



REVIEW ARTICLE

# An emerging role for BAG3 in gynaecological malignancies

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BAG3, a member of the BAG family of co-chaperones, is a multidomain protein with a role in several cellular processes, including the control of apoptosis, autophagy and cytoskeletal dynamics. The expression of *bag3* is negligible in most cells but can be induced by stress stimuli or malignant transformation. In some tumours, BAG3 has been reported to promote cell survival and resistance to therapy. The expression of BAG3 has been documented in ovarian, endometrial and cervical cancers, and studies have revealed biochemical and functional connections of BAG3 with proteins involved in the survival, invasion and resistance to therapy of these malignancies. BAG3 expression has also been shown to correlate with the grade of dysplasia in squamous intraepithelial lesions of the uterine cervix. Some aspects of BAG3 activity, such as its biochemical and functional interaction with the human papillomavirus proteins, could help in our understanding of the mechanisms of oncogenesis induced by the virus. This review aims to highlight the potential value of BAG3 studies in the field of gynaecological tumours.

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## BACKGROUND

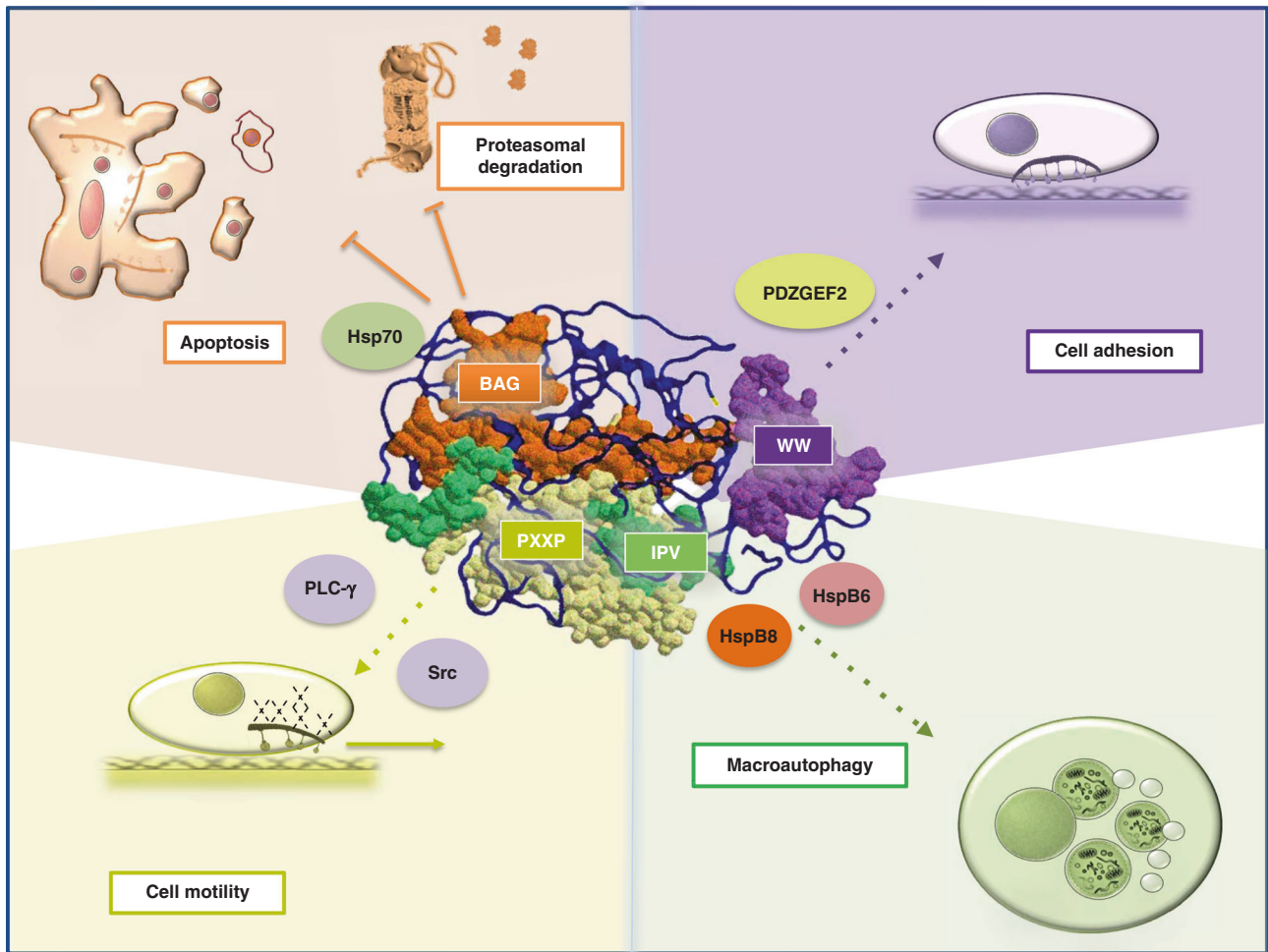
BAG3 belongs to a family of co-chaperone proteins that interact with the ATPase domain of the heat shock protein (HSP) 70 through a structural domain known as the Bcl-2-associated athanogene (BAG) domain. Only a few cell types—for example, skeletal and cardiac myocytes—express *bag3* constitutively, but its expression can be regulated by HSF1 and induced by several stress stimuli, such as high temperature and heavy metals,<sup>1</sup> retinal light damage,<sup>2</sup> HIV-1 infection,<sup>3</sup> serum deprivation,<sup>4</sup> electromagnetic fields exposure,<sup>5</sup> transient forebrain ischemia,<sup>6</sup> in many other cells (leukocytes, epithelial and glial cells, retinal cells). Following the observations that allow to classify BAG3 as a stress protein, it is not surprising that skeletal and cardiac muscle, subjected to continuous mechanical stress, express constitutively the protein due to their physiological function. On the other hand, the constitutive expression of *bag3* has been reported in several tumours, such as pancreatic ductal adenocarcinomas (PDACs),<sup>7,8</sup> melanomas,<sup>9,10</sup> hepatocellular carcinomas,<sup>11</sup> lung cancers,<sup>12</sup> colorectal carcinomas,<sup>13</sup> astrocytomas and glioblastomas,<sup>6</sup> leukemias,<sup>14–18</sup> thyroid carcinomas,<sup>19</sup> ovarian carcinomas,<sup>20,21</sup> breast cancers,<sup>22</sup> prostate cancers,<sup>23</sup> endometrioid endometrial adenocarcinomas.<sup>24</sup> In tumour cells BAG3 plays a role in cell survival, tumour progression and resistance to therapy, and in other functions as autophagy, protein quality control, angiogenesis, cytoskeleton organization and cell motility,<sup>25–28</sup> through the interaction with several intracellular and extracellular partners. In the context of gynaecological tumours, BAG3-mediated mechanisms constitute a new area of study. Some of the known partners of the protein have a recognised role in the growth and spread of these malignancies, and their response to therapy. BAG3's regulatory

