BJ www.biochemj.org

BJ Disease

Biochem. J. (2010) **425**, 303–311 (Printed in Great Britain) doi:10.1042/BJ20091318

REVIEW ARTICLE

The N-terminal RASSF family: a new group of Ras-association-domain-containing proteins, with emerging links to cancer formation

Victoria SHERWOOD[1], Asha RECINO, Alex JEFFRIES, Andrew WARD and Andrew D. CHALMERS[2]

Centre for Regenerative Medicine, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, U.K.

The RASSF (Ras-association domain family) has recently gained several new members and now contains ten proteins (RASSF1–10), several of which are potential tumour suppressors. The family can be split into two groups, the classical RASSF proteins (RASSF1–6) and the four recently added N-terminal RASSF proteins (RASSF7–10). The N-terminal RASSF proteins have a number of differences from the classical RASSF members and represent a newly defined set of potential Ras effectors. They have been linked to key biological processes, including cell death, proliferation, microtubule stability, promoter methylation, vesicle trafficking and response to hypoxia. Two members of the N-terminal RASSF family have also been highlighted as potential tumour suppressors. The present review will summarize what is known about the N-terminal RASSF proteins, addressing their function and possible links to cancer formation. It will also compare the N-terminal RASSF proteins with the classical RASSF proteins and ask whether the N-terminal RASSF proteins should be considered as genuine members or imposters in the RASSF family.

Key words: N-terminal Ras-association domain family (RASSF7–10), tumour suppressor, ubiquitin fold.

INTRODUCTION

Ras proto-oncogenes form part of a superfamily of small GTPases comprising of five families: *Ras*, *Rho*, *Rab*, *Ran* and *Arf* [1]. They play a pivotal role in a myriad of cellular processes, including cell growth, apoptosis, adhesion, migration and differentiation [2,3]. Unsurprisingly, defects in Ras signalling can result in disease progression, in particular oncogenesis. Indeed, Ras mutations, resulting in signalling aberrations, frequently occur in human tumours, particularly in pancreatic and lung adenocarcinomas [see the COSMIC (Catalogue of Somatic Mutation in Cancer) database at http://www.sanger.ac.uk/genetics/CGP/cosmic/]. Ras proteins carry out their diverse functions by binding to a broad range of Ras effectors and blocking these interactions has been highlighted as an important therapeutic opportunity that could be exploited for cancer treatments [4]. However, this requires a better understanding of the effector pathways utilized by Ras [4].

Each Ras effector contains one of a number of Ras-binding domains, an example is the RA [RalGDS (Ral guanine nucleotide dissociation stimulator)/AF6/MLLT4 (mixed-lineage leukaemia translocated to 4) Ras association] domain. This conserved domain is the defining feature of RASSF (Ras association domain family) members. The family now contains ten members (RASSF1–10) which are split into two groups, the classical (RASSF1–6) and the N-terminal (RASSF7–10) RASSF proteins [5]. Members of the classical RASSF proteins have been implicated in a range of biological processes, including the regulation of cell death, cell cycle control and microtubule stability, and they are generally regarded as tumour suppressors. This has prompted great interest in these proteins and there are excellent reviews which mainly focus on the classical RASSF family [6–8] and, in particular, RASSF1A [9–11]. Recently, four other proteins have been added to the family [5] and renamed RASSF7–10 (Table 1). These N-terminal RASSF proteins represent a new group of potential Ras effectors and they may have important biological functions, some of which could well be distinct from previously studied Ras effectors. They may also have a role in cancer progression. In the present review we will focus on the N-terminal RASSF proteins. We will summarize what is known about this newly described group of proteins and ask if there is any evidence to suggest a role for these proteins in cancer formation. We will also address the question of whether they should be considered as long-lost members or imposters in the RASSF family.

RASSF PROTEINS ARE DEFINED BY THE PRESENCE OF A RA DOMAIN/UBIQUITIN FOLD

The defining feature of the RASSF proteins is the presence of a RA domain. This domain was identified by comparing sequences from different Ras-binding proteins [12] and is present in over 50 human proteins [see the SMART (Simple Modular Architecture Research Tool) database at http://smart.embl-heidelberg.de/]. However, the RA domain nomenclature is potentially misleading as it implies that a protein with this domain will bind Ras. In fact, the binding affinities of RA domains for members of the Ras family show a huge variation and not all members will bind Ras

Abbreviations used: ALL, acute lymphocytic leukaemia; BRCA1, breast cancer 1; C1, protein kinase C-conserved region; CDC20, cell division cycle protein 20; FBLN1, fibulin-1; FERM, 4.1/ezrin/radixin/moesin; HRC-1, H-Ras1 cluster 1; JNK, c-Jun N-terminal kinase; LATS, large tumour suppressor; MOAP1, modulator of apoptosis 1; MST, mammalian STE20-like kinase; NDR, nuclear Dbf2-related; PAM, peptidylglycine α-amidating monooxygenase; P-CIP1, PAM C-terminal interactor 1; PI3K, phosphoinositide 3-kinase; RA, RalGDS (Ral guanine nucleotide dissociation stimulator)/AF6/MLLT4 (mixed-lineage leukaemia translocated to 4) Ras-association; RASSF, Ras-association domain family; RB, Ras-binding; SARAH, salvador, RASSF and hippo; SMART, Simple Modular Architecture Research Tool.

[1]Present address: Cell and Experimental Pathology, Lund University, Malmö University Hospital, S-205 02 Malmö, Sweden

[2]To whom correspondence should be addressed (email ac270@bath.ac.uk).

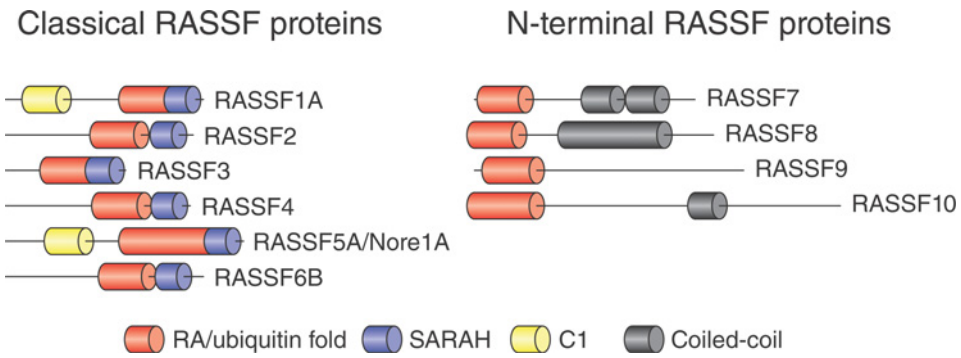

Figure 1 N-terminal RASSF proteins are structurally distinct from the classical RASSF proteins

The N-terminal RASSF proteins comprise a recently identified set of RA-domain/ubiquitin-fold-containing proteins. Their domain architecture is distinct from the classical RASSF proteins suggesting they should be considered as a separate group. Sequences used for the domain analysis (RefSeq accession numbers are given) are as follows: hsRASSF1A, NP_009113; hsRASSF2, NP_055552; hsRASSF3, NP_835463; hsRASSF4, NP_114412; hsRASSF5/splice variant NORE1A , NP_872604; hsRASSF6B (NP_958834; hsRASSF7, NP_003466; hsRASSF8, NP_009142; P-CIP1/RASSF9, AAD03250; and RASSF10 (NP_001073990, the short version described in [103]). hs, *Homo sapiens*.

[13,14]. A good example of a RA domain which does not bind Ras is found in the class IX myosin protein, Myr5 [15]. All RA domains are believed to form a similar three dimensional structure called an ubiquitin fold [16]; however, the RA domain in Myr5 lacks the positively charged amino acids which are required for Ras binding [15]. It is not surprising that only a subset of RA domains bind Ras, as the sequences of different RA domains are highly divergent [12]. There are also other ubiquitin-fold-containing proteins, such as FERM (4.1/ezrin/radixin/moesin)-domain-containing proteins and ubiquitin, which do not interact with Ras [16]. Another possible cause of confusion is the fact that other Ras effectors such as Raf and PI3K (phosphoinositide 3-kinase) interact with Ras through a RB (Ras-binding) domain. Despite the difference in nomenclature this domain also forms an ubiquitin fold [16]. Thus Raf, PI3K, RASSF proteins, FERM domain proteins and ubiquitin all share a common structural domain and can be considered part of an ubiquitin-fold family [13]. The variation in ability to bind Ras means that a key step in studying RA domain/ubiquitin fold proteins, such as the RASSF family members, is to establish whether the proteins function as Ras effectors, something which will be discussed below.

