Published in final edited form as:
Biochim Biophys Acta. 2009 January ; 1795(1): 25–36. doi:10.1016/j.bbcan.2008.09.001.

BAR the door: cancer suppression by amphiphysin-like genes

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Abstract

The evolutionarily conserved amphiphysin-like genes *Bin1* and *Bin3* function in membrane and actin dynamics, cell polarity, and stress signaling. Recent genetic studies in mice discriminate non-essential roles in endocytic processes commonly ascribed to amphiphysins from essential roles in cancer suppression. *Bin1* acts in default pathways of apoptosis and senescence that are triggered by the *Myc* and *Raf* oncogenes in primary cells, and *Bin1* gene products display a 'moonlighting function' in the nucleus where several other 'endocytic' proteins are also found. Together, genetic investigations in yeast, flies, and mice suggest that amphiphysin-like adapter proteins may suppress cancer by helping integrate cell polarity signals generated by actin and vesicle dynamics with central regulators of cell cycle arrest, apoptosis, and immune surveillance.

Keywords

tumor suppressor; modifier; lung cancer; vesicle trafficking; indoleamine 2,3-dioxygenase; c-myc; ras; rho

Introduction

BAR (Bin/Amphiphysin/Rvs) adapter proteins have been implicated in many cellular processes, including endocytosis, vesicle fusion and trafficking, specialized membrane organization, actin organization, cell polarity, stress signaling, transcription, and tumor suppression [1]. The signature fold that characterizes this group of adapter proteins, termed the BAR domain [2], mediates oligomerization to a banana-shaped dimer that can bind curved membranes, small GTPases, and other proteins [1,3,4]. Biochemical and structural studies indicate that BAR domains deform and tubulate membranes and can facilitate interactions with the actin cytoskeleton [3,5]. However, like many other proteins implicated in membrane dynamics and endocytosis [6], certain BAR proteins also localize to the nucleus [2,7-9]. BAR adapter proteins are now recognized to be part of a larger superfamily of structurally related proteins that includes the so-called F-BAR and I-BAR proteins [10-13].

Bin1 and *Bin3* are the archetypal members of the BAR adapter gene family that are conserved throughout evolution from yeast to man. Figure 1 presents the primary structure of several

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ubiquitous and tissue-specific members of the BAR adapter family. Like most other genes that are evolutionarily well conserved, *Bin1* and *Bin3* are expressed ubiquitiously in mammalian cells with certain splice variants expressed in neurons, muscle cells or tumor cells (Fig. 2 and Suppl. Fig. 1). *Bin1*, also known as *Amphiphysin II (Amph II)*, *Amphl*, *ALP*, or *SH3P9*, was described by several groups who cloned various splice isoforms on the basis of their amphiphysin-like structure, SH3 domain, or binding to the c-Myc or c-Abl oncoproteins [2, 14-18]. Although *Bin1* was identified after *Amph I* the expression of the latter gene is primary restricted to neurons such that *Bin1* is the germane amphiphysin-like function in most cells (Fig. 2). Of the >10 isoforms of Bin1 described, the ubiquitous and muscle-specific isoforms localize to nucleus as well as cytosol, a property essential for anticancer functions [8,19-31]. A recent study suggests that cell polarity is a key determinant in controlling Bin1 nuclear localization [32].

Bin3 was identified on the basis of structural similarity to its homolog *RVS161* in budding yeast [33]. *Bin3* is expressed oppositely to *Amph I*. Whereas *Amph I* is expressed mainly in the brain, *Bin3* is expressed in all tissues but poorly in the brain (Fig. 2). Alternate splice isoforms which differentially include 5' exons of *Bin3* exist but nothing is known about their specific roles as yet. Bin3 proteins localize to vesicular membranes that are distinct from lysosomal membranes but partially overlap with mitochondrial membranes [34] (Fig. 3).

Although not similarly conserved in evolution, there are other BAR adapter proteins that reinforce interest in this gene family in cancer and their canonical roles in membrane dynamics, actin regulation, and signaling processes (Fig. 1 and Suppl. Fig. 1). Three ubiquitous expressed BAR family members of particular interest in cancer are *Bif-1*, *APPL*, and *Tuba*. Bif-1, also known as the vesicular protein endophilin B1 or the product of the *SH3GLB1* gene, encodes Bax-interacting factor-1, a pro-apoptotic adapter protein that binds to Bax and promotes its conformational change at sites of mitochondrial scission, each of which are important to trigger apoptosis [35-37]. Of note to discussions below, Bif-1 interacts with Bin1 [38]. *APPL* encodes nuclear-shuttling adapter proteins that bind Rab5 and Akt2 on vesicles and that function in the EGF pathway to coordinate signaling and transcriptional repression via binding to the chromatin modification complex NuRD/Mi-2 in the nucleus [9,39,40]. *Tuba* (aka *DNMBP*) encodes an adapter protein that stimulates N-WASP-dependent actin assembly and is critical to drive the invasive motility of cancer cells [41,42]. In contrast to Bif-1, APPL, Bin1 and Bin3, all of which exert tumor suppressor functions, Tuba may contribute positively to malignant progression by supporting invasion.

Several non-conserved BAR family genes of some interest to cancer are the tissue-specific genes *Amphiphysin* (*Amph I*), which is normally expressed only in neurons, and *Graf* and *Snx6*, which are expressed mainly in hematopoietic cells [43-45]. Amph I proteins are thought to contribute to synaptic vesicle recycling. In rare cases of breast and lung cancer, Amph I has been identified as paraneoplastic autoantigen underlying the neurological condition Stiff-man syndrome [46]. Although the meaning of this relationship is unclear, it is intriguing given evidence that the closely related amiphiphysin-like gene *Bin1* has an essential role in supporting tumoral immune surveillance [27]. While amphiphysin-like orthologs in yeast and *Drosophila* are referred to as amphiphysin homologs in the literature, considerations of structural and functional homology make it clear that they likely derived from *Bin1* rather than *Amph I*, the latter of which probably arose later in evolution. *Graf* (*ARHGAP26*) encodes a Rho GTPase activating protein (RhoGAP) that binds to the integrin effector kinase FAK and regulates Cdc42/Rho activity [47]. Prooncogenic chromosomal fusions involving *Graf* occur in juvenile myelomonocytic leukemia [44], perhaps helping deregulate FAK signals needed to drive cancer cell survival, motility, and crosstalk with the inflammatory microenvironment [48,49]. Snx6 is a vesicle trafficking protein that interacts with TGF-ß receptors and is delivered to the nucleus by Pim oncoproteins that are required for Abl-mediated cell transformation

[43,50,51]. Given the propensity of BAR adapter proteins to form heterodimers, the tissue-specific members of this family may associate with Bin1 or Bin3 to specify or integrate their functions in certain settings. For example, BAR domain-medated heterodimers of Bin2-Bin1 are found in hematopoietic cells and Amph I-Bin1 are found in neurons [22,52], although the essential functions of each has yet to be understood.

Excellent recent reviews of the canonical function of BAR adapter proteins in membrane deformation, vesiculation processes, and actin organization have been published elsewhere [1,11]. The purpose of the present review is to summarize the literature on amphiphysin-like genes in cancer, which has not been done before. These genes has been considered mainly in light of their roles in actin dynamics and endocytotic processes however, genetic studies in fission yeast, flies, and mice argue that amphiphysin-like genes are non-essential for endocytosis. We consider less studied but evolutionarily conserved roles of these genes in cell polarity and stress signaling that relate to cancer. Cell polarity signaling is closely integrated with vesicle trafficking and actin dynamics, such that amphiphysin-like proteins have an integrative role. In this review, we focus on the role of conserved amphiphysin-like genes in cell polarity signaling and stress signaling that are presently less studied or understood at present but proposed to be linked to their essential functions in cancer suppression.

