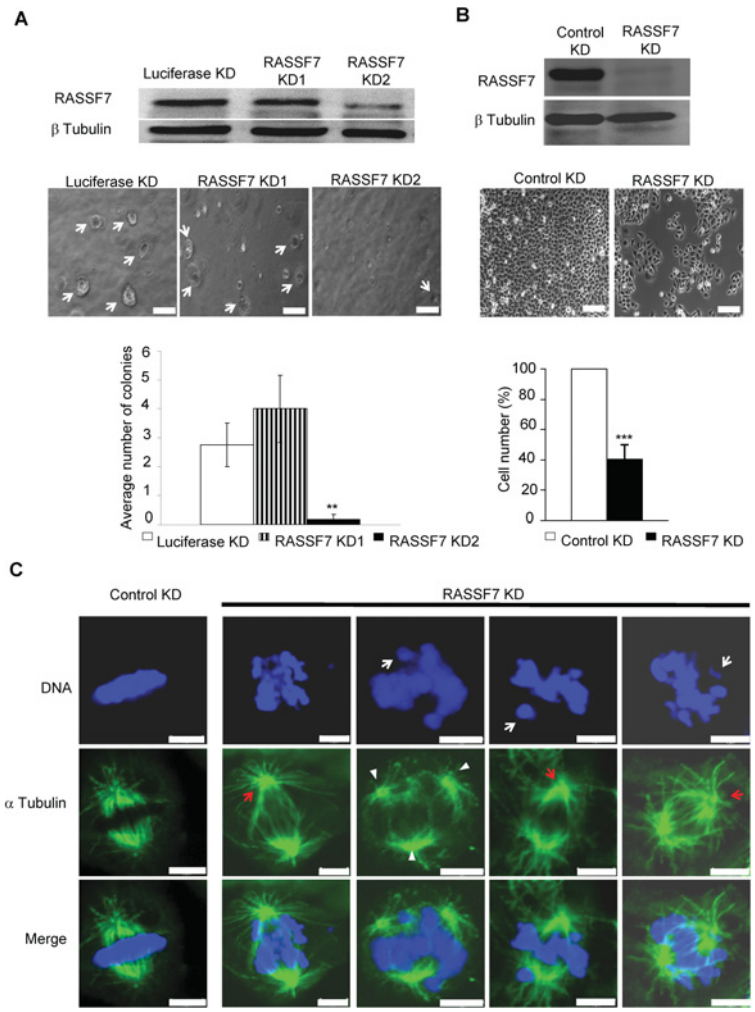

Figure 2 ***RASSF7* knockdown caused a reduction in cell number and defects in mitosis**

(**A**) *RASSF7* siRNA oligonucleotides KD2, but not KD1, blocked *RASSF7* expression and prevented H1792 cells from forming colonies (arrows). Quantification is based on the number of colonies bigger than 50 μm (**$P < 0.01$ for RASSF7 KD2 compared with Luciferase KD and RASSF7 KD1). (**B**) shRNA knockdown of *RASSF7* reduced HeLa cell numbers. The difference between control and RASSF7-depleted cells was 2.6 ± 0.6-fold (***$P < 0.001$), based on five independent experiments. This was not due to variations in the selection process as it was observed when cells were selected, replated at the same density and then allowed to grow (1.9 ± 0.4-fold reduction). (**C**) *RASSF7*-knockdown HeLa cells exhibit defects in spindle formation (red arrows highlight the more radial microtubules) and chromosomal congression, with an increase in cells which had failed to align their DNA (63 % compared with 18 % in controls, $n = 300$, $P < 0.01$) and an increase in cells with lagging chromosomes (21.5 % compared with 6 % in controls, $n = 300$, $P < 0.01$, white arrows). There was also a small increase in tripolar spindles (32.6 % compared with 23.7 % in controls, $n = 300$, $P < 0.05$, arrowheads). Scale bars, 100 μm (**A** and **B**) and 5 μm (**C**).