relationships with these interacting partners therefore requires further investigation.

In this article, we will address, after a general review of the known role of BAG3 in tumours, recent clues that come from studies in gynaecological cancers. This is indeed a new field, that appears worthy of deeper investigations. High BAG3 protein levels in cancer cells, mainly exert oncogenic functions by supporting several key characteristics of all malignant diseases. Indeed, BAG3 is able to influence distinct cellular and molecular pathways that finally promotes cancer cells survival, proliferation, invasion of other anatomic sites and resistance to therapy. This eclectic functionality relies on the ability of the protein to interact with several partners through its numerous functional domains embedded in its structure, thus influencing the cancer cell fate. The BAG domain located at the C-terminus of the protein binds Hsp70 and interferes with the HSP70-mediated delivery of Bcl-2 anti-apoptotic proteins,<sup>25</sup> or the mitochondrial translocation of BAX pro-apoptotic protein.<sup>14</sup> Moreover, a WW domain can bind to proline-rich repeats of interactors, as PDZGEF2 (Guanine nucleotide exchange factor 2),<sup>29</sup> that plays a central role in cell-cell adhesion and in the cell anchorage to extracellular matrix. Also a proline-rich (PxxP) domain is able to interact with SH3 domains of PLC $\gamma$ , Src and other proteins that are master regulators of cancer development.<sup>30</sup> More recently, the discovery of two conserved IPV (Ile-Pro-Val) motifs, drew the attention on BAG3 as an essential player in cellular macroautophagy, dependent on the interaction with the small heat shock proteins HSPB8 and HSPB6<sup>31</sup> (Fig. 1). Finally, not less important than apoptosis inhibition, its ability to switch on the cytoprotective autophagy effects, promotes the