Yeast amphiphysin-like orthologs function in cell polarity and stress signaling

Bin1 and *Bin3* orthologs have been investigated extensively in budding yeast *S. cerevisiae*, where they are termed respectively *RVS167* and *RVS161*, and also to a lesser extent in fission yeast *S. pombe*, where they are termed respectively *hob1+* and *hob3+*. *RVS* genes were identified initially in budding yeast by a screen for mutants that exhibited reduced viability upon nutrient starvation [53,54], revealing an essential function in stress signaling. *RVS* genes were also identified in screens for mutants that were defective in actin delocalization and internalization of endocytic vesicles [55], highlighting functions in actin and vesicular dynamics. More recently, a generalized function in vesicle trafficking has been defined based on evidence of a essential role in supporting ER-Golgi transport [56]. In addition to the extensive genetic and biochemical evidence linking *RVS* function to actin organization and vesiculation processes, suppressor screens reveal that *rvs* mutants can be rescued by sphingolipid alterations [1,57]. The Rvs167p and Rvs161p proteins function in a stable heterodimeric complex mediated by BAR interaction [58-60]. However, a similar situation does not exist in mammalian cells, fission yeast, or *Drosophila* (which appears to lack a *Bin3* homolog), where, as discussed further below, functional homologies diverge and analogous stable complexes are not seen. RVS167 is phosphorylated by the Pho85p kinase that helps integrate cell polarity and cell cycle control in budding yeast [61,62]. In mammalian cells, the cell cycle-related kinase Cdk5 is an ortholog of Pho85p that phosphorylates Amph I protein in neurons [63,64]. This is a specific connection insofar as Cdk5 does not phosphorylate Bin1 (Amph II) proteins which form heterodimers with Amph I in neurons (J. DuHadaway and G.C.P., unpublished observations). Like Amph I, Cdk5 is implicated in synaptic vesicle trafficking and its expression is confined mainly to neurons where it is controlled by the neuron-specific Cdk5 regulatory partner p35 [65-67]. Together these findings suggest that *RVS167* may encompass functions in vesiculation processes akin to *Amph I* more than *Bin1*.

Rvs mutants exhibit a variety of cell polarity defects marked by aberrant budding patterns and actin organization [54,68]. *Rvs167* mutants accumulate late secretory vesicles at polar sites of membrane and cell wall construction [69]. During vegetative growth, Rvs167p is located at cortical actin patches but during mating it moves to the so-called shmoo tip of the yeast cell where cell-cell fusion occurs [70]. Rvs167p likely delivers Rvs161p to the cell fusion region, where Rvs161p binds to Fus2p to exert an actin-independent function needed for successful fusion and mating [71]. Reinforcing an important role in coordinating cell polarity-informed

processes, protein-protein interaction studies argue that *RVS167* is a nodal point for integrating cell polarity signaling [72].

One difficulty that arises in interpreting the information gained from budding yeast studies is that neither *Bin1* nor *Amph I* are able to genetically complement the defects produced by deletion of *RVS167* in budding yeast [73]. This speaks to an important difference in function not revealed by studies in budding yeast. The functional divergence along with regulatory differences prompted investigations of the BAR family genes in the fission yeast *S. pombe*. This yeast is diverged similarly from humans and budding yeast, but for certain processes such as cell division cycle control it is closer to mammalian cells. Similar to budding yeast, the fission yeast genome includes two BAR adapter family orthologs termed *hob1+* and *hob3+* that are closely related to *Bin1* and *Bin3* [33,73].

hob1+ was defined as the homolog of *Bin1* in *S. pombe* by sequence similarity but also by functional complementation [33,73]. In support of the notion of some functional drift in BAR adapters during evolution, mutations in *RVS167* and *hob1+* produced somewhat different phenotypes in the two yeasts. Upon nutrient starvation or genotoxic stress produced by treatment with the DNA damaging agent phleomycin, *hob1*Δ mutant cells exhibited a *cdc25*-like phenotype marked by abnormal cell elongation and defective growth arrest followed by cell death [73]. In contrast, *hob1*Δ mutant lacked apparent defects in endocytosis or osmolar sensitivity in the manner of *rvs167* mutant cells in budding yeast. Growth control could be rescued by *Bin1* but not *Amph I* or *RVS167* [73]. Thus, a specific homology existed between *Bin1* and *hob1+* that was not shared with *Amph I* despite its structural similarity [73]. The concept that *hob1+* acts differently than *RVS167* in how it influences actin organization and cell polarity has been extended by a further study [74]. Recently, analysis of the survival defect in *hob1*Δ mutant cells after phleomycin treatment suggests that *hob1+* supports a Rad6/Set1-mediated pathway of chromatin modification that drives transcriptional repression [75]. In another study, evidence of genetic interaction between *hob1+* and *pku70+* encoding the DNA end-binding Ku70 was obtained, prompted by findings that Bin1 binds to Ku in mammalian cells [76]. Regarding the relationship between Bin1 and c-Myc in mammalian cells, these findings are interesting in light of evidence that c-Myc binds Ku and that c-Myc antagonizes the Rad/Set1 transcriptional repression pathway which is supported by *hob1+* in fission yeast [77,78]. Together, these findings are consistent with other evidence that in fission yeast the cell division cycle is more conserved to mammalian cells than in budding yeast.

hob3+ was defined as the homolog of *Bin3* through sequence similarity but also through functional complementation [33]. *hob3*Δ mutant cells lacked any evident defects in endocytosis [33]. In contrast, under normal growth conditions many cells exhibited a profound disruption in polarized actin organization and an elongated, multinucleated phenotype associated with accumulation of cell wall material at sites of defective cell division [33]. This phenotype was strongly exacerbated by nutrient starvation, deepening the phenotype, but only limited effects on viability were seen. A comparison of *hob3+*, *Rvs161*, and *Bin3* revealed that these genes could complement each other in budding and fission yeasts, arguing that their functional homology was conserved more closely than the *Bin1/Amph* homologs in evolution. *hob3*Δ mutant cells did not display sensitivity to phleomycin, however, loss of *hob3+* relieved the senstitivity of *hob1*Δ cell to phleomycin, arguing that *hob3+* may interact with *hob1+* at some level in this response [73]. However, neither the Hob1p and Hob3p proteins nor the Bin1 and Bin3 proteins appear to interact in a stable complex like Rvs167 and Rvs161 (A. Ramalingam and G.C.P., unpublished observations). Notably, the defect in cell division in *hob3*Δ cells reflects a need for Hob3p to recruit and activate the Rho small GTPase Cdc42p to sites of cell division where it is required to mediate cytokinesis [79]. Included in the Hob3p-Cdc42p complex is a guanine nucleotide exchange factor termed Gef1p that Hob3p supports to stimulate Cdc42p activity in this role. There are two pathways leading to Cdc42 activation in

cytokinesis in fission yeast but the Hob3p-Gef1p pathway is non-essential unless the second Cdc42 activating pathway is absent or defective, indicating a redundancy in this mechanism of cell division. The spatial regulation of Cdc42p in cytokinesis depends upon actin-dependent polarity cues [80]. Overall, genetic studies on the *hob* genes indicate that they coordinate cell polarity and cell division under under conditions of cell stress. In summary, fission yeast studies argue that the essential functions of amphiphysin-like genes that are evolutionarily conserved to mammals relate more to cell polarity signaling than endocytotic trafficking.