(Figure 3D, arrowheads). The active Aurora staining was present at the centrosomes (Figures 3C and 3D, arrows), but strongly reduced at the kinetochore (Figures 3C and 3D, arrowheads) in the *RASSF7* knockdowns, with only 9.3 % of cells showing strong Aurora B staining compared with 83.8 % in the controls ($n = 240$, $P < 0.001$), suggesting a failure in Aurora B activation. This loss appeared to be restricted to metaphase as the activation of Aurora B during cytokinesis appeared normal (see Supplementary Figure S3A at http://www.BiochemJ.org/bj/430/bj4300207add.htm). To confirm the loss of Aurora B activity, the phosphorylation of an Aurora B target, CENP-A [30], was analysed. Phospho-CENP-A levels were reduced (Figure 3E), with only 22 % of cells showing strong staining compared with 93.4 % of control cells ($n = 210$, $P < 0.001$), providing further evidence that Aurora B is not activated normally in the absence of RASSF7. The loss of Aurora B activity is likely to contribute to the mitotic defects seen in *RASSF7* knockdowns and, consistent with this hypothesis, inhibiting Aurora B causes chromosomal congression defects similar to those in *RASSF7*-knockdown cells [31,32].

To begin to understand the mechanistic basis for these mitotic defects, we analysed the subcellular localization of RASSF7. *Xenopus* rassf7 fusion proteins localize to the centrosome [15] and a GFP-tagged human RASSF7 protein also localized to the centrosome in HeLa cells (see Supplementary Figure S4A at http://www.BiochemJ.org/bj/430/bj4300207add.htm). To establish the localization of endogenous RASSF7, HeLa cells were stained with an anti-RASSF7 antibody. This showed strong staining which co-localized with γ-tubulin, a component of the pericentriolar material (Figure 4A) and this staining was specific as the signal was not present in *RASSF7*-knockdown cells (see Supplementary Figure S4B). RASSF7 staining was found at the centrosome in interphase cells and throughout all stages of mitosis (Figure 4A), showing that RASSF7 is not recruited to the centrosome during mitosis, but localizes there in dividing and