THE CLASSICAL AND N-TERMINAL RASSF PROTEINS HAVE DIFFERENT DOMAIN ARCHITECTURES

The RA domain/ubiquitin fold of classical RASSF members is found near the C-terminal of the protein, adjacent to a protein–protein interaction domain called the SARAH domain (Figure 1). This domain is named after the three types of proteins that contain it; salvador (also known as WW45 in vertebrates), RASSF and hippo [MST1/2 (mammalian STE20-like kinase 1/2) in vertebrates] [17]. SARAH domains have two α-helices, which form a novel dimeric anti-parallel helix [18]. Dimerization between SARAH domains allows salvador, RASSF and hippo to form homo- and hetero-dimers. RASSF1 and 5 also contain a DAG (diacylglycerol/phorbol ester)-binding domain (Figure 1), known as C1 (protein kinase C conserved region). In RASSF5 (also known as Nore1) the C1 domain can form an intramolecular complex with the RA domain/ubiquitin fold and, when free, bind the lipid phosphatidylinositol 3-phosphate [19].

The N-terminal RASSF proteins have different domain architecture to the classical RASSFs (Figure 1). The RA domain/ubiquitin fold of the N-terminal members is located at the opposite end to the C-terminal location found in the classical RASSF proteins. The RA domains/ubiquitin folds of the two groups also have quite different sequences which form phylogenetically distinct groups (Figure 2). In addition to the differences in the RA domains/ubiquitin folds the N-terminal RASSF members lack an identifiable SARAH motif [5,17]. However, some caution may be required on this point. The SMART database predicts that RASSF7, 8 and 10 have extensive coiled-coil regions, which, like SARAH domains, can form dimers mediated by hydrophobic residues [20]. Structural studies are required to confirm there is no similarity between the coiled-coils of the N-terminal RASSF proteins and SARAH domains of the classical proteins.

The *RASSF7, 8* and *10* genes are all located close to members of the *Ras* family in the genome [5,21,22]. This suggests that the N-terminal RASSF proteins may have co-evolved with members of the Ras family. We have not found a similar association between the classical *RASSF* genes and members of the *Ras* family, so this unusual juxtaposition of a *Ras* gene and a potential Ras effector represents another distinction between the two groups. The separation between N-terminal and C-terminal RASSF genes is not a recent event, *Drosophila* and *Caenorhabditis elegans* have both classical (*dmRASSF* [23] and *T24F1*.3 respectively [24]) and N-terminal RASSF (Table 1 and [5]) homologues. The differences between classical and N-terminal RASSF proteins prompted us to suggest they are distinct families, with the N-terminal RASSF proteins representing a new group of RA-domain/ubiquitin-fold-containing proteins [5].

CLASSICAL RASSF PROTEINS ACT AS TUMOUR SUPPRESSORS

The focus of the present review is the N-terminal RASSF proteins; however, before covering these proteins in detail we summarize what is known about the six classical RASSF (RASSF1–6) proteins. This is not intended to replace comprehensive reviews of the family [6–8], but to allow a comparison with the N-terminal RASSF members.

RASSF1 was originally identified in a yeast two-hybrid screen and the gene was found to reside at chromosome 3p21.3 [25], a region long suspected to contain at least one tumour suppressor [26]. The expression of one of the *RASSF1* transcripts, *RASSF1A*, was found to be repressed by promoter hypermethylation in lung tumours [25]. Subsequent studies found that *RASSF1A* was inactivated by methylation in a wide range of tumours (e.g. see [27]) and it quickly emerged that *RASSF1A* is one of the

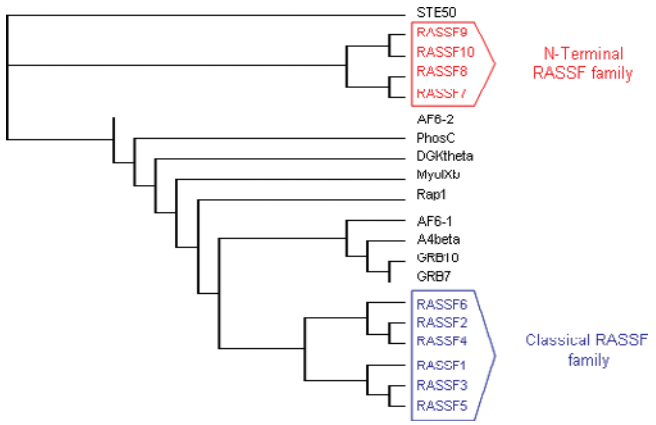

Figure 2 The RA domains of the classical and N-terminal RASSF proteins are phylogenetically distinct

The phylogeny of the RA domains from RASSF1–10, ten other RA domains from nine other proteins (AF6 has two RA domains), and a yeast outlier was inferred. The analysis was carried out by profile-aligning the RA domains to the alignment of RA domains from the SMART database, using ClustalW. Phylogenetic inference was then carried out using neighbour-joining, parsimony and maximum-likelihood methods using the PHYLIP3.67 software package. There was some variation in the tree topologies, but with each method the RA domains from the classical and N-terminal proteins clustered in two well-separated monophyletic groups. The tree here shows the maximum-likelihood inference using the Jones–Taylor–Thornton model. RA domains from the following sequence were used (RefSeq accession numbers are given unless otherwise stated): RASSF1A, NP_009113.3; RASSF2, NP_055552.1; RASSF3, NP_835463.1; RASSF4, NP_114412.2; RASSF5, NP_872604.1; RASSF6, NP_803876.1; RASSF7, NP_003466.1; RASSF8, NP_009142.2; RASSF9, GenBank® AAD03250.1; RASSF10, NP_001073990.1. STE50, Swiss-Prot P25344; Rap1 GenBank® AF478469_1; A4beta [amyloid β (A4) precursor protein-binding], GenBank® EAW86096.1; GRB10, Swiss-Prot Q13322; MYOIXb (myosin-IXb), Swiss-Prot Q13459; GRB7, NP_005301.2; DGKtheta (diacylglycerol kinase θ) Swiss-Prot P52824; PhosC (phospholipase C, ε1), Uni-Prot Q5VWL4; AF6-1, GenBank® BAA32485.1.

most frequently methylated genes in cancer [9–11]. Restoring expression of *RASSF1A* reduces tumour growth and knocking out *Rassf1A* in mice causes an increased frequency of tumour formation [28,29]. These studies provide convincing evidence that *RASSF1A* is a tumour suppressor, which is inactivated in a wide range of cancers. Inactivation by promoter methylation occurs in several other members of the classical RASSF family in human tumour cells, including *RASSF2*, *4* and *5* [6–8], suggesting that they may also be tumour suppressors. Understanding why RASSF1A and other members of the classical RASSF family act as tumour suppressors has been far from straightforward due to the variety of biological roles they possess. Classical RASSF proteins have been linked to a range of processes, particularly the regulation of apoptosis, cell-cycle progression and microtubule stability.

CLASSICAL RASSF PROTEINS ARE KEY REGULATORS OF APOPTOSIS

RASSF family members have been linked to promoting apoptosis through a number of effectors [6–8]. One group of effectors are the pro-apoptotic kinases MST1 and MST2, which bind members of the classical RASSF family [24,30,31]. Hippo, the *Drosophila* homologue of MST1, forms part of an important tumour suppressor network which is crucial for growth control [32,33]. Hippo functions by regulating the kinase warts which, in turn, regulates the transcriptional activator yorkie, which controls apoptosis-associated genes. Recent work shows that RASSF1A-induced apoptosis acts via a similar pathway, which involves MST2 activating LATS (large tumour suppressor), causing the release of YAP (yes-associated protein 1) which promotes transcription of p73 [34]. NDR (nuclear Dbf2-related) kinases, which are related to LATS kinases, can also function downstream of MST1 to promote apoptosis [35]. In addition to MST1/2, classical RASSF proteins bind another positive regulator of apoptosis, MOAP1 (modulator of apoptosis 1) [36–38]. After death receptor signalling, MOAP1 and RASSF1A are recruited to the death receptor where the interaction of RASSF1A with MOAP1 allows MOAP1 to activate Bax and promote apoptosis [36,39].

CLASSICAL RASSF PROTEINS ARE IMPORTANT FOR MICROTUBULE STABILITY AND CELL-CYCLE PROGRESSION

A second function of the classical RASSF proteins is to regulate the cell-cycle. Expression of RASSF1A blocks cell-cycle progression at a number of stages, including G_1, G_2–M and in prometaphase [40–43]. The RASSF1A-induced arrest in G_1 is associated with reduced activity of JNK (c-Jun N-terminal kinase) [44] and AP1 (activator protein-1) [45], which both promote cell-cycle progression. RASSF1A also up-regulates expression of the cyclin-dependent kinase inhibitor $p21^{Cip1/Waf1}$ [46]. These effects are likely to be mediated by a number of effectors. RASSF1A can bind MDM2 (murine double minute 2) and DAAX (death-domain-associated protein). This prevents degradation of the tumour suppressor p53, which would allow p53 to promote cell-cycle arrest [47]. RASSF1A can also bind and increase the activity of the transcription factor p120(E4F), a transcriptional repressor of cyclin A2 [48,49].