Drosophila ortholog of *Bin1* is dispensable for endocytotic processes but essential to localize the cell polarity complex Scribble/Discs Large/Lethal Giant Larvae (Scr/Dlg/Lgl)

The fruit fly *Drosophila* encodes a gene termed *Amphiphysin* (*dAmph*) that is structurally related to the mammalian *Bin1* and *Amph I* genes. In contrast, no *Bin3* ortholog is found in flies. Mutants that lack *dAmph* are viable, lacking evident defects in endocytosis or neurotransmission, but they exhibit an altered synaptic physiology and are flightless due to a muscle defect [81,82]. Consistent with a non-essential role in endocytosis the fly protein DAmph does not bind clathrin, however, it tubulates lipids and is localized to the specialized T tubule system in muscle cells [81,82]. Additionally, it is broadly expressed at actin-rich membrane domains in many other cell types, such as at the apical membranes in polarized epithelial cells, the apical rhabdomere membranes of photoreceptor neurons and the postsynaptic density of neuromuscular junctions [82]. Mutant flies have a severely disorganized T-tubule/sarcoplasmic reticulum system, defining an essential function in organizing the membraneous compartments of the excitation-contraction coupling machinery in muscles [81]. Thus, the fly gene *dAmph* is implicated in muscle function like the mammalian *Bin1* gene consistent with the notion that they are functional homologs [25,30,31].

In support of an important role in cell polarity, mutant fly larvae and adults exhibit defects in cellular locomotion and mislocalization of the cell polarity complex composed of the Scribble (Scr), Discs Large (Dlg), and Lethal Giant Larvae (Lgl) proteins [82]. In flies the Scr/Dlg/Lgl complex acts as a tumor suppressor [83]. Notably, inactivation of this complex cooperates with Ras to drive formation of invasive tumors in the same way that inactivation of *Bin1* cooperates with Ras to drive invasive tumors in mice [84-86]. It is not known if inactivation of the *dAmph* gene similarly cooperates with Ras [28,85]. However, even aside from considerations of homology, this question is interesting to consider in light of other information. In epithelial cells, vesicle trafficking maintains cell polarity that relies upon Scr/Dlg/Lgl function. Intriguingly, investigations of cell competition in *Drosophila* wing epithelial cells suggest a potential role for vesicle dynamics in coordinating cell division and survival. In particular, one study found that Myc can be phenocopied by Rab5 in cells that outcompete neighboring cells [87]. In mammalian cells, Bin1 binds to Rin2 and Rin3, two Rab GTP exchange factors (RabGEFs) that bind Ras and stimulate Rab5 activity [88]. Additionally, Bin1 binds to Myc and inhibits its oncogenic activity [2,28,85]. If Bin1 restricts the activities of Myc and Rab5, in the latter case by sequestering RabGEFs, then inactivation of Bin1 may cooperate with Ras by relieving restraints to Myc and Rab5 needed to license the division of polarized cells. In summary, studies of the *Bin1*-like gene *dAmph* in *Drosophila* reinforce the concept that the key evolutionarily conserved function of amphiphsyin-like genes relates primarily to cell polarity signaling, with hints to the mechanism of how its action may integrate polarity with cell growth and survival controls.

Bin1 exerts a tumor suppressor function that is widely attenuated in human cancer

Various isoforms of Bin1 that are produced by alternate RNA splicing were discovered in different laboratories on the basis of similarity to *Amph I*, the presence of an SH3 domain, or the ability to bind the Myc and Abl oncoproteins [89]. These diverse efforts, reflecting the complexity of Bin1 splicing in mammalian cells, has led to a complex nomenclature in the

literature for the gene and its isoforms (which have also been termed Amphiphysin II or 2, Amph II or 2, Amphl, ALP, and SH3P9). In this review we have used HUGO-approved nomenclature and the NCBI Entrez Gene nomenclature to refer to genes and splice isoforms [90]. When not specifically defined in the text, Bin1 refers to the two ubiquitious isoforms 9 and 10 (aka Bin1-10 and Bin1-10-13), which localize in cells with preference to the nucleus [31,91,92] or cytosol [16,91-93], respectively. CNS-specific Bin1 isoforms 1-7 are exclusively cytosolic and include the clathrin-binding domains found in Amph I proteins [14,15,92,94]. The muscle-specific isoform 8 localizes to T tubules or nucleus [31,92,95]. Lastly, cancer-specific variants of the ubiquitous isoforms 9 and 10, which we refer to as Bin1+12A isoforms due to aberrant inclusion of the CNS-specific exon 12A [18], are exclusively cytosolic and lack tumor suppressor activity [22]. Notably, exon 12A missplicing in the cancer-specific Bin1 +12A isoforms results form activation of the oncogenic RNA splicing factor SF2/ASF [96]. Bin1+12A isoforms are observed in many tumor cells and tumor cell lines, representing one of the most common missplicing events occuring in human cancer generally [97,98].

Our group initially identified Bin1 through its ability to bind to c-Myc and inhibit its primary cell transforming activity with Ras [2]. The robust antitransforming activity of Bin1 displayed in classical primary cell assays of oncogene co-transformation is dependent on an intact Myc binding domain (MBD), present only in the muscle isoform 8 and ubiquitous isoform 9 which can localize to the nucleus [2,7,91,92]. Further studies confirming the c-Myc-Bin1 interaction have provided evidence that the SH3 domain in Bin1 contributes to Myc binding and that the cytosolic isoform 10 lacking an intact MBD binds Myc poorly [26,89]. Myc Box regions I and II, which are crucial for the oncogenicity and transcriptional activity of all Myc proteins, are also essential for Bin1 binding [2]. Bin1 suppresses the transcriptional transactivation activity of c-Myc and the BAR domain is sufficient for transcriptional repression when independently tethered to a promoter by the yeast Gal4 DNA binding domain [20]. T58 mutations in the Myc Box I region, which are found in all viral *myc* genes and many human tumors [99], permit Myc to escape the antitransforming effects of Bin1 in primary cells [2]. Moreover, inhibition of Myc-Bin1 interaction in cells via MBD overexpression promotes transformation and blunts apoptosis by Myc in primary cells [2,19]. This effect is specific, because while the MBD is essential for Bin1 to suppress the co-transforming activity of Myc, it is non-essential to suppress the co-transforming activity of adenovirus E1A or mutant p53 plus Ras [2,8], which appears to involve a distinct Rb-dependent mechanism [100]. The anti-transforming and pro-apoptotic effects of Bin1 are strongest in primary cells; during establishment of rodent cells in tissue culture, Bin1 tends to be attenuated at the level of expression, missplicing, or both (J. DuHadaway, A. Muller, and G.C.P., unpublished observations). This phenomenon is interesting as it parallels the disabling of polarity signaling which occurs in primary cells as they become established into cell lines in tissue culture. Supporting the *in vivo* relevance of Bin1 for helping restrict the oncogenicity of Myc, loss of Bin1 cooperates with Ras to drive malignant progression in mice [85]. Taken together, these findings offer genetic evidence of an critical role for Bin1 in helping safeguard normal cells from the powerful oncogenic effects of Myc overexpression.

In a separate line of work, Bin1 isoform 10 was identified through interaction with the tyrosine kinase Abl [16]. This interaction was dependent on the SH3 domains in each protein and it was later confirmed that all Bin1 isoforms can bind Abl in cells (D. Sakamuro and G.C.P., unpublished observations). In the initial study, overexpression of isoform 10 was found to cause an Abl-dependent morphological transformation of established NIH3T3 fibroblasts, suggesting that isoform 10 might mediate cytoskeletal functions of Abl kinase [16]. This finding was not extended, however, and later studies conducted in primary fibroblasts indicated that isoform 10 could suppress tumor formation by oncogene-transformed primary cells [28]. One study suggested that Bin1 isoform 10 (termed there Amph IIm) was critical for phagocytosis in macrophages [93], however, this finding was based on a weakly controlled

dominant negative strategy and it was later refuted by studies employing genetically null cells [52]. Thus, not only Bin1 isoform 10 but all Bin1 isoforms are dispensable for phagocytosis.