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**Fig. 1** BAG3 structural domains/motifs involved in the regulatory protein–protein interactions of major cellular pathways. (Orange = BAG domain; Purple = WW domain; Green, Ile-Pro-Val (IPV) motif; Yellow = Proline-rich domain (PxxP)). Key functional/interaction domains of BAG3, major BAG3 interactors and associated cellular events are depicted.

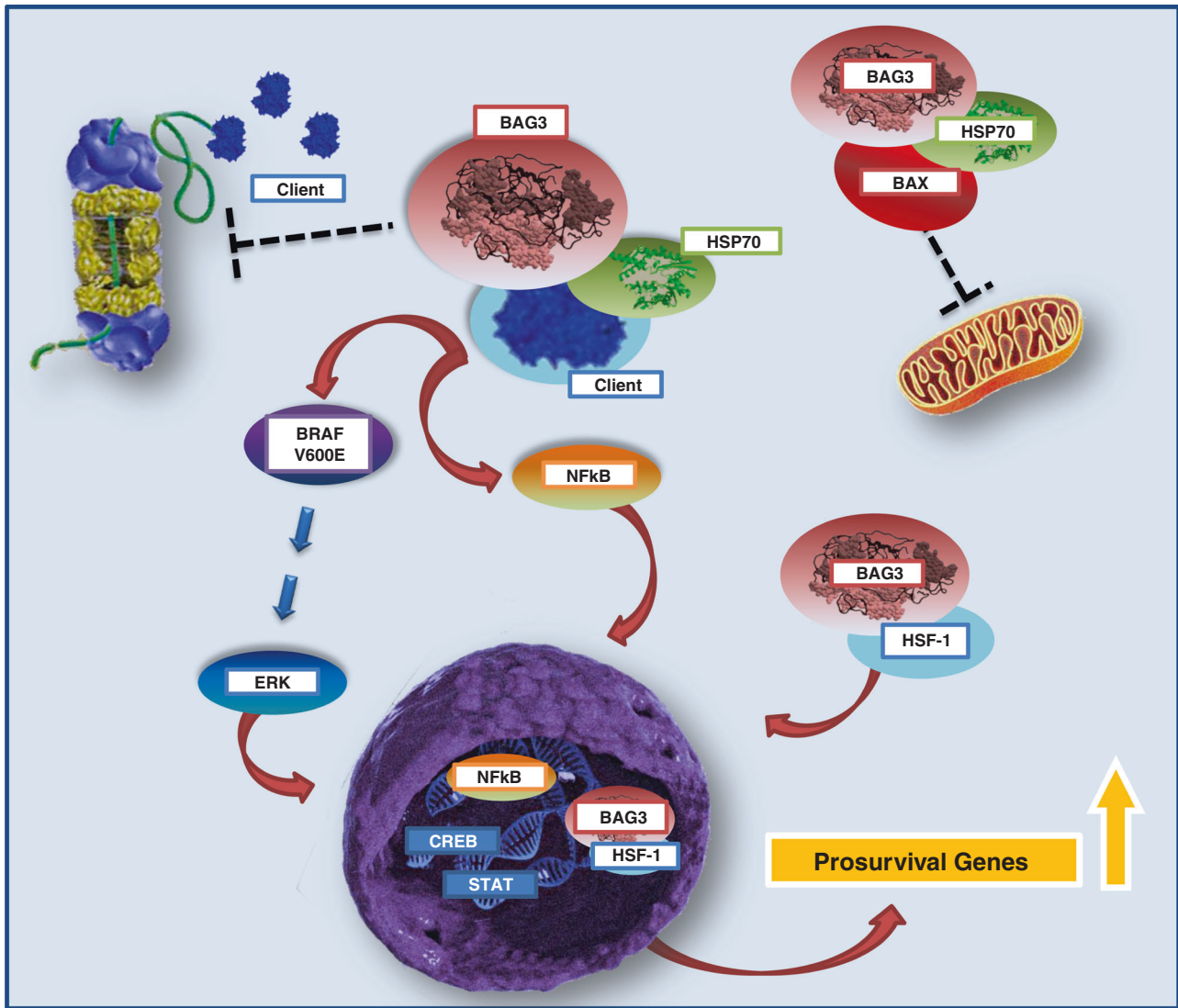
emergence of drug resistance in cancer cells, especially after targeted therapies,<sup>32</sup> as demonstrated in triple-negative breast cancer treated with doxorubicin or 5-fluorouracil, or in rhabdomyosarcoma treated with ST80/Bortezomid.<sup>25</sup> The activity of BAG3 underlying drug resistance has been investigated in different tumour models. In melanoma and osteosarcoma BAG3 has been found to protect IKK $\gamma$ , a subunit of the IKK complex that activates NF- $\kappa$ B, from HSP70-mediated delivery to the proteasome, resulting in increased NF- $\kappa$ B activation and suppression of etoposide-induced apoptosis.<sup>4</sup> This action is plausibly due to its ability to compete with BAG1 in binding to HSP70, and thus inhibit the interaction of HSP70 with the proteasome and the degradation of its client proteins.<sup>26</sup> Similarly, BAG3 stabilises several anti-apoptotic proteins, such as Bcl-2 family proteins (e.g. Mcl-1, Bcl-x and Bcl-2 itself) which, at high expression levels counteract the pro-apoptotic effects of anticancer drugs, including platinum compounds in ovarian cancer and small cell lung cancer, vemurafenib in melanoma, Bcl-2 antagonists in neuroblastoma and androgen receptor negative prostate cancer cells.<sup>26</sup>