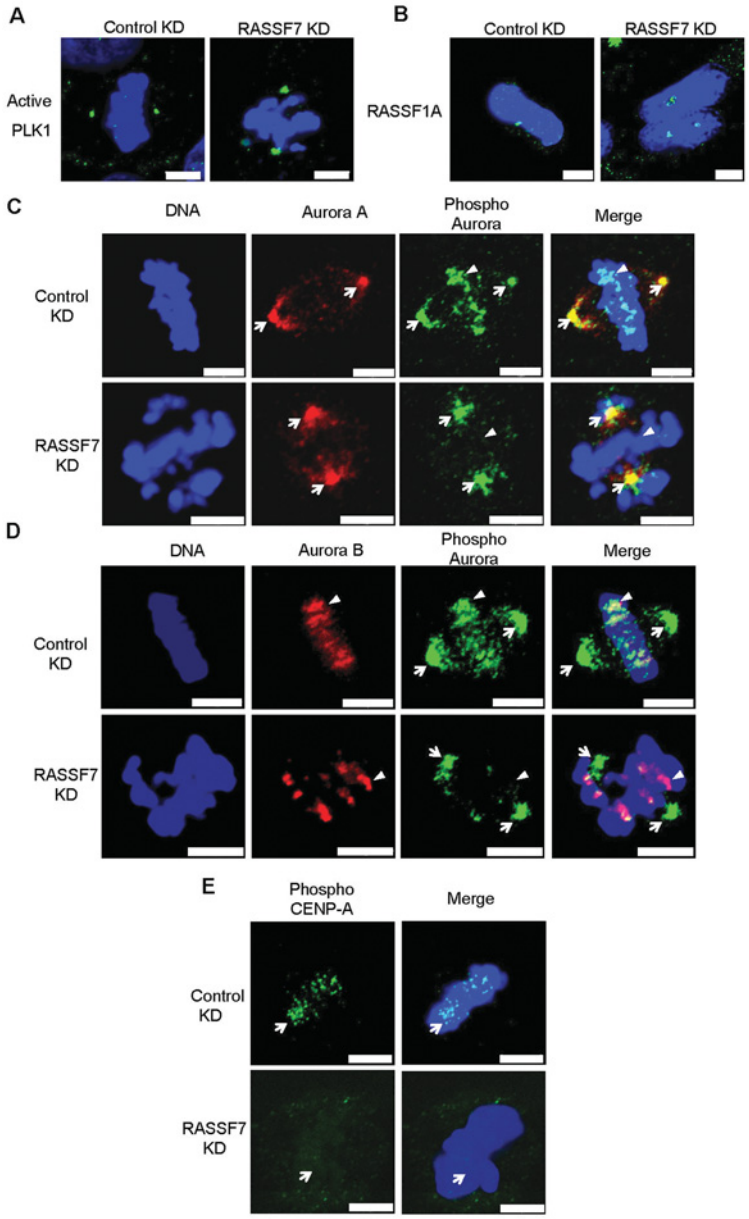

Figure 3 Analysis of mitotic signalling proteins in *RASSF7*-knockdown HeLa cells

(**A**) Active PLK1 (green) appeared normal. (**B**) RASSF1A (green) appeared normal. (**C** and **D**) Aurora A (red, arrows) and Aurora B (red, arrowheads) appeared to localize normally. Phospho-Aurora at the centrosomes (green, arrows) appeared normal, but phospho-Aurora at the kinetochore (green, arrowheads) was reduced (9.3 % of cells showed strong Aurora B staining compared with 83.8 % in the controls, $n = 240$, $P < 0.001$) suggesting that activation of Aurora B fails. (**E**) Phospho-CENP-A (green, arrow) was reduced (22 % of cells showed strong staining compared with 93.4 % of control cells, $n = 210$, $P < 0.001$). Blue shows nuclear staining. Scale bars, 5 μm.

non-dividing cells. The amount of RASSF7 protein was also not increased in mitotic cells (see Supplementary Figure S1B), so, despite RASSF7 having a role in mitosis, RASSF7 protein levels do not appear to be regulated during the cell cycle. The localization of RASSF7 at the centrosome was not affected by microtubule depolymerization with nocodazole (Figure 4B), indicating that human RASSF7 is an integral centrosomal component whose localization is microtubule-independent. Our previous data with *Xenopus* rassf7 suggested that its localization was microtubule-dependent [15]; this discrepancy could well be due to the use of a fusion protein in the *Xenopus* experiments.

The localization of RASSF7 at the centrosome and the spindle defects seen in the knockdowns suggest that RASSF7 might have a role in regulating microtubules. To test whether RASSF7 is required for microtubule polymerization, we investigated whether microtubule regrowth was affected by the loss of RASSF7. Cells were treated with nocodazole and subsequent microtubule regrowth was examined. The initial nucleation of microtubules in knockdown cells appeared normal (5 min), but after 15 min, 53 % of *RASSF7*-knockdown cells showed a delay in microtubule regrowth compared with 14 % of controls ($n = 100$, $P < 0.001$) (Figure 4C, arrows). The microtubules in *RASSF7*-knockdown cells also appeared more bent and looped than controls (Figure 4C, arrows). A similar delay in microtubule regrowth was seen at 30 min. After 60 min, the number of microtubules in the *RASSF7*-knockdown cells resembled the controls (Figure 4C), showing that