Bin1 losses at the level of expression or missplicing occur frequently in human tumors of the breast, prostate, brain, colon, skin, brain, and lung, and efforts to restore expression in tumor cell lines causes growth inhibition and/or cell suicide [2,21-24,29,101-103]. These effects are specific as Bin1 does not similarly compromise the growth or survival of normal primary cells or nontransformed cells [20,22,24,31]. The human gene is located at human 2q14, within a mid-2q region deleted ~42% of metastatic prostate cancer that encompasses *Bin1* [23,104]. As noted above, *Bin1* is often attenuated by missplicing of CNS-specific exon 12A [22] and Bin1 +12A isoforms can no longer access the nucleus [20-22,24,102], bind to Myc [26], or activate cell suicide [20-22,24,102]. The high frequency of Bin1 missplicing in many human tumors and tumor cell lines that has been documented [97,98] means that cancer microarray expression analyses defining Bin1 expression must be interpreted carefully, given the likelihood that tumors may accumulate mRNAs encoding Bin1+12A isoforms which lack tumor suppressor activity [22].

Several studies document a role for *Bin1* in programmed cell death (PCD) in transformed or tumor cell lines [19-22,24,28,85,102,105]. In panels of melanomas and breast carcinomas where *Bin1* was misspliced or attenuated in expression, ectopic expression of Bin1 isoform 8 triggered PCD associated with cell detachment, rounding, and DNA degradation [22,24]. These effects were not correlated with Myc overexpression. In contrast, in neuroblastomas *Bin1* was found to be misspliced or attenuated only in neuroblastomas with amplified N-Myc, and ectopic expression facilitated apoptosis by serum deprivation or cytotoxic drug treatment [29,102]. A detailed analysis of PCD induced in human hepatocarcinoma cells indicated that *Bin1* engaged a caspase-independent process characterized by cell shrinkage, substratum detachment, vacuolated cytoplasm, and limited DNA degradation with nuclear margination [20]. PCD induction was relieved by mutation of the BAR domain or by exon 12A missplicing. p53 was dispensable and PCD was not blocked by either Bcl-2 or inhibition of the Fas pathway. In contrast, serine protease inhibitors delayed DNA degradation and SV40 large T antigen completely blocked PCD [20]. The latter finding was consistent with earlier evidence that co-transformation of primary cells by T antigen+Ras is refractory to suppression by Bin1 [2,8]. Autophagy was ruled out based on the lack of autophagic vesicles in electron micrographs and the lack of sensitivity the autophagic inhibitor 3-methyl-adenine, but this should be be re-evaluated given recent advances in the field. Notably, electron micrography revealed a close resemblance between the PCD phenotype triggered by Bin1 and the non-apoptotic PCD triggered in cells by Myc when caspases are inhibited [106].

Bin1 is essential for default pathways to apoptosis or senescence that are triggered by oncogenes in primary cells where cell polarity signaling is intact

Precedents exist for tumor suppressor genes that support multiple apoptosis pathways while nevertheless triggering caspase-independent PCD when expressed in tumor cells, for example, the tumor suppressor gene *Pml* [107,108]. In the case of Bin1, this dichotomy might be rectified by recognizing that in tumor cells polarity signaling and apoptotic signaling are both grossly altered. For example, in cells where polarity is intact, Myc-mediated apoptosis is influenced by the integrity of the polarity factor Lkb1 [109,110] or the polarity complex Dgl/Lgl/Scr (S. Muthuswamy, pers. comm.). Indeed, genes such as Myc or adenovirus E1A that ‘epithelialize’ mesenchymal cells alter their sensitivity to apoptosis [111-113]. In frank tumor cells where apoptosis and polarity pathways are both altered, restoring a polarity function that normally licenses a default pathway of apoptosis triggered by oncogenic stress might instead manifest only as a non-apoptotic PCD. In this context, it is intriguing to note that studies of the proteomic response to Myc have revealed a cytoskeletal regulatory function involving integrins and actin

regulators which play important roles in polarity signaling [114]. Overall, hints of the potential connections between Myc, Bin1 and polarity complexes such as Dgl/Lgl/Scr reinforce the notion that polarity signaling may license a default pathway of apoptosis that is triggered by oncogene stress in primary cells. Such pathways are vitally important in primary cells, in contrast to established cell lines which with rare exceptions have grossly impaired cell polarity signaling.

In support of this concept, other studies indicate that *Bin1* provides a critical support to default pathways of apoptosis or senescence triggered by oncogenes in primary cells. In chick embryo cells, a classical model of one-step transformation by Myc, blocking Bin1 expression or Myc-Bin1 interaction did not affect cell proliferation or transformation but it specifically inhibited Myc-mediated apoptosis [19,115]. Investigations in this system implicated *Bin1* in the autocrine production of a secreted factor required for Myc to mediate apoptosis [115]. Similar effects were obtained in primary baby rat kidney (BRK) cells immortalized by c-Myc [19]. In contrast, in established or tumor cell lines, where polarity signaling is expected to be impaired, efforts to ablate Bin1 did not alter Myc-mediated apoptosis. While Myc can sensitize cells to apoptosis by a variety of mechanisms [116], one generally critical step seems to be its ability to trigger a configurational change in Bax needed to allow it to insert into the mitochondria outer membrane, where Bax must go to trigger cytochrome c release, caspase activation, and cell suicide [117,118]. Precisely how Myc influences Bax configuration remains obscure at present. Notably, a recent report implicates the BAR adapter protein Bif-1 (endophilin B1) in activating the Bax configurational change that is needed for its mitochondrial recruitment [36]. In light of the evidence that Bif-1 and Bin1 can interact with each other [38], it is conceivable that the titration of Bin1 by Myc or other factors might relieve a restraint to Bif-1, thereby facilitating its liberation to promote Bax activation. In any case, the evidence that Bin1 is germane only in primary cell settings is consistent with the notion that its ability to facilitate apoptosis may rely upon intact cell polarity signaling at some level.

Additional studies performed in primary mouse cells where *Bin1* has been deleted reinforce the idea that it supports a default pathway of apoptosis which is sensitized by oncogenic stress. Cell transformation by Myc or E1A is well-known to sensitize cells to apoptosis by tumor necrosis factor (TNF) or related factors such as TRAIL [118-121]. Similar effects occur in polarized cells, where TNF cooperates with Myc to trigger apoptosis [109]. In primary cells transformed by E1A+Ras, which are very sensitive to TNF, deletion of *Bin1* was sufficient to abolish apoptosis [28]. This loss of sensitization was associated with precocious nuclear localization and DNA binding activity of NF-κB [28]. Together these results implied that *Bin1* supported the apoptotic sensitivity of transformed cell by restricting NF-κB activity at some level. While the mechanism was not defined, effects on the canonical pathway of NF-κB were ruled out. The concept that *Bin1* supports a ‘death sensitization pathway’ was extended in another study which illustrated a specific requirement in apoptosis induced by farnesyl transferase inhibitors [105], a class of targeted therapeutic drugs with a well-documented selectivity for transformed cells [122]. Together, these studies reinforced the conclusion that *Bin1* supports a default pathway apoptosis that is triggered by oncogenic stress in primary cells.