The RASSF1A-induced arrest in mitosis is tightly associated with the ability of RASSF1A to associate with microtubules. RASSF1A can bind and stabilize microtubules [41,43,50,51], probably via interacting with a number of microtubule-associated proteins [50,52]. Once bound to microtubule-associated proteins RASSF1A appears to function as a scaffold, recruiting multiple regulators of mitosis. Current data suggests these may include MST1/2, CDC20 (cell division cycle protein 20), Aurora-A and Ran. MST1 could signal through the hippo pathway (see above) to regulate mitotic progression [53]. RASSF1A can also bind and inhibit CDC20, which activates the anaphase-promoting complex [54], although it should be noted that this interaction is controversial [55]. RASSF1A is phosphorylated by the mitotic kinase, Aurora-A [56], and can regulate the activity of Aurora-A [57]. Finally it has recently been shown that Ran can act as a RASSF1A effector to regulate microtubule stability [58]. In addition to mitosis, RASSF1A has been linked to cell migration, which is consistent with a role in regulating microtubules [59].

Other members of the classical RASSF family have been linked to cell-cycle progression. An example is RASSF5/Nore1, which shows striking similarities to RASSF1A. RASSF5/Nore1 can associate with microtubules [60] and suppress growth by a mechanism which involves p53 activating the expression of $p21^{Cip1}$ [61]. In summary, classical RASSF proteins have been linked to apoptosis, cell-cycle control and the regulation of microtubule stability, all of which may contribute to the tumour suppressor function of these proteins.

CLASSICAL RASSF PROTEINS AND RAS

The presence of a RA domain/ubiquitin fold suggests that the classical RASSF proteins will act as Ras effectors. However, as discussed above, not all RA domains bind Ras, and for many of

Table 1 N-terminal RASSF family

The Table summarizes the nomenclature used for the N-terminal RASSF proteins. The genes marked with an asterisk (*) are not predicted to have an RA domain according to the SMART database; however, they do have sequence similarity over the RA domain region of the vertebrate N-terminal RASSF protein and thus can be considered as potential homologues. CG5053 can be considered a homologue of both RASSF7 and 8; K05B2.2 is the only *C. elegans* homologue of all N-terminal RASSF proteins.

N-terminal RASSF member	Chromosome	Alternative names	Potential *Drosophila* homologue	Potential *C. elegans* homologue
RASSF7	11p15.5	HRC-1, C11orf13	CG5053	K05B2.2*
RASSF8	12p12.3	HoJ-1 (Human carcinoma associated HoJ-1), C12orf2		
RASSF9	12q21.31	PAM, P-CIP1, PAMC1	CG13875*	
RASSF10	11p15.2	Similar to peptidylglycine α-amidating monooxygenase/C-terminal interactor 1	CG32150*	

the classical RASSF proteins it is not clear whether they function as Ras effectors in order to mediate the processes described above. RASSF5/Nore1 is perhaps the best documented Ras effector of the RASSF family. The splice variant RASSF5A/Nore1A was identified as a Ras-interacting protein by yeast two-hybrid screens and the endogenous protein interacts with Ras following addition of EGF (epidermal growth factor) [62]. RASSF5A/Nore1A is also the first member of the RASSF family to have the crystal structure of its RA domain/ubiquitin fold determined [63]. This was carried out in complex with Ras and demonstrated that the region which interacts with Ras is extended compared with other Ras effectors. This lengthened interface provides the RASSF5A/Nore1A–Ras complex with a prolonged lifetime compared with other Ras effectors. However, a physiological role for a RASSF5A/Nore1A–Ras complex has yet to be identified. The splice variant RASSF5B/Nore1B (also known as RAPL) is a Ras effector with a well-documented physiological role in T-cell signalling, where it associates with the Ras protein Rap1 [64].

RASSF1A can bind Ras in a GTP-dependent manner [65]. However, it binds with a much lower affinity than RASSF5/Nore1 [63,66]. This raises the question, is RASSF1A a genuine Ras effector? An andogenous RASSF1A–Ras complex has been described [66a], but, similar to the RASSF5A/Nore1A–Ras complex, the physiological role of this complex is not known. A recent twist to this story is that RASSF1A can bind to the small GTPase Ran [58] and regulate microtubule organization (discussed above). The binding appears to be direct and can be seen with endogenous protein. Ran is not a member of the Ras family but is part of the larger superfamily of related small GTPases [1]. This suggests that RASSF1A functions by binding Ran in addition to, or instead of, binding Ras. It also raises the possibility that RASSF1A and other RASSF proteins might bind other small GTPases in addition to Ras and Ran. Future work is required to untangle the biology of the classical RASSF proteins and the role that Ras and other small GTPases play in their function.

THE N-TERMINAL RASSF FAMILY

The difference in domain architecture and sequence of the RA domains prompted us to propose that the N-terminal RASSF proteins are a distinct family from the classical RASSF proteins [5]. This makes the decision to add them to the RASSF family look questionable. However, one crucial benefit of the renaming is to group the N-terminal RASSF proteins together for the first time. This makes it possible to compare what is known about each member. To achieve this we have searched the literature for studies relating to each N-terminal RASSF protein. We used the 11 different names which have been given to the vertebrate members (Table 1); it is important to point out that many of the references cited in the present review use the older nomenclature. Given the importance of the work on the *Drosophila* classical RASSF protein [23], we have also looked at the three potential N-terminal RASSF members in *Drosophila*. In the following sections we will summarize what is known about each of the N-terminal RASSF proteins and, where appropriate, relate that back to what is known about the classical RASSF proteins.

RASSF7: THE FIRST RASSF PROTEIN TO BE DESCRIBED?

The *RASSF7* gene was originally identified by a study which set out to sequence genes which are located close to *H-Ras* in the genome [21]. The authors found an unstudied gene and called it *HRC-1* (H-Ras1 cluster 1). *HRC-1* was recently renamed *RASSF7*, presumably because the protein it encodes contains an RA domain/ubiquitin fold and was not part of a recognized family. The hypothesis of the authors who identified *RASSF7*/*HRC-1* was that it might be a growth regulator because it was close to *H-Ras* in the genome [21]. They also suggested, on the basis of Southern blotting experiments, that RASSF7/HRC-1 might be part of a large family of related proteins. This was six years before RASSF5/Nore1 was identified as a potential Ras effector [62] and it is only recently that the authors' predictions about RASSF7 have begun to be confirmed.

The genomic position of *RASSF7* places it in close proximity to the *HRAS1* minisatellite which is immediately downstream of *H-Ras*. Rare alleles of this minisatellite were shown to be associated with cancer risk [67,68], and it was proposed that altered expression of *RASSF7* might contribute to the increased risk [69]. This generated a great deal of interest in the region; however, subsequent studies using improved technology failed to find a link [70,71] and the idea that rare alleles of the minisatellite are associated with cancer risk has fallen from favour.

RASSF7: A HYPOXIC-RESPONSE GENE WHICH IS UP-REGULATED IN CERTAIN CANCERS

The advent of genomic screening technology has made it possible to 'interrogate' the entire genome for genes which are misregulated in cancer. Several microarray studies have shown that *RASSF7* is up-regulated in cancer (Table 2). One example is in pancreatic cancer. Two independent studies have found that *RASSF7* expression is increased in pancreatic ductal adenocarcinoma relative to normal tissue [72–74]. In addition to ductal adenocarcinoma, *RASSF7* has increased expression in a second type of pancreatic cancer, islet cell tumours [75]; the study in fact selected *RASSF7* as a key gene whose expression can be used to identify islet cell tumours. *RASSF7* expression

Table 2 Aberrant expression of the N-terminal RASSFs in cancer cells

The Table presents a summary of studies reporting aberrant expression of N-terminal RASSF members in cancer. There are other examples in the Oncomine database (http://www.oncomine.org/), but we have only included those where we can find reference to the N-terminal member in the primary paper or supplementary material. It is important to note that these papers often use the alternative gene names described in Table 1.