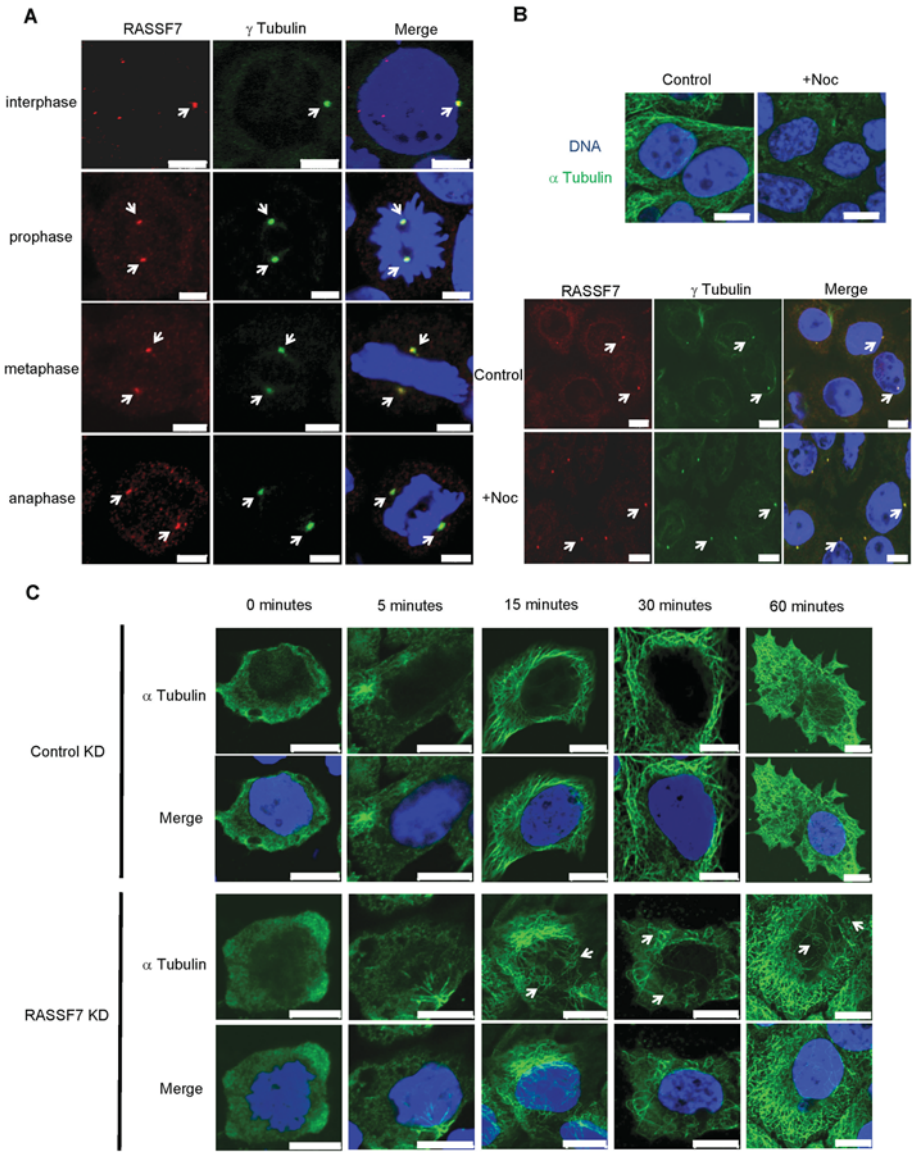

Figure 4 RASSF7 localizes to the centrosome and knocking down its expression causes defective microtubule regrowth in HeLa cells

(**A**) Co-localization of RASSF7 with the centrosomal marker γ-tubulin (arrows). (**B**) Microtubules (α-tubulin/green) were disrupted by treatment with nocodazole (300 ng/ml) for 1 h at 37 °C (top panels). This did not affect RASSF7 localization at the centrosomes which were stained for γ-tubulin (arrows, bottom panels). (**C**) Microtubule-regrowth assay. Microtubules were disrupted with nocodazole (300 ng/ml) for 1 h and subsequent regrowth was monitored at different time points. *RASSF7* knockdowns had fewer microtubules (after 15 and 30 min) and more bent microtubules (arrows). Quantification showed that, after 15 min, 53 % of knockdown cells had a delay in regrowth compared with 14 % of controls ($n = 100$, $P < 0.001$). Blue shows nuclear staining. Scale bars, 5 μm (**A**) and 10 μm (**B** and **C**).