More recently, *Bin1* was found to be essential for a default pathway of senescence that is also triggered by oncogenes in primary cells. Specifically, *Bin1* was identified in a large-scale screen for genes that are required by the Ras effector kinase B-Raf to induce senescence in primary human fibroblasts or melanocytes [123]. This study screened ~28,000 genes by an siRNA-based method, identifying *Bin1* in a set of 17 genes that also included *p53* and other tumor suppressor genes known to be critical mediators of cell cycle arrest and senescence. This study has important implications for unraveling the precise suppressor functions of *Bin1*, insofar as other genes identified in the screen might be expected to participate in common or overlapping default pathways of senescence triggered by oncogene stress. In this context, it is

interesting to note that the screen also identified *SmarcB1*, which encodes the SWI/SNF family transcriptional co-factor Ini1/SNF5 that binds Myc and mediates it transactivation activity [124]. Similar to its role in default pathways of apoptosis, the essential role revealed for *Bin1* in oncogene-induced senescence is essential in primary cells where cell polarity signaling is enabled and helps restrict cancer at its earliest stages.

Bin1 in muscle development: potential integration of stress and polarity signaling roles

The finding that *Bin1* is needed for default pathways of apoptosis or senescence under conditions of oncogenic stress may prompt a re-interpretation of earlier studies of the role of *Bin1* in myogenesis, obtained originally in models where differentiation is triggered by growth factor deprival. In mouse C2C12 myoblasts, a commonly accepted model of muscle terminal differentiation, inhibiting *Bin1* expression prevents upregulation of the cell cycle kinase inhibitor p21WAF and entry to terminal cell cycle arrest, which in turn prevents cell fusion events that form myotubes [30,31,95]. Moreover, a genetic suppressor screen for genes that block C2C12 differentiation identified only *Bin1* and the retinoblastoma protein gene *Rb* [25], the loss of which produces a muscle phenotype [125]. The notion that Bin1 might participate in Rb-mediated cell cycle arrest has received an additional line of support recently [100]. Given its high levels of expression in skeletal muscle, these findings have tended to encourage the interpretation that *Bin1* is crucial for terminal cell differentiation in muscle. However, more recent observations in *Bin1* null mice call this interpretation into question since no defect in skeletal muscle is evident [52]. Thus, one interpretation of the findings of C2C12 studies which are consistent with other findings is that *Bin1* may participate in cell fate decisions that are triggered by a growth-related stress, in this case by growth factor deprival [31].

This interpretation does not discount other evidence that *Bin1* has a non-redundant function in muscle [95], and in fact, a recent genetic study indicates that germ-line mutations in human *BIN1* cause a rare myopathy known as centronuclear myopathy [126]. Myopathies are marked by muscle weakness and this particular disease is associated with abnormal centralization of nuclei in muscle fibers formed by cell-cell fusion during myoblast differentation. Specifically, in three families exhibiting autosomal recessive inheritance, five individuals were found to harbor homozygous mutations in *BIN1*, all of which were implicated in disrupting recruitment or tubulation activity at membranes [126]. While these defects were interpreted to underlie nuclei mislocalization and muscle function, the impact of the mutations on polarity signaling was not considered, which could conceivably be relevant to the subcellular movement and positioning of nuclei in muscle fibers.

This study noted no cases of cancer among the five affected individuals, however, all but one died in infancy or at an early age, largely precluding an assessment of the effects of *BIN1* alteration on cancer. In multicellular organisms, germ line and somatic alterations differ in their phenotypic consequences. Germ line mutations in suppressor genes can cause developmental abnormalities, but even if this is not the case, such mutations may not manifest their effects on cancer until later in adulthood (as studies of *Bin1* in the mouse would suggest [86]). Thus, for practical purposes, early deaths associated with germ line mutations in *BIN1* would seem to prevent an evaluation of their effect on cancer. In contrast, as discussed above, numerous studies have documented somatic alterations in Bin1 expression or splicing in cancer that are associated with poor outcomes and/or deficiencies in suppressor activities. Moreover, as discussed below, genetic ablation studies in the mouse establish that *Bin1* loss-of-function is sufficient to cause cancer and to drive cancer progression [85,86]. Thus, functions of *Bin1* in muscle and cancer suppression may be non-redundant and distinct. Nevertheless, in considering findings from C2C12 myoblasts, one can re-interpret the results in a manner that

is consistent with an emerging theme for *Bin1* as an essential mediator of apoptosis and senescence pathways that help restrict the cancerous effects of 'rogue' oncogenic signals.

Bin1 and *Bin3* have essential functions in cancer suppression

Four amphiphysin-like genes – the ubiquitous *Bin1* and *Bin3* and the tissue-specific *Amph I* and *Bin2* – representing a subset of the BAR adapter family in mammals can be defined on the basis of close structural relatedness and biochemical interactions. Deletion of *Bin1* in mice causes developmentally lethality, but analysis of a conditional mutant has permitted investigations of cancer susceptibility using mosaic or tissue-specific strategies to delete the gene under circumstances where viability is maintained [52,85,86]. While the consequences of *Bin2* deletion have not been described, inactivation of *Bin3* or *Amph I* does not affect viability or fecundity, permitting a direct evaluation of the essential functions of these genes [127, 128]. Figure 4 summarizes the phenotypes observed in knockout mice lacking *Bin1*, *Amph I*, or *Bin3*.

Bin1 deletion in the mouse produces a perinatal lethality associated with the development of cardiomyopathy in the embryo [52]. While precluding studies of cancer susceptibility, this phenotype was interesting in light of the evidence of a cardiomyopathy susceptibility locus in humans that maps to chromosome 2q14-22 where the *Bin1* gene can be found [129]. Extensive investigations of mutant embryo cells revealed no apparent defects in endocytosis, phagocytosis, actin organization, proliferation, or survival [52]. Thus, in contrast to its homologs in yeast [1], *Bin1* seems to be non-essential for these processes. While *Bin1* is highly expressed in the CNS and in skeletal muscle, where it has been implicated in myoblast differentiation [25,31,95], no histological deficiencies are apparent in the brain or skeletal muscle, however, sluggish movement is apparent in neonates consistent with a muscle or neuromuscular deficiency [52]. In brain, *Bin1* deletion associates with a concomitant loss of Amph I protein, arguing that Amph I is unstable in the absence of the ability to heterodimerize with Bin1 [Amph II] proteins in that tissue. This effect exaggerates the loss of amphiphysin-like function in brain without any obvious impact on development. In contrast, cardiac hypertrophy was evident in *Bin1* null animals where the myofibrils of ventricular cardiomyocytes were severely disorganized. Interestingly, immunohistochemical analysis revealed that Bin1 proteins were predominantly cytoplasmic in skeletal muscle cells but predominantly nuclear in cardiac muscle cells [52], suggesting that nuclear interactions might contribute distally to the formation of a proper myofibril architecture in cardiomyocytes. While it is conceivable that compensary mechanisms act to limit effects of *Bin1* loss in the brain or skeletal muscle, no upregulation of the amphiphysin-like genes *Amph I*, *Bin2*, or *Bin3* was observed in the mutant embryo tissues. Although some studies have argued that *Bin1* is essential for endocytosis or phagocytosis [93,130], evaluations in nullizygous cells do not support this conclusion; the inconsistent findings obtained in the earlier studies may reflect the promiscuous and weakly controlled dominant inhibitory strategies (e.g. SH3 domain overexpression). More recent investigations in yeast and mammalian cells suggest a role in vesicle trafficking processes but where essential sites of function have not yet been established [60,131]. However, in general, genetic knockout studies of *Amph I* and *Bin1* in mice tend to reinforce the conclusions of *Drosophila* and fission yeast studies arguing that amphiphysin-like functions are non-essential for endocytotic processes.