Gene	Tumour type	Change	Reference
RASSF7	Pancreatic adenocarcinoma	Up-regulated	[73,74]
	Islet cell tumour	Up-regulated	[75]
	Endometrial carcinoma	Up-regulated	[76]
	Ovarian clear cell carcinoma	Amplified and up-regulated	[77]
RASSF8	Lung adenocarcinoma	Down-regulated	[22]
	Male germ cell tumours	Down-regulated	[94]
RASSF10	T-cell ALL	Down-regulated by promoter methylation	[103]

is also increased in endometrial cancer [76]. Similar to the situation in islet cell tumours, *RASSF7* showed a large increase in expression and was selected as one of the top 50 genes that distinguish malignant from normal endometrium [76]. Finally *RASSF7* lies in a genomic region which is amplified in ovarian clear cell carcinoma and its expression is increased in these cancers, correlating with the genomic amplification [77].

Interestingly, recent work offers plausible explanations as to why *RASSF7* may be up-regulated in cancer samples. Hypoxia, which occurs in solid tumours, is known to cause a large number of gene-expression changes [78] and *RASSF7* expression was found to be up-regulated by hypoxia in the MCF7 breast cancer cell line [79] and in human umbilical vein endothelial cells [80]. This predicts that the hypoxic environment found in solid tumours would cause an increase in *RASSF7* expression. Furthermore, *RASSF7* is also down-regulated by the tumour suppressor, BRCA1 (breast cancer 1), suggesting its expression would be increased in cancer cells which have lost BRCA1 function [81].

RASSF7 IS REQUIRED FOR CELL DEATH AND PROLIFERATION

An important question is what role RASSF7 plays in these cancerous cells. There is currently no evidence to suggest that increased expression of RASSF7 promotes cancer formation. However, RASSF7 function has been linked to some key biological processes including the regulation of cell death and proliferation. RASSF7 has been shown to be required for necroptosis [82], a regulated form of necrosis which is distinct from apoptosis. A large-scale siRNA (small interfering RNA) screen was carried out to find proteins required for necroptosis and this identified RASSF7 and RASSF8.

We identified *Xenopus RASSF7* in a microarray screen [83] and subsequently found that in *Xenopus* RASSF7 is essential for cell-cycle progression and cell survival [5]. In cells where RASSF7 is knocked-down, mitotic spindles fail to form and cells arrest in mitosis. This causes nuclear fragmentation and apoptosis. Consistent with a role in mitotic progression, *Xenopus* RASSF7 is localized at the centrosome. However, *Xenopus* RASSF7 is not a core component of the centrosome, rather it appears to be enriched at the centrosome because it interacts with the minus ends of microtubules. Preliminary results from a large-scale screen suggests that the *Drosophila* homologue may also be required for cell proliferation as knockdown by RNA interference caused a reduction in the mitotic index and weak spindle defects [84].

RASSF7, like other RASSF proteins, contains no catalytic domain so to understand its function it is crucial to identify the proteins it interacts with. Yeast two-hybrid studies have identified potential binding partners for human RASSF7. These include CHMP1B (chromatin modifying protein 1B), which is associated with endosomal membrane trafficking, and DISC1 (disrupted in schizophrenia 1), which interestingly interacts with microtubules [85,86].

RASSF8 IS LOCATED IN A GENOMIC REGION ASSOCIATED WITH LUNG CANCER RISK

The genomic sequence for *RASSF8* was first deposited into the NCBI (National Center for Biotechnology Information) database by Hoon and Yuzuki in 1996 (accession number Q8NHQ8) and was called human carcinoma-associated HoJ-1. However, there appears to have been no publication associated with this submission. Subsequently, *RASSF8* was characterized as a gene involved in a chromosomal translocation that is associated with a complex type of synpolydactyly [87,88]. *RASSF8* was found to be located on chromosome 12 and referred to as *C12orf2*. The chromosomal translocation fused *RASSF8* with the *FBLN1* (fibulin-1) gene (22q13.3). It is believed that this disrupts a *FBLN1* splice variant, causing the synpolydactyly. C12orf2 was then renamed RASSF8, presumably because it contains a RA domain/ubiquitin fold and was not part of a recognized family. This occurred at the same time as HRC-1 was renamed RASSF7 and both proteins were added to the RASSF family.

RASSF8 is located approx. 700 kb from the *K-Ras2* gene [22] and both genes lie in a region called Pals1. This region has been identified as a major susceptibility locus in a mouse model for lung carcinogenesis [89]. There are a number of genes in this region, but it is mutations in the *K-Ras2* gene that are believed to be responsible for the increased risk [90]. The homologous region in humans has also been associated with increased lung adenocarcinoma risk [91]. However, in humans it is not clear whether it is *K-Ras2* that is responsible. Analysis of the region in a Japanese population, identified the D12S1034 microsatellite as being most tightly associated with lung cancer risk [92]. The D12S1034 locus showed a bigger difference between cases and controls than the microsatellite adjacent to *K-Ras2*. This argues that in some human cancers, susceptibility may be due to a mutation in a gene adjacent to D12S1034 rather than in *K-Ras2* itself. *RASSF8* lies within 20 kb of D12S1034 making it a good candidate gene, particularly as RASSF8 has also been described as a potential tumour suppressor in lung cancer (see below). However, common polymorphisms in *RASSF8* are not associated with cancer risk in an Italian population [93]. Thus it is not clear currently if there is a link between *RASSF8* and the increased lung cancer risk associated with the Pals1 region.

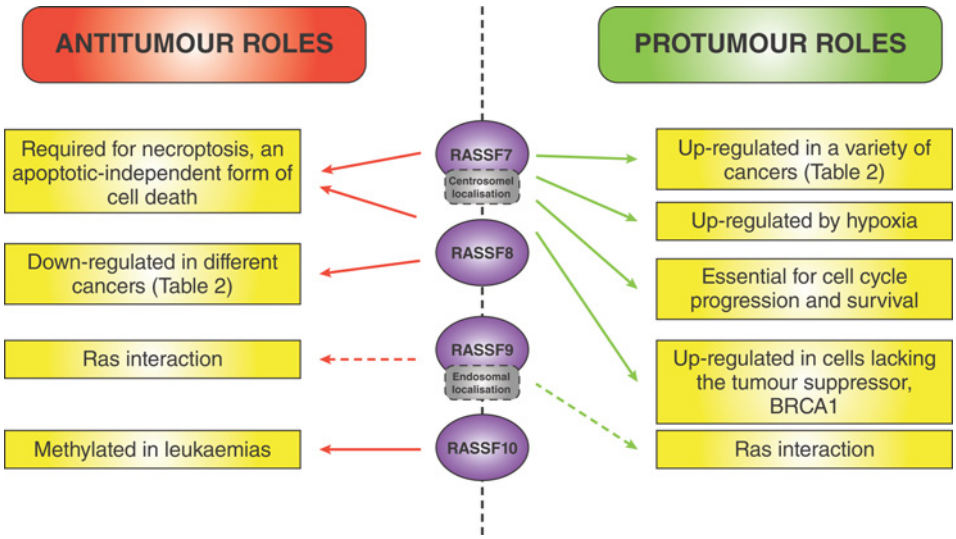

Figure 3 Emerging evidence suggests a possible link between the N-terminal RASSF proteins and cancer progression

A summary of the evidence suggesting that the N-terminal RASSFs may play a role in tumorigenesis. Data consistent with potential antitumorigenic roles are indicated by red arrows and evidence suggesting pro-tumorigenic roles are indicated by green arrows. Broken arrows highlight links which may be anti- or pro-tumorigenic. The cellular localization is given where it is known. Full details and references are provided in the main text.

RASSF8 IS A POTENTIAL TUMOUR SUPPRESSOR

There are several lines of evidence to suggest that RASSF8 may be a tumour suppressor (altered expression levels are summarized in Table 2). The best characterized example is in lung cancer [22]. In lung adenocarcinoma *RASSF8* transcript levels were reduced compared with normal tissue. Overexpression of RASSF8 protein in lung cancer cell lines also inhibited anchorage-independent growth, which has been correlated with tumour progression and metastasis. In addition to lung adenocarcinoma, *RASSF8* expression is also down-regulated in male germ cell tumours [94], despite the fact that the gene lies in a genomic region which shows gain in almost 100 % of these cancers. Finally, *RASSF8* was identified as a candidate gene involved in leukaemia and lymphoma formation in a study on retroviral-induced blood cancers in mice [95]. This model assumes that oncogenes and tumour suppressors often lie near common retroviral insertion sites. A genomic region next to *RASSF8* was targeted seven times, making it one of the most frequently hit sites in the study. This suggests that misregulation of *RASSF8* may contribute to leukaemia and lymphoma formation, so it is interesting that *RASSF8* has higher expression in human haematopoietic stem cells and is required for blood cell development in zebrafish [96]. It is not known why RASSF8 might be a tumour suppressor, but it is interesting that, similar to RASSF7, it is required for cell death by necroptosis [82]. This form of cell death may be particularly important in cells with deficiencies in their apoptotic machinery, such as tumour cells, so a role in necroptosis would be consistent with a tumour suppressor function.