RASSF7-knockdown cells eventually produce similar numbers of microtubules to control cells. However, the microtubules still appeared less straight than the control microtubules (Figure 4C, arrows). This assay demonstrates that RASSF7 is a key regulator of microtubule growth and provides an explanation for the spindle defects seen in mitosis. The broad expression of *RASSF7* and its localization to the centrosomes of interphase cells suggests that RASSF7 may also regulate microtubules in non-dividing cells.

A role in regulating microtubules can explain the defects in spindle formation seen in the knockdowns, but why is RASSF7, which localizes at the centrosome, required for the activation of Aurora B at the kinetochores? Aurora B activation requires the recruitment of INCENP [33], but *RASSF7* knockdown did not compromise the localization of this protein (see Supplementary Figure S3B). Interestingly, Aurora B activation also requires contact with microtubules [34,35]. We propose that the defect in spindle formation seen in the *RASSF7*-knockdown cells might stop microtubules from correctly interacting with Aurora B and cause the failure in Aurora B activation. This argues that the primary role of RASSF7 is in regulating microtubule growth and that mis-regulation of this process is responsible for the spindle defects and the loss of Aurora B activation. The aberrant spindle and loss of Aurora B would then both contribute to the dramatic failure in chromosomal congression seen in *RASSF7*-knockdown cells.

In summary, the present study provides the first functional analysis of human RASSF7 and shows that down-regulation of RASSF7 impairs cell growth and causes defects in mitosis, including abnormal spindle assembly, loss of Aurora B activation and a failure in chromosomal congression. We propose that these

mitotic defects are caused because RASSF7 localizes to the centrosome and regulates microtubule dynamics.

AUTHOR CONTRIBUTION

Asha Recino co-ordinated the study, designed experiments, collected and analysed the majority of the data and wrote the paper; Victoria Sherwood designed experiments, and collected and analysed data for preliminary experiments; Amy Flaxman assisted in data collection and analysis; Wendy Cooper designed experiments, and collected and analysed data; Farida Latif designed experiments and analysed data; Andrew Ward designed experiments and analysed data; Andrew Chalmers oversaw the project, designed experiments, analysed data and wrote the paper.

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SUPPLEMENTARY ONLINE DATA

Human RASSF7 regulates the microtubule cytoskeleton and is required for spindle formation, Aurora B activation and chromosomal congression during mitosis