#### CELL SURVIVAL

The pro-survival activity of BAG3 in primary tumours was shown for the first time in B cells from patients with chronic lymphocytic leukaemia (B-CLL)<sup>14</sup> Consistent with this activity, downregulating

BAG3 levels in neoplastic B lymphocytes from B-CLL patients using antisense oligodeoxynucleotides, induced apoptosis and enhanced the pro-apoptotic effect of fludarabine.<sup>14</sup> Analogous results were obtained in neoplastic cells from patients with acute lymphoblastic leukaemia (ALL),<sup>15</sup> and the pro-apoptotic effect of BAG3 silencing was also subsequently observed in several other tumours, such as thyroid carcinoma,<sup>33</sup> melanomas,<sup>34</sup> small cell lung carcinoma,<sup>12</sup> glioblastoma,<sup>6</sup> prostate,<sup>35</sup> ovarian<sup>36,37</sup> and pancreatic cancer.<sup>8</sup>

The anti-apoptotic activity of BAG3 is conferred through more than one mechanism and is cell-context-dependent. One mechanism through which BAG3 confers an anti-apoptotic effect involves it binding to a number of anti-apoptotic proteins, such as inhibitor of  $\kappa$ B kinase (IKK)  $\gamma$  (IKK $\gamma$ ) and BRAF<sup>V600E</sup>, and preventing their delivery by HSP70 to the proteolytic proteasome.<sup>4,19,38–40</sup> Hsp70-client protein delivery to the proteasome is usually mediated by HSP70 binding to BAG1, which associates with the proteasome via a ubiquitin-like domain at its amino terminus; increased levels of BAG3 can compete with BAG1 for HSP70 binding, thereby preventing the association of the HSP70-client complex with the proteasome.<sup>41,42</sup> Another mechanism for preventing apoptosis is mediated by the ability of BAG3 to regulate the localisation of the pro-apoptotic protein BAX. Under apoptotic conditions, BAX localises at the mitochondrial outer membrane, where it promotes apoptosis by inducing



**Fig. 2 BAG3 interactors in apoptosis.** BAG3 interactors and downstream effectors in cellular processes involved in the inhibition of cellular apoptosis.