Mass spectrometry and yeast two-hybrid screens have identified a number of potential binding partners for RASSF8, including the scaffolding protein, 14-3-3γ, which binds phosphoproteins to modulate their function [97], FRMD4A (FERM-domain-containing 4A), a protein that links membrane domains to actin, and PSMD4 [proteasome (prosome, macropain) 26S subunit, non-ATPase, 4], a component of the proteosome [98]. These potential binding partners offer interesting leads for future work aimed at understanding why RASSF8 may function as a tumour suppressor.

RASSF9 IS A RAS-BINDING PROTEIN THAT HAS BEEN LINKED TO VESICLE TRAFFICKING

RASSF9 was first identified by a yeast two-hybrid screen as a protein that interacted with the cytoplasmic domain of PAM (peptidylglycine α-amidating monooxygenase) [99]. On the basis of this interaction it was originally named P-CIP1 (PAM C-terminal interactor 1). PAM is a transmembrane protein found in secretory vesicles of neurons and endocrine cells, where it catalyses the α-amidation of bioactive peptides such as oxytocin and vasopressin. This modification is essential for the activity of these peptides [100]. We realized that P-CIP1 contains an RA domain/ubiquitin fold and is closely related to RASSF7 and 8 and suggested it should be renamed RASSF9 [5]. The binding of RASSF9/P-CIP1 to PAM was confirmed and RASSF9/P-CIP1 was found to associate with recycling endosomes [101]. This led to the model that it might bind the cytoplasmic domain of PAM during recycling of the enzyme [101], an interaction that may be regulated by phosphorylation, as the cytoplasmic domain of PAM is known to be multiply phosphorylated [102]. However, *RASSF9* mRNA is expressed much more widely than that of *PAM*, so RASSF9 might have additional roles and perhaps binds other transmembrane proteins.

Interestingly RASSF9 is the one member of the N-terminal RASSF proteins which has been shown to bind Ras proteins. Pull-down experiments with RASSF9 and Ras family GTPases showed that RASSF9 binds N-Ras, K-Ras and R-Ras [14]. An issue that remains to be addressed is whether RASSF9 binds endogenous Ras proteins, or other small GTPases, something which has not been straightforward to answer for the classical RASSF proteins (see above).

RASSF10 IS A CANDIDATE TUMOUR SUPPRESSOR IN CHILDHOOD LEUKAEMIA

We discovered a predicted protein was similar in sequence to RASSF9 and named this protein RASSF10 [5]. This gene was completely unstudied prior to recent work showing that it is a

candidate tumour suppressor in childhood leukaemias [103]. The transcript of *RASSF10* was characterized and found to be shorter than the predicted version. The protein encoded by the shorter version is more similar to RASSF7–9 and is used in Figure 1. *RASSF10* contains a large CpG island and, given the frequent inactivation of classical RASSFs by promoter hypermethylation (see above), the authors examined the methylation status of this gene in childhood leukaemia. They found that *RASSF10* was frequently methylated in leukaemia cell lines (100%) and T-cell ALL (acute lymphocytic leukaemia) (88%), but not in normal bone marrow and blood samples. *RASSF10* was also rarely methylated in B-cell ALL (16%). Inhibiting this methylation caused an up-regulation of expression in the leukaemia cell lines. These results strongly suggest that *RASSF10* expression is inhibited by promoter methylation in a high percentage of T-cell ALL, raising the possibility that RASSF10 might function as a tumour suppressor in these cancers. ESTs (expressed sequence tags) for *RASSF10* are present in a number of tissues [103] and it will be interesting to see whether *RASSF10* is methylated in tumours derived from these tissues.

The function of RASSF10 remains unstudied in vertebrates. There is a potential *Drosophila* homologue (Table 1). However, little is known about this gene except that it is expressed in precursors of the peripheral nervous system [104], and knocking down its function impairs hedgehog signalling [105]. RASSF10 offers exciting opportunities for future study.

CONCLUDING REMARKS

The N-terminal RASSF proteins have a different domain architecture from the classical RASSF proteins and so we proposed that they should be considered as a separate family [5]. Donninger and colleagues came to a similar conclusion for RASSF7 and 8, suggesting that they are a separate sub-family distinct from the 'true' RASSF proteins [10]. If the N-terminal and classical RASSF proteins are members of different families then one might expect that there will be little overlap between their biology. However, RASSF7 and RASSF1A show similar centrosomal localization and mitotic defects when knocked-down, and RASSF10 and members of the classical RASSF family both show promoter hypermethylation. These similarities might suggest that the N-terminal RASSF proteins are genuine RASSF proteins. However, we feel the differences between them still outweigh the similarities and that the N-terminal RASSF proteins are not true RASSF proteins, but a separate family. Emerging evidence presented in this review suggests that the N-terminal RASSF proteins might play a role in tumour formation (summarized in Figure 3). There is now an exciting opportunity to study this new group of proteins in more detail and confirm whether they are important in oncogenic progression.

ACKNOWLEDGEMENTS

We apologize to those whose work we could not cite due to space restrictions. We thank Dr Eric O'Neill (Gray Institute for Radiation Oncology and Biology, University of Oxford, Oxford, U.K.) and Dr Paul Whitley (University of Bath, Bath, U.K.) for critical comments on the manuscript prior to submission.

FUNDING

The work of our laboratory is supported by a Marie Curie PhD studentship [grant number MCEST-CT-2005-019822 to A.R.]; a Medical Research Council Co-operative Group Grant [grant number 66812 to A.W.]; a Medical Research Council Fellowship [grant number G120/844 to A.C.]; and by Cancer Research U.K. [grant number C26932/A9548].