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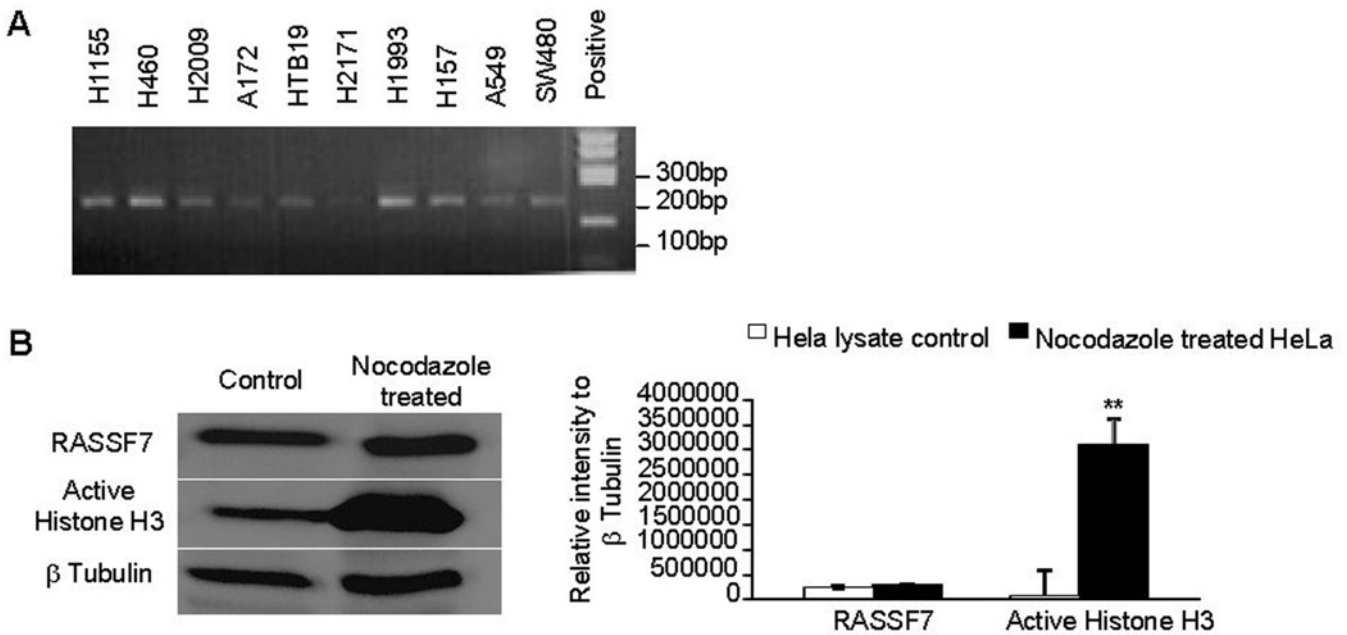

Figure S1 Expression of *RASSF7*

(**A**) Methylation of the *RASSF7* promoter. Analysis of the methylation status of the 5′ CpG island associated with the *RASSF7* gene was carried out using COBRA (combined bisulfite restriction analysis). The full-length PCR product was 206 bp and complete digestion with BstUI would generate products less than 86 bp. Efficient enzyme activity is indicated by complete digestion of plasmid DNA (positive control). No methylation was observed in any of the cancer cell lines analysed (20 lung, 12 breast, eight colorectal, eight kidney, five glioma and four neuroblastoma). Data from ten cell lines are presented. Sequencing of samples identified the correct PCR product and confirmed the unmethylated state. (**B**) Expression of RASSF7 protein in mitosis. HeLa cell lysates were enriched with M-phase cells through nocodazole treatment (75 ng/ml) for 18 h followed by mitotic shake off. There was no significant change in RASSF7 protein expression levels. Expression of the positive control, active histone H3 expression, increased as expected. $^{**}P<0.01$ compared with corresponding controls.

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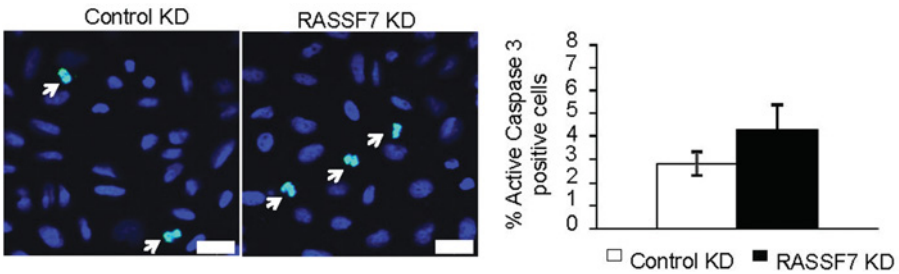

Figure S2 ***RASSF7*** **knockdown did not cause a significant increase in apoptosis**

RASSF7 depletion in HeLa cells did not significantly increase the number of active caspase 3-positive cells (green, highlighted by arrows) compared with controls. More than 500 cells were counted for each sample from three independent experiments. Blue shows nuclear staining. Scale bar, 20 μm.