More recently, a conditional cre-loxP strategy was developed to directly test the hypothesis that *Bin1* suppresses cancer in tissue-specific or mosaic mutant settings where cardiac development and viability were preserved [85,86]. Based upon the frequent attenuations of *Bin1* expression found in human breast carcinomas [24], the effects of mammary gland-specific deletion were investigated on the initiation and progression of breast cancer [85]. *Bin1* loss delayed the explosive outgrowth and later involution of the glandular ductal network that

occurs during pregnancy, however, this effect was not associated with increased susceptibility to breast cancer during the lifetime of a parous female. In contrast, when breast cancer was initiated by treatment with the classical *ras*-activating carcinogen DMBA, *Bin1* loss strongly accentuated the formation of poorly differentiated tumors characterized by low tubule formation, high mitotic index, and a high degree of nuclear pleomorphism. Analysis of epithelial tumor cell populations indicated that *Bin1* loss provided significant benefits to proliferation, survival, and invasive capacity, in support of similar correlations in human breast carcinoma [101]. These effects were specific, because in parallel experiments *Bin1* loss did not accentuate progression of tumors initiated by an overexpressed c-*myc* transgene, which on its own produced poorly differentiated and aggressive tumors [85]. Together, these findings extend the notion of some functional overlap between *Bin1* loss and c-*myc* activation in breast cancer, given that *ras* activation can cooperate with either event to drive breast tumor progression [86,132]. Clearly, this overlap is partial, because *Bin1* loss does not fully phenocopy c-*myc* activation. Thus, it can be inferred that the extent of the overlap relates only to a subset of functions that are specifically germane to malignant progression in cooperation with *ras* . Here it is worth noting that while deregulating c-*myc* expression is sufficient to prevent cell cycle exit and to drive tumorigenesis, while all human cancers deregulate c-*myc* some also overexpress it, implying that overexpression confers additional benefits in cancer. With this in mind, a model was proposed in which *Bin1* loss partly or fully phenocopies the benefits gained by c-*myc* overexpression in cooperation with *ras* activation [85].

A subsequent study conducted in mosaic mice extended the evidence that *Bin1* is essential to suppress cancer during aging [86]. Mosaic mice were generated in animals where Cre recombinase is expressed only very early in development in oocytes and preimplantation embryos including at the one-cell stage zygote [133]. Loss of *Bin1* was associated with an increased incidence of inflammation and the development of premalignant and malignant lesions in a variety of tissues during aging. By the age of 18-20 months, mosaic null mice displayed an increased incidence of myocarditis, an inflammatory condition of the heart, and pancreatitis, a known risk factor for pancreatic cancer. Additionally, mosaic mice displayed a significantly increased incidence of prostatitis or prostate hyperplasia, atypia or intraepithelial neoplasia [86]. These findings were notable, given the frequent loss of heterozygosity and expression of *Bin1* documented in human cases of metastatic prostate cancer [23]. None of these features were apparent in control animals of a similar age. These findings suggested that by promoting inflammation, which contributes to the genesis and progression of many age-associated epithelial cancers, *Bin1* attenuation might contribute to cancers in the prostate, pancreas, or other tissues if appropriate initiating lesions are present. More dramatically, >50% of mosaic null animals examined displayed frank carcinomas of the lung or liver by 18 months of age [86]. In support of a negative modifier role in progression, in young mice where colon carcinoma was initiated by exposure to a carcinogen, *Bin1* loss was associated with increased tumor invasiveness. One other notable phenotype was an extended period of fecundity in female mosaic null mice, which retained reproductive capability to the unusually old age of 17 months [86]. Elderly age is the top risk factor for cancer, but few genes that modify cancer incidence during aging are known. Thus, one important implication of this study was the identification of *Bin1* as an important modifier of cancer susceptibility during aging. Along with the broad evidence that its attenuation occurs often in human malignancy [2,21-24,29, 85,91,101-103], these findings establish directly that *Bin1* has an essential and non-redundant function in cancer suppression, perhaps including by restricting inflammation.

Amph I is expressed specifically in the central nervous system (CNS) and mice lacking this gene exhibit increased susceptibility to epileptic-like seizures upon reaching adulthood. *Amph I* null mice also show learning deficiencies in support of a role in brain function [128]. However, *Amph I* deletion has little effect on synaptic vesicle recycling [128]. This finding is inconsistent with others based on dominant negative strategies to disrupt *Amph I* function, for example, by

overexpressing the Amph I SH3 domain [134], possibly reflecting the pleiotropic and non-specific effects of dominant negative mutants which can arise (especially with promiscuous binding domains such as the SH3 domain). More recently, syndaptin I was defined as a physiologically relevant partner to dynamin in synaptic vesicle endocytosis and synaptic transmission rather than Amph-I [135]. Thus, while the learning deficiencies and increased seizures seen in *Amph I* null mice point to important essential functions in the CNS the molecular basis for these effects remain somewhat unclear. As mentioned above, Amph I acts as a paraneoplastic autoimmune antigen in rare occult cases of lung and breast cancer, suggesting that aberrant expression of Amph I in these cancers might contribute a target for immune surveillance during cancer development. In support of some link, remission of neurological symptoms has been documented to occur in patients after tumor excision and therapy [136]. Prompted by evidence of Amph I-Bin1 interaction, the role of *Bin1* in suppressing breast and lung cancer, and the role of *Bin1* in supporting tumoral immune surveillance (discussed below), it may be interesting to compare the susceptibility of *Amph I* null mice to breast or lung carcinogenesis to assess their propensity for tumoral immune escape [137].

Bin3 deletion causes ocular cataracts and increased lymphomas during aging [127]. In young animals, the lens is profoundly affected with vacuoles arising in cortical fibers and a near total loss of F-actin occuring in lens fiber cells but not lens epithelial cells. Young animals up to one year of age displayed no other overt phenotypes. However, reminiscent of the observations in *Bin1* mosaic mice, in aging animals loss of *Bin3* was associated with an increased incidence of lymphoma formation. This finding is of clinical interest, because the human *Bin3* gene is located at chromosome 8p21.3 within a region of frequent deletions in non-Hodgkin's lymphoma and various epithelial tumors where a tumor suppressor gene has yet to be defined [138-140]. High-resolution chromosomal deletion analyses have center on a ~1 Mb region that includes *Bin3* among 10 other genes (Fig. 5). While several TRAIL family receptors within this region were originally considered good suppressor candidates in non-Hodgkins lymphoma [138], they have been ruled out as relevant recently whereas loss of heterozygosity of *Bin3* has been confirmed (J. Martinez-Climent, pers. comm.). In younger *Bin3* null mice that are treated with carcinogens, an increase is observed in the incidence of lung cancer was observed. Cellular analyses suggested that *Bin3* was dispensable for normal cell proliferation, phagocytosis, cytosolic actin organization, or susceptibility to oncogenic transformation. In contrast, *Bin3* deletion was found to increase the proliferation and motility of primary cells transformed by the SV40 T antigen and Ras oncogenes [127]. Together, the findings indicate that *Bin3* has an essential function in cancer suppression.