REFERENCES

1 Takai, Y., Sasaki, T. and Matozaki, T. (2001) Small GTP-binding proteins. Physiol. Rev. **81**, 153–208
2 Cully, M. and Downward, J. (2008) SnapShot: Ras Signaling. Cell **133**, 1292–1292.e1
3 Hancock, J. F. and Parton, R. G. (2005) Ras plasma membrane signalling platforms. Biochem. J. **389**, 1–11
4 Tanaka, T. and Rabbitts, T. H. (2008) Interfering with protein–protein interactions: potential for cancer therapy. Cell Cycle **7**, 1569–1574
5 Sherwood, V., Manbodh, R., Sheppard, C. and Chalmers, A. D. (2008) RASSF7 is a member of a new family of Ras association domain-containing proteins and is required for completing mitosis. Mol. Biol. Cell **19**, 1772–1782
6 Avruch, J., Xavier, R., Bardeesy, N., Zhang, X. F., Praskova, M., Zhou, D. and Xia, F. (2009) Rassf family of tumor suppressor polypeptides. J. Biol. Chem. **284**, 11001–11005
7 Richter, A. M., Pfeifer, G. P. and Dammann, R. H. (2009) The RASSF proteins in cancer; from epigenetic silencing to functional characterization. Biochim. Biophys. Acta **1796**, 114–128
8 van der Weyden, L. and Adams, D. J. (2007) The Ras-association domain family (RASSF) members and their role in human tumourigenesis. Biochim. Biophys. Acta **1776**, 58–85
9 Dammann, R., Schagdarsurengin, U., Seidel, C., Strunnikova, M., Rastetter, M., Baier, K. and Pfeifer, G. P. (2005) The tumor suppressor RASSF1A in human carcinogenesis: an update. Histol. Histopathol. **20**, 645–663
10 Donninger, H., Vos, M. D. and Clark, G. J. (2007) The RASSF1A tumor suppressor. J. Cell. Sci. **120**, 3163–3172
11 Hesson, L. B., Cooper, W. N. and Latif, F. (2007) The role of RASSF1A methylation in cancer. Dis. Markers **23**, 73–87
12 Ponting, C. P. and Benjamin, D. R. (1996) A novel family of Ras-binding domains. Trends Biochem. Sci. **21**, 422–425
13 Wohlgemuth, S., Kiel, C., Kramer, A., Serrano, L., Wittinghofer, F. and Herrmann, C. (2005) Recognizing and defining true Ras binding domains I: biochemical analysis. J. Mol. Biol. **348**, 741–758
14 Rodriguez-Viciana, P., Sabatier, C. and McCormick, F. (2004) Signaling specificity by Ras family GTPases is determined by the full spectrum of effectors they regulate. Mol. Cell. Biol. **24**, 4943–4954
15 Kalhammer, G., Bahler, M., Schmitz, F., Jockel, J. and Block, C. (1997) Ras-binding domains: predicting function versus folding. FEBS Lett. **414**, 599–602
16 Herrmann, C. (2003) Ras-effector interactions: after one decade. Curr. Opin. Struct. Biol. **13**, 122–129
17 Scheel, H. and Hofmann, K. (2003) A novel interaction motif, SARAH, connects three classes of tumor suppressor. Curr. Biol. **13**, R899–R900
18 Hwang, E., Ryu, K. S., Paakkonen, K., Guntert, P., Cheong, H. K., Lim, D. S., Lee, J. O., Jeon, Y. H. and Cheong, C. (2007) Structural insight into dimeric interaction of the SARAH domains from Mst1 and RASSF family proteins in the apoptosis pathway. Proc. Natl. Acad. Sci. U.S.A. **104**, 9236–9241
19 Harjes, E., Harjes, S., Wohlgemuth, S., Muller, K. H., Krieger, E., Herrmann, C. and Bayer, P. (2006) GTP-Ras disrupts the intramolecular complex of C1 and RA domains of Nore1. Structure **14**, 881–888
20 Grigoryan, G. and Keating, A. E. (2008) Structural specificity in coiled-coil interactions. Curr. Opin. Struct. Biol. **18**, 477–483
21 Weitzel, J. N., Kasperczyk, A., Mohan, C. and Krontiris, T. G. (1992) The HRAS1 gene cluster: two upstream regions recognizing transcripts and a third encoding a gene with a leucine zipper domain. Genomics **14**, 309–319
22 Falvella, F. S., Manenti, G., Spinola, M., Pignatiello, C., Conti, B., Pastorino, U. and Dragani, T. A. (2006) Identification of RASSF8 as a candidate lung tumor suppressor gene. Oncogene **25**, 3934–3938
23 Polesello, C., Huelsmann, S., Brown, N. H. and Tapon, N. (2006) The *Drosophila* RASSF homolog antagonizes the hippo pathway. Curr. Biol. **16**, 2459–2465
24 Khokhlatchev, A., Rabizadeh, S., Xavier, R., Nedwidek, M., Chen, T., Zhang, X. F., Seed, B. and Avruch, J. (2002) Identification of a novel Ras-regulated proapoptotic pathway. Curr. Biol. **12**, 253–265
25 Dammann, R., Li, C., Yoon, J. H., Chin, P. L., Bates, S. and Pfeifer, G. P. (2000) Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat. Genet. **25**, 315–319
26 Lerman, M. I. and Minna, J. D. (2000) The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. Cancer Res. **60**, 6116–6133
27 Agathanggelou, A., Honorio, S., Macartney, D. P., Martinez, A., Dallol, A., Rader, J., Fullwood, P., Chauhan, A., Walker, R., Shaw, J. A. et al. (2001) Methylation associated inactivation of RASSF1A from region 3p21.3 in lung, breast and ovarian tumours. Oncogene **20**, 1509–1518
28 van der Weyden, L., Tachibana, K. K., Gonzalez, M. A., Adams, D. J., Ng, B. L., Petty, R., Venkitaraman, A. R., Arends, M. J. and Bradley, A. (2005) The RASSF1A isoform of RASSF1 promotes microtubule stability and suppresses tumorigenesis. Mol. Cell. Biol. **25**, 8356–8367

29 Tommasi, S., Dammann, R., Zhang, Z., Wang, Y., Liu, L., Tsark, W. M., Wilczynski, S. P., Li, J., You, M. and Pfeifer, G. P. (2005) Tumor susceptibility of Rassf1a knockout mice. Cancer Res. **65**, 92–98

30 Praskova, M., Khoklatchev, A., Ortiz-Vega, S. and Avruch, J. (2004) Regulation of the MST1 kinase by autophosphorylation, by the growth inhibitory proteins, RASSF1 and NORE1, and by Ras. Biochem. J. **381**, 453–462

31 Cooper, W. N., Hesson, L. B., Matallanas, D., Dallol, A., von Kriegsheim, A., Ward, R., Kolch, W. and Latif, F. (2009) RASSF2 associates with and stabilizes the proapoptotic kinase MST2. Oncogene **28**, 2988–2998

32 Harvey, K. and Tapon, N. (2007) The Salvador-Warts-Hippo pathway: an emerging tumour-suppressor network. Nat. Rev. Cancer **7**, 182–191

33 Saucedo, L. J. and Edgar, B. A. (2007) Filling out the Hippo pathway. Nat. Rev. Mol. Cell. Biol. **8**, 613–621

34 Matallanas, D., Romano, D., Yee, K., Meissl, K., Kucerova, L., Piazzolla, D., Baccarini, M., Vass, J. K., Kolch, W. and O'Neill, E. (2007) RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein. Mol. Cell. **27**, 962–975

35 Vichalkovski, A., Gresko, E., Cornils, H., Hergovich, A., Schmitz, D. and Hemmings, B. A. (2008) NDR kinase is activated by RASSF1A/MST1 in response to Fas receptor stimulation and promotes apoptosis. Curr. Biol. **18**, 1889–1895

36 Baksh, S., Tommasi, S., Fenton, S., Yu, V. C., Martins, L. M., Pfeifer, G. P., Latif, F., Downward, J. and Neel, B. G. (2005) The tumor suppressor RASSF1A and MAP-1 link death receptor signaling to Bax conformational change and cell death. Mol. Cell. **18**, 637–650

37 Vos, M. D., Dallol, A., Eckfeld, K., Allen, N. P., Donninger, H., Hesson, L. B., Calvisi, D., Latif, F. and Clark, G. J. (2006) The RASSF1A tumor suppressor activates Bax via MOAP-1. J. Biol. Chem. **281**, 4557–4563

38 Allen, N. P., Donninger, H., Vos, M. D., Eckfeld, K., Hesson, L., Gordon, L., Birrer, M. J., Latif, F. and Clark, G. J. (2007) RASSF6 is a novel member of the RASSF family of tumor suppressors. Oncogene **26**, 6203–6211

39 Foley, C. J., Freedman, H., Choo, S. L., Onyskiw, C., Fu, N. Y., Yu, V. C., Tuszynski, J., Pratt, J. C. and Baksh, S. (2008) Dynamics of RASSF1A/MOAP-1 association with death receptors. Mol. Cell. Biol. **28**, 4520–4535

40 Shivakumar, L., Minna, J., Sakamaki, T., Pestell, R. and White, M. A. (2002) The RASSF1A tumor suppressor blocks cell cycle progression and inhibits cyclin D1 accumulation. Mol. Cell. Biol. **22**, 4309–4318

41 Liu, L., Tommasi, S., Lee, D. H., Dammann, R. and Pfeifer, G. P. (2003) Control of microtubule stability by the RASSF1A tumor suppressor. Oncogene **22**, 8125–8136

42 Song, M. S., Song, S. J., Ayad, N. G., Chang, J. S., Lee, J. H., Hong, H. K., Lee, H., Choi, N., Kim, J., Kim, H., Kim, J. W., Choi, E. J., Kirschner, M. W. and Lim, D. S. (2004) The tumour suppressor RASSF1A regulates mitosis by inhibiting the APC–Cdc20 complex. Nat. Cell Biol. **6**, 129–137

43 Rong, R., Jin, W., Zhang, J., Sheikh, M. S. and Huang, Y. (2004) Tumor suppressor RASSF1A is a microtubule-binding protein that stabilizes microtubules and induces G2/M arrest. Oncogene **23**, 8216–8230

44 Whang, Y. M., Kim, Y. H., Kim, J. S. and Yoo, Y. D. (2005) RASSF1A suppresses the c-Jun-NH2-kinase pathway and inhibits cell cycle progression. Cancer Res. **65**, 3682–3690

45 Deng, Z. H., Wen, J. F., Li, J. H., Xiao, D. S. and Zhou, J. H. (2008) Activator protein-1 involved in growth inhibition by RASSF1A gene in the human gastric carcinoma cell line SGC7901. World J. Gastroenterol. **14**, 1437–1443

46 Thaler, S., Hahnel, P. S., Schad, A., Dammann, R. and Schuler, M. (2009) RASSF1A mediates p21Cip1/Waf1-dependent cell cycle arrest and senescence through modulation of the Raf–MEK–ERK pathway and inhibition of Akt. Cancer Res. **69**, 1748–1757

47 Song, M. S., Song, S. J., Kim, S. Y., Oh, H. J. and Lim, D. S. (2008) The tumour suppressor RASSF1A promotes MDM2 self-ubiquitination by disrupting the MDM2–DAXX–HAUSP complex. EMBO J. **27**, 1863–1874