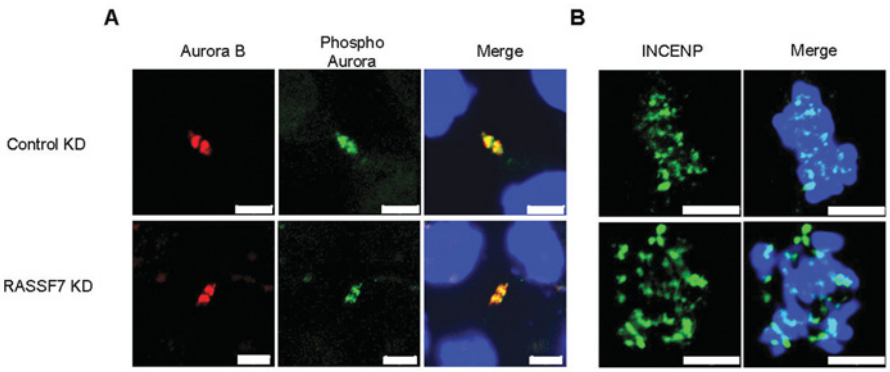

Figure S3 **Aurora B activity during mitosis**

(**A**) Aurora B phosphorylation appears normal during cytokinesis in *RASSF7*-knockdown HeLa cells. (**B**) INCENP (green), which is required for Aurora B activation, maintains its correct localization during metaphase in *RASSF7*-knockdown HeLa cells. Blue shows nuclear staining. Scale bar, 5 μm.

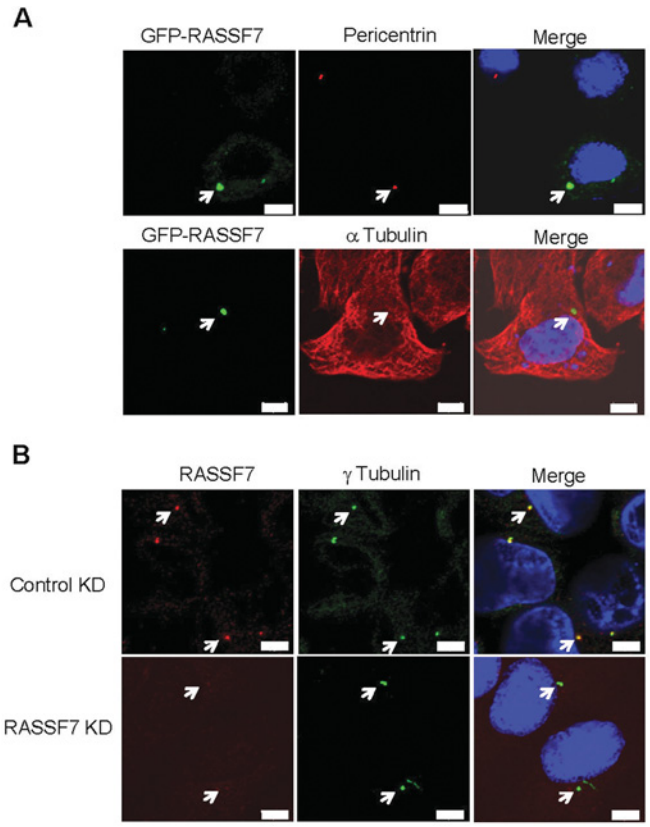

Figure S4 Localization of RASSF7

(**A**) A GFP–RASSF7 fusion protein localizes to the centrosomes (arrows), marked with pericentrin, when expressed at low levels in HeLa cells. When expressed at high levels, GFP–RASSF7 formed large aggregates (results not shown). We did not see any stabilization of microtubules by RASSF7 (high or low levels), reminiscent of the striking phenotype seen after expressing RASSF1A. (**B**) The endogenous centrosomal RASSF7 staining is lost in *RASSF7*-knockdown HeLa cells (arrows). Blue shows nuclear staining. Scale bar, 5 μm.

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