Bin1 supports tumoral immune surveillance via indoleamine 2,3-dioxygenase (IDO)

Tumor graft studies of oncogenically transformed primary epithelial cells revealed unexpectedly that in addition to its cell-intrinsic suppressive effects *Bin1* also exerts a powerful support to T cell-mediated immune surveillance [27]. Specifically, it was found that primary keratinocytes co-transformed with c-Myc+Ras (MR KECs) formed more aggressive tumors in the absence of *Bin1* but only if the mouse host was competent for T cell immunity. In syngeneic animals, MR KECs expressing *Bin1* formed only small, indolent nodules, whereas MR KECs lacking *Bin1* formed large aggressive tumors that grew to an average size of >30-fold larger over the same period. This difference depende upon T cell immunity in the host animal, because *Bin1* loss in the transformed cells conferred no advantage to tumor growth in either immunocompromised nude mice that are deficient for T cell function or in syngeneic mice that were depleted of CD4+CD8+ T cells [27]. Thus, *Bin1* restricted the formation of epithelial tumors in a cell-extrinsic manner that was mediated by an ability to promote T cell-mediated anti-tumor immunity. Mechanistic investigations traced this effect to transcriptional repression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO). In support

of the hypothesis that IDO upregulation mediates the benefits of *Bin1* loss to tumor outgrowth, pharmacological inhibition of IDO with 1-methyl-tryptophan (1MT) specifically blocks the *in vivo* growth of *Bin1*−/− MR KECs and this effect is dependent upon intact T cell immunity in the host [27]. Thus, *Bin1* loss facilitated immune escape due to increased expression of IDO, a potent T cell suppressor that is frequently overexpressed in human tumors where it contributes to immune tolerance [141-143].

This finding that IDO is under the genetic control of *Bin1* is important, because while immune escape is a fundamental trait of cancer and a critical feature of progression, relatively little is known about how it develops [144]. Since *Bin1* supports immune surveillance by restricting IDO, a positive selection would exist for cells that have attenuated *Bin1* and elevated IDO as one means to promote immune escape and progression. This connection does not rely upon cell transformation insofar as the same regulatory relationship was seen in primary monocytes where IDO is normally expressed [137]. IDO dysregulation caused by *Bin1* loss is mediated by the increased activity of STAT1 and NF-κB, a finding that is interesting in light of the evidence that *Bin1* may influence the nuclear localization efficiency of these transcription factors [27,145]. IDO expression also appears to be elevated as a result of *Bin3* loss (J. DuHadaway and G.C.P., unpublished observations). Thus, IDO may represent a generalized integration point for BAR adapter signaling by *Bin1* or *Bin3*. Together, these findings have provided a major stimulus in the evaluation of IDO inhibitors as potential anti-tumor agents, as reviewed elsewhere [28,144,146,147], and they have prompted further genetic investigations of the role of IDO in cancer and the its relationship to the cancer suppressor functions encoded by *Bin1* and *Bin3*.

Future Perspectives

With the growing use of transgenic mouse models for cancer genetics studies, it is becoming clear that genes that modify oncogenesis may have strong effects on dormancy versus progression in cancer, a gateway that in humans would be expected to strongly affect clinical outcomes. Thus, further studies of *Bin1* and *Bin3* and other BAR adapter proteins and their critical signaling pathways in cancer may lead to insights into how cell polarity signals can govern cell proliferation survival, motility, and immune response, and how these signaling pathways break down during tumorigenesis and malignant progression. Signaling pathways that mediate cell polarity and immune escape represent hot emerging areas of signal transduction research in cancer, and the link revealed between these processes through *Bin1* studies is an especially interesting development. In any case, given that the purposes of basic research is to develop new hypotheses, work on BAR adapter pathways in cancer seem like to provide valuable jumping-off points to new insights into the cellular pathophysiology of cancer and its treatment.

Supplementary Material

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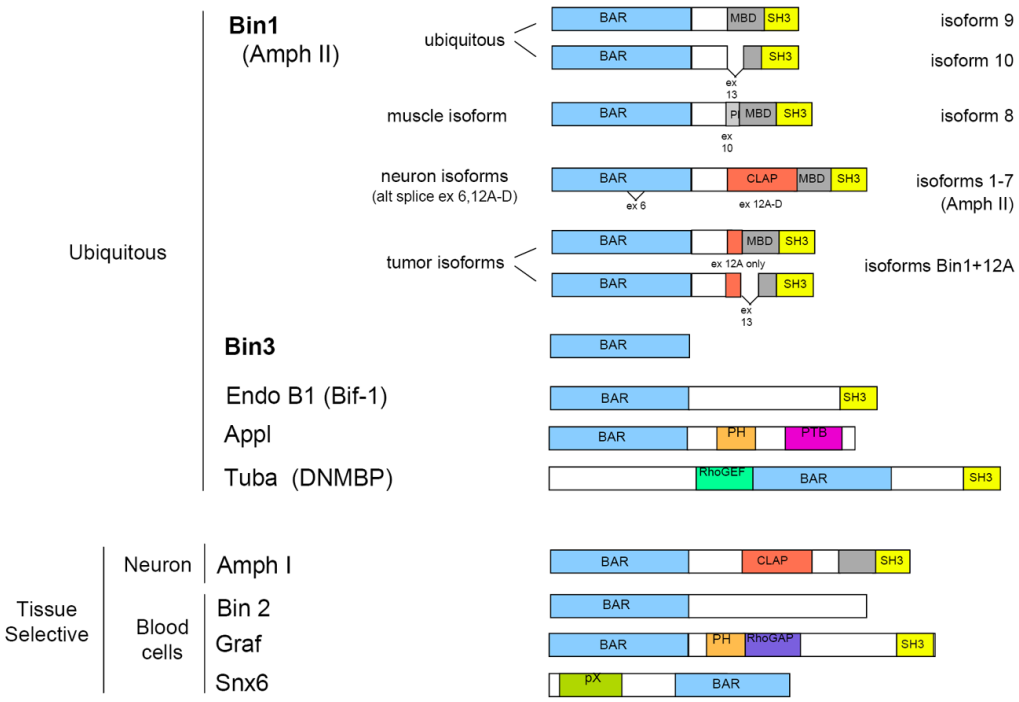

Figure 1. BAR family of adapter proteins

The primary structure of amphiphysin-like proteins and other selected ubiquitous and tissue-specific members of the BAR adapter family with relevance to cancer are presented. The organization and nomenclature of the various splice isoforms of Bin1 discussed in the text are noted. Exons 6, 10, 13, and 12A-D as defined by Wechsler-Reya et al. are alternately spliced [137]. While alternate splice isoforms for the other genes are known there has been generally little if any functional analysis reported, in contrast to Bin1 isoforms of Bin1. SH3, Src homology 3 domain; MBD, Myc binding domain; CLAP, clathrin-AP2 binding region; PH, pleckstrin homology region (PI binding); PTB, phosphotyrosine binding region; RhoGEF, Rho guanine nucleotide exchange function; RhoGAP, Rho guanine nucleotide activating protein function; pX, phox homology region (PI binding).

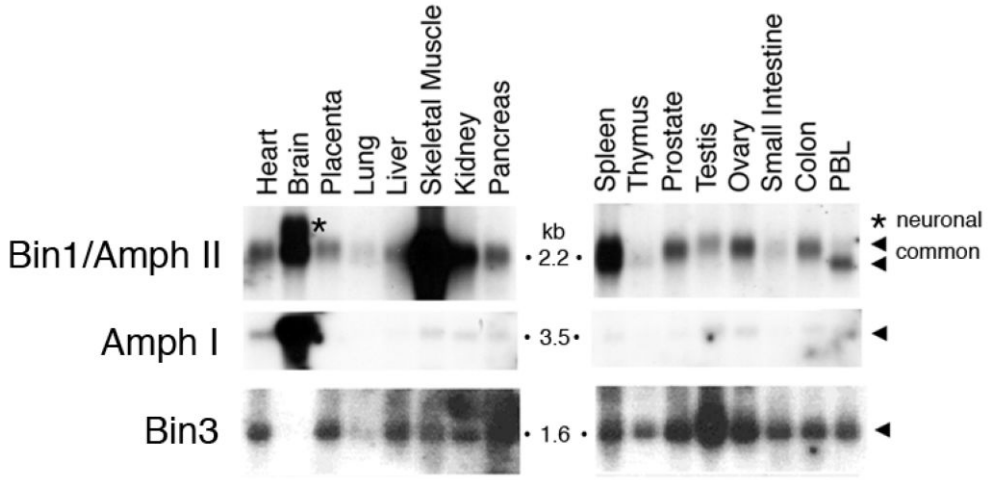

Figure 2. Patterns of *Bin1*, *Amph I*, and *Bin3* expression in mouse tissues

Northern analysis was performed of total RNAs isolated from the adult murine tissues indicated. Figure is adapted from Routhier et al. [92].