48 Ahmed-Choudhury, J., Agathanggelou, A., Fenton, S. L., Ricketts, C., Clark, G. J., Maher, E. R. and Latif, F. (2005) Transcriptional regulation of cyclin A2 by RASSF1A through the enhanced binding of p120E4F to the cyclin A2 promoter. Cancer Res. **65**, 2690–2697

49 Fenton, S. L., Dallol, A., Agathanggelou, A., Hesson, L., Ahmed-Choudhury, J., Baksh, S., Sardet, C., Dammann, R., Minna, J. D., Downward, J. et al. (2004) Identification of the E1A-regulated transcription factor p120 E4F as an interacting partner of the RASSF1A candidate tumor suppressor gene. Cancer Res. **64**, 102–107

50 Dallol, A., Agathanggelou, A., Fenton, S. L., Ahmed-Choudhury, J., Hesson, L., Vos, M. D., Clark, G. J., Downward, J., Maher, E. R. and Latif, F. (2004) RASSF1A interacts with microtubule-associated proteins and modulates microtubule dynamics. Cancer Res. **64**, 4112–4116

51 Vos, M. D., Martinez, A., Elam, C., Dallol, A., Taylor, B. J., Latif, F. and Clark, G. J. (2004) A role for the RASSF1A tumor suppressor in the regulation of tubulin polymerization and genomic stability. Cancer Res. **64**, 4244–4250

52 Song, M. S., Chang, J. S., Song, S. J., Yang, T. H., Lee, H. and Lim, D. S. (2005) The centrosomal protein RAS association domain family protein 1A (RASSF1A)-binding protein 1 regulates mitotic progression by recruiting RASSF1A to spindle poles. J. Biol. Chem. **280**, 3920–3927

53 Guo, C., Tommasi, S., Liu, L., Yee, J. K., Dammann, R. and Pfeifer, G. P. (2007) RASSF1A is part of a complex similar to the *Drosophila* Hippo/Salvador/Lats tumor-suppressor network. Curr. Biol. **17**, 700–705

54 Song, M. S. and Lim, D. S. (2004) Control of APC–Cdc20 by the tumor suppressor RASSF1A. Cell Cycle **3**, 574–576

55 Liu, L., Baier, K., Dammann, R. and Pfeifer, G. P. (2007) The tumor suppressor RASSF1A does not interact with Cdc20, an activator of the anaphase-promoting complex. Cell Cycle **6**, 1663–1665

56 Rong, R., Jiang, L. Y., Sheikh, M. S. and Huang, Y. (2007) Mitotic kinase Aurora-A phosphorylates RASSF1A and modulates RASSF1A-mediated microtubule interaction and M-phase cell cycle regulation. Oncogene **26**, 7700–7708

57 Liu, L., Guo, C., Dammann, R., Tommasi, S. and Pfeifer, G. P. (2008) RASSF1A interacts with and activates the mitotic kinase Aurora-A. Oncogene **27**, 6175–6186

58 Dallol, A., Hesson, L. B., Matallanas, D., Cooper, W. N., O'Neill, E., Maher, E. R., Kolch, W. and Latif, F. (2009) RAN GTPase is a RASSF1A effector involved in controlling microtubule organization. Curr. Biol. **19**, 1227–1232

59 Dallol, A., Agathanggelou, A., Tommasi, S., Pfeifer, G. P., Maher, E. R. and Latif, F. (2005) Involvement of the RASSF1A tumor suppressor gene in controlling cell migration. Cancer Res. **65**, 7653–7659

60 Moshnikova, A., Frye, J., Shay, J. W., Minna, J. D. and Khokhlatchev, A. V. (2006) The growth and tumor suppressor NORE1A is a cytoskeletal protein that suppresses growth by inhibition of the ERK pathway. J. Biol. Chem. **281**, 8143–8152

61 Calvisi, D. F., Donninger, H., Vos, M. D., Birrer, M. J., Gordon, L., Leaner, V. and Clark, G. J. (2009) NORE1A tumor suppressor candidate modulates p21CIP1 via p53. Cancer Res. **69**, 4629–4637

62 Vavvas, D., Li, X., Avruch, J. and Zhang, X. F. (1998) Identification of Nore1 as a potential Ras effector. J. Biol. Chem. **273**, 5439–5442

63 Stieglitz, B., Bee, C., Schwarz, D., Yildiz, O., Moshnikova, A., Khokhlatchev, A. and Herrmann, C. (2008) Novel type of Ras effector interaction established between tumour suppressor NORE1A and Ras switch II. EMBO J. **27**, 1995–2005

64 Kinashi, T. (2007) Integrin regulation of lymphocyte trafficking: lessons from structural and signaling studies. Adv. Immunol. **93**, 185–227

65 Vos, M. D. and Clark, G. J. (2005) RASSF FAMILY Proteins and Ras transformation. Methods Enzymol. **407**, 311–322

66 Ortiz-Vega, S., Khokhlatchev, A., Nedwidek, M., Zhang, X. F., Dammann, R., Pfeifer, G. P. and Avruch, J. (2002) The putative tumor suppressor RASSF1A homodimerizes and heterodimerizes with the Ras-GTP binding protein Nore1. Oncogene **21**, 1381–1390

66a Calvisi, D. F., Ladu, S., Gorden, A., Farina, M., Conner, E. A., Lee, J. S., Factor, V. M. and Thorgeirsson, S. S. (2006) Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology **130** 1117–1128

67 Krontiris, T. G., Devlin, B., Karp, D. D., Robert, N. J. and Risch, N. (1993) An association between the risk of cancer and mutations in the HRAS1 minisatellite locus. N. Engl. J. Med. **329**, 517–523

68 Krontiris, T. G., DiMartino, N. A., Colb, M. and Parkinson, D. R. (1985) Unique allelic restriction fragments of the human Ha-ras locus in leukocyte and tumour DNAs of cancer patients. Nature **313**, 369–374

69 Weitzel, J. N., Ding, S., Larson, G. P., Nelson, R. A., Goodman, A., Grendys, E. C., Ball, H. G. and Krontiris, T. G. (2000) The HRAS1 minisatellite locus and risk of ovarian cancer. Cancer Res. **60**, 259–261

70 Firgaira, F. A., Seshadri, R., McEvoy, C. R., Dite, G. S., Giles, G. G., McCredie, M. R., Southey, M. C., Venter, D. J. and Hopper, J. L. (1999) HRAS1 rare minisatellite alleles and breast cancer in Australian women under age forty years. J. Natl. Cancer Inst. **91**, 2107–2111

71 Tamimi, R. M., Hankinson, S. E., Ding, S., Gagalang, V., Larson, G. P., Spiegelman, D., Colditz, G. A., Krontiris, T. G. and Hunter, D. J. (2003) The HRAS1 variable number of tandem repeats and risk of breast cancer. Cancer Epidemiol. Biomarkers Prev. **12**, 1528–1530

72 Brandt, R., Grutzmann, R., Bauer, A., Jesnowski, R., Ringel, J., Lohr, M., Pilarsky, C. and Hoheisel, J. D. (2004) DNA microarray analysis of pancreatic malignancies. Pancreatology **4**, 587–597

73 Friess, H., Ding, J., Kleeff, J., Fenkell, L., Rosinski, J. A., Guweidhi, A., Reidhaar-Olson, J. F., Korc, M., Hammer, J. and Buchler, M. W. (2003) Microarray-based identification of differentially expressed growth- and metastasis-associated genes in pancreatic cancer. Cell. Mol. Life Sci. **60**, 1180–1199

74 Logsdon, C. D., Simeone, D. M., Binkley, C., Arumugam, T., Greenson, J. K., Giordano, T. J., Misek, D. E., Kuick, R. and Hanash, S. (2003) Molecular profiling of pancreatic adenocarcinoma and chronic pancreatitis identifies multiple genes differentially regulated in pancreatic cancer. Cancer Res. **63**, 2649–2657

75 Lowe, A. W., Olsen, M., Hao, Y., Lee, S. P., Taek Lee, K., Chen, X., van de Rijn, M. and Brown, P. O. (2007) Gene expression patterns in pancreatic tumors, cells and tissues. PLoS ONE **2**, e323

76 Mutter, G. L., Baak, J. P., Fitzgerald, J. T., Gray, R., Neuberg, D., Kust, G. A., Gentleman, R., Gullans, S. R., Wei, L. J. and Wilcox, M. (2001) Global expression changes of constitutive and hormonally regulated genes during endometrial neoplastic transformation. Gynecol. Oncol. **83**, 177–185

77 Tan, D. S., Lambros, M. B., Rayter, S., Natrajan, R., Vatcheva, R., Gao, Q., Marchio, C., Geyer, F. C., Savage, K., Parry, S. et al. (2009) PPM1D is a potential therapeutic target in ovarian clear cell carcinomas. Clin. Cancer Res. **15**, 2269–2280

78 Kenneth, N. S. and Rocha, S. (2008) Regulation of gene expression by hypoxia. Biochem. J. **414**, 19–29

79 Camps, C., Buffa, F. M., Colella, S., Moore, J., Sotiriou, C., Sheldon, H., Harris, A. L., Gleadle, J. M. and Ragoussis, J. (2008) hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. Clin. Cancer Res. **14**, 1340–1348

80 Liang, G. P., Su, Y. Y., Chen, J., Yang, Z. C., Liu, Y. S. and Luo, X. D. (2009) Analysis of the early adaptive response of endothelial cells to hypoxia via a long serial analysis of gene expression. Biochem. Biophys. Res. Commun. **384**, 415–419
81 Welcsh, P. L., Lee, M. K., Gonzalez-Hernandez, R. M., Black, D. J., Mahadevappa, M., Swisher, E. M., Warrington, J. A. and King, M. C. (2002) BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. Proc. Natl. Acad. Sci. U.S.A. **99**, 7560–7565
82 Hitomi, J., Christofferson, D. E., Ng, A., Yao, J., Degterev, A., Xavier, R. J. and Yuan, J. (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell **135**, 1311–1323
83 Chalmers, A. D., Lachani, K., Shin, Y., Sherwood, V., Cho, K. W. and Papalopulu, N. (2006) Grainyhead-like 3, a transcription factor identified in a microarray screen, promotes the specification of the superficial layer of the embryonic epidermis. Mech. Dev. **123**, 702–718
84 Goshima, G., Wollman, R., Goodwin, S. S., Zhang, N., Scholey, J. M., Vale, R. D. and Stuurman, N. (2007) Genes required for mitotic spindle assembly in *Drosophila* S2 cells. Science **316**, 417–421
85 Morris, J. A., Kandpal, G., Ma, L. and Austin, C. P. (2003) DISC1 (disrupted-in-schizophrenia 1) is a centrosome-associated protein that interacts with MAP1A, MIPT3, ATF4/5 and NUDEL: regulation and loss of interaction with mutation. Hum. Mol. Genet. **12**, 1591–1608
86 Tsang, H. T., Connell, J. W., Brown, S. E., Thompson, A., Reid, E. and Sanderson, C. M. (2006) A systematic analysis of human CHMP protein interactions: additional MIT domain-containing proteins bind to multiple components of the human ESCRT III complex. Genomics **88**, 333–346
87 Debeer, P., Schoenmakers, E. F., Thoelen, R., Holvoet, M., Kuittinen, T., Fabry, G., Fryns, J. P., Goodman, F. R. and Van de Ven, W. J. (2000) Physical map of a 1.5 mb region on 12p11.2 harbouring a synpolydactyly associated chromosomal breakpoint. Eur. J. Hum. Genet. **8**, 561–570
88 Debeer, P., Schoenmakers, E. F., Twal, W. O., Argraves, W. S., De Smet, L., Fryns, J. P. and Van De Ven, W. J. (2002) The fibulin-1 gene (FBLN1) is disrupted in a t(12;22) associated with a complex type of synpolydactyly. J. Med. Genet. **39**, 98–104
89 Gariboldi, M., Manenti, G., Canzian, F., Falvella, F. S., Radice, M. T., Pierotti, M. A., Della Porta, G., Binelli, G. and Dragani, T. A. (1993) A major susceptibility locus to murine lung carcinogenesis maps on chromosome 6. Nat. Genet. **3**, 132–136
90 To, M. D., Perez-Losada, J., Mao, J. H., Hsu, J., Jacks, T. and Balmain, A. (2006) A functional switch from lung cancer resistance to susceptibility at the Pas1 locus in Kras2LA2 mice. Nat. Genet. **38**, 926–930
91 Manenti, G., De Gregorio, L., Pilotti, S., Falvella, F. S., Incarbone, M., Ravagnani, F., Pierotti, M. A. and Dragani, T. A. (1997) Association of chromosome 12p genetic polymorphisms with lung adenocarcinoma risk and prognosis. Carcinogenesis **18**, 1917–1920
92 Yanagitani, N., Kohno, T., Sunaga, N., Kunitoh, H., Tamura, T., Tsuchiya, S., Saito, R. and Yokota, J. (2002) Localization of a human lung adenocarcinoma susceptibility locus, possibly syntenic to the mouse Pas1 locus, in the vicinity of the D12S1034 locus on chromosome 12p11.2-p12.1. Carcinogenesis **23**, 1177–1183
93 Falvella, F. S., Spinola, M., Manenti, G., Conti, B., Pastorino, U., Skaug, V., Haugen, A. and Dragani, T. A. (2007) Common polymorphisms in D12S1034 flanking genes RASSF8 and BHLHB3 are not associated with lung adenocarcinoma risk. Lung Cancer **56**, 1–7
94 Korkola, J. E., Houldsworth, J., Chadalavada, R. S., Olshen, A. B., Dobrzynski, D., Reuter, V. E., Bosl, G. J. and Chaganti, R. S. (2006) Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with *in vivo* differentiation of human male germ cell tumors. Cancer Res. **66**, 820–827
95 Weiser, K. C., Liu, B., Hansen, G. M., Skapura, D., Hentges, K. E., Yarlagadda, S., Morse Iii, H. C. and Justice, M. J. (2007) Retroviral insertions in the VISION database identify molecular pathways in mouse lymphoid leukemia and lymphoma. Mamm. Genome **18**, 709–722
96 Eckfeldt, C. E., Mendenhall, E. M., Flynn, C. M., Wang, T. F., Pickart, M. A., Grindle, S. M., Ekker, S. C. and Verfaillie, C. M. (2005) Functional analysis of human hematopoietic stem cell gene expression using zebrafish. PLoS Biol. **3**, e254
97 Jin, J., Smith, F. D., Stark, C., Wells, C. D., Fawcett, J. P., Kulkarni, S., Metalnikov, P., O'Donnell, P., Taylor, P., Taylor, L. et al. (2004) Proteomic, functional, and domain-based analysis of *in vivo* 14–3–3 binding proteins involved in cytoskeletal regulation and cellular organization. Curr. Biol. **14**, 1436–1450
98 Rual, J. F., Venkatesan, K., Hao, T., Hirozane-Kishikawa, T., Dricot, A., Li, N., Berriz, G. F., Gibbons, F. D., Dreze, M., Ayivi-Guedehoussou, N. et al. (2005) Towards a proteome-scale map of the human protein-protein interaction network. Nature **437**, 1173–1178
99 Alam, M. R., Caldwell, B. D., Johnson, R. C., Darlington, D. N., Mains, R. E. and Eipper, B. A. (1996) Novel proteins that interact with the COOH-terminal cytosolic routing determinants of an integral membrane peptide-processing enzyme. J. Biol. Chem. **271**, 28636–28640
100 Prigge, S. T., Mains, R. E., Eipper, B. A. and Amzel, L. M. (2000) New insights into copper monooxygenases and peptide amidation: structure, mechanism and function. Cell. Mol. Life Sci. **57**, 1236–1259
101 Chen, L., Johnson, R. C. and Milgram, S. L. (1998) P-CIP1, a novel protein that interacts with the cytosolic domain of peptidylglycine alpha-amidating monooxygenase, is associated with endosomes. J. Biol. Chem. **273**, 33524–33532
102 Rajagopal, C., Stone, K. L., Francone, V. P., Mains, R. E. and Eipper, B. A. (2009) Secretory granule to the nucleus: role of a multiply phosphorylated intrinsically unstructured domain, J. Biol. Chem. 18, 25723–25734
103 Hesson, L. B., Dunwell, T. L., Cooper, W. N., Catchpoole, D., Brini, A. T., Chiaramonte, R., Griffiths, M., Chalmers, A. D., Maher, E. R. and Latif, F. (2009) The novel RASSF6 and RASSF10 candidate tumour suppressor genes are frequently epigenetically inactivated in childhood leukaemias. Mol. Cancer **8**, 42
104 Reeves, N. and Posakony, J. W. (2005) Genetic programs activated by proneural proteins in the developing *Drosophila* PNS. Dev. Cell. **8**, 413–425
105 Nybakken, K., Vokes, S. A., Lin, T. Y., McMahon, A. P. and Perrimon, N. (2005) A genome-wide RNA interference screen in *Drosophila melanogaster* cells for new components of the Hh signaling pathway. Nat. Genet. **37**, 1323–1332

Received 25 August 2009/23 September 2009; accepted 24 September 2009
Published on the Internet 23 December 2009, doi:10.1042/BJ20091318