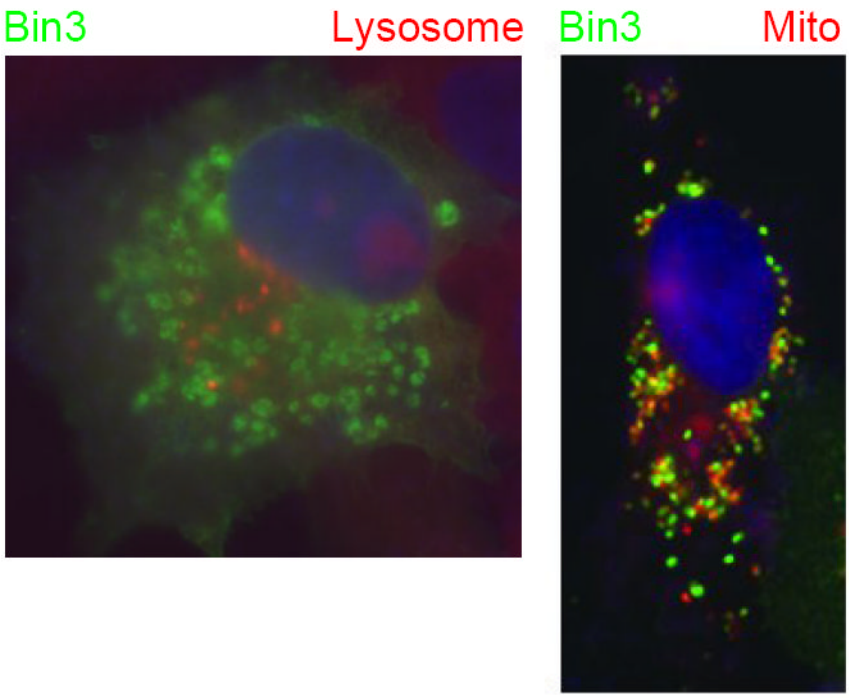

Figure 3. Bin3 localizes to vesicular membranes that overlap partially with mitochondria but not lysosomes

COS cells transfected with a human *Bin3* expression vector were fixed and processed for indirect immunofluorescence with a Bin3 antibody [33] and simultaneously stained with DAPI to visualize nuclei plus LysoTracker or MitoTracker, to visualize lysosomes or mitochondria, respectively. Figure is adapted from Ramalingam et al. [34].

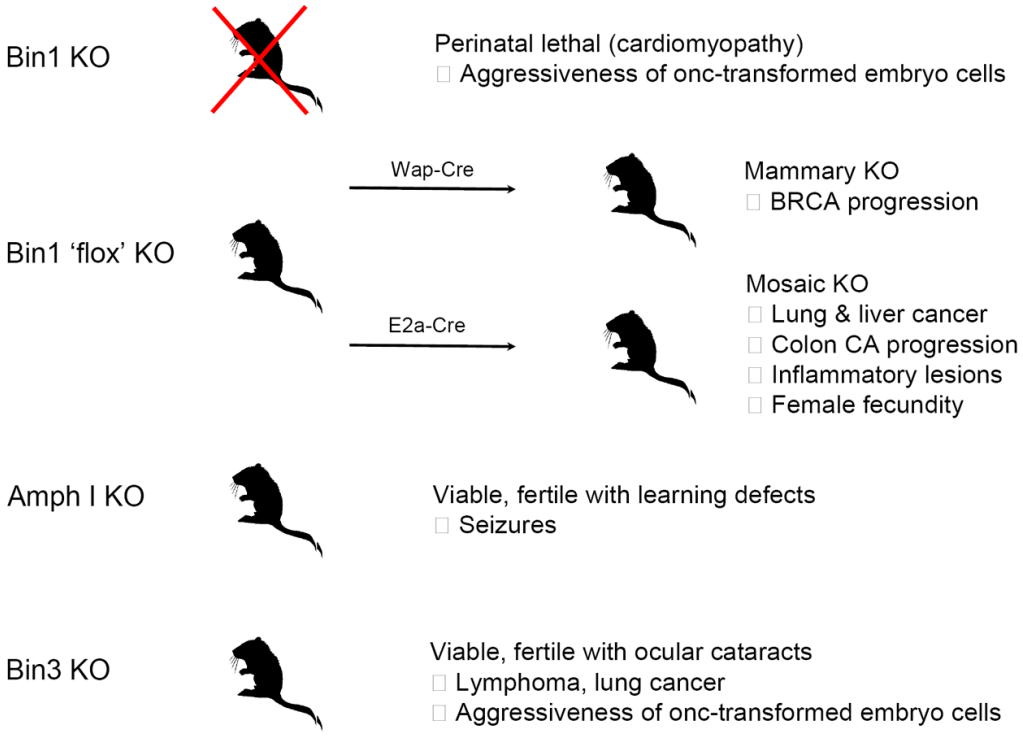

Figure 4. Phenotypes caused by deletion of amphiphysin-like genes in the mouse

See text for details. Deletion of the amphiphysin-like *Bin2* gene in the mouse has yet to be reported.

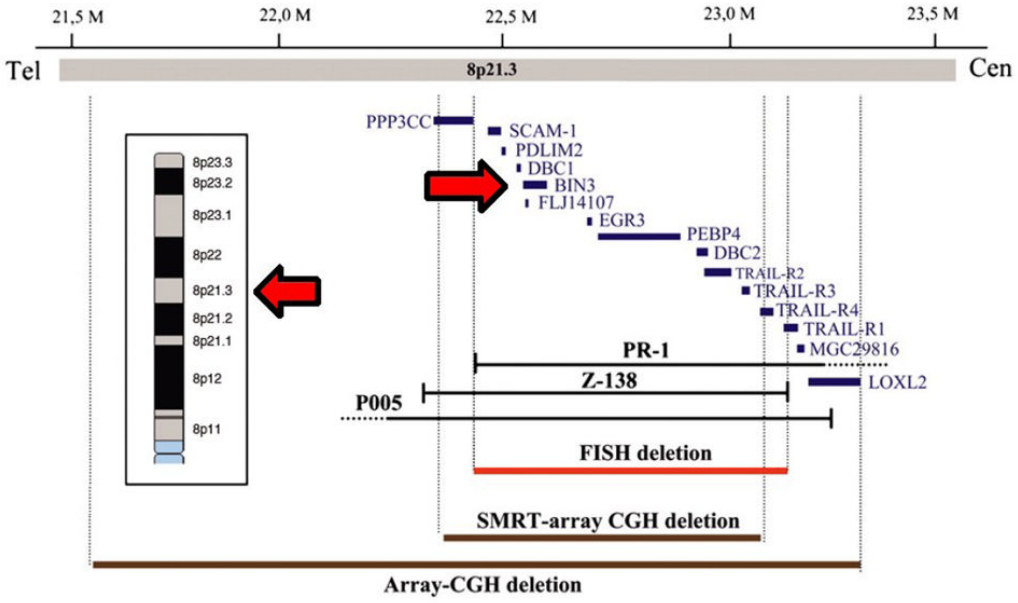

Figure 5. Bin3 maps to a hotspot for deletions at chromosome 8p21.3 in non-Hodgkin's lymphoma and other tumors

Figure is adapted from Rubio-Moscardo et al. [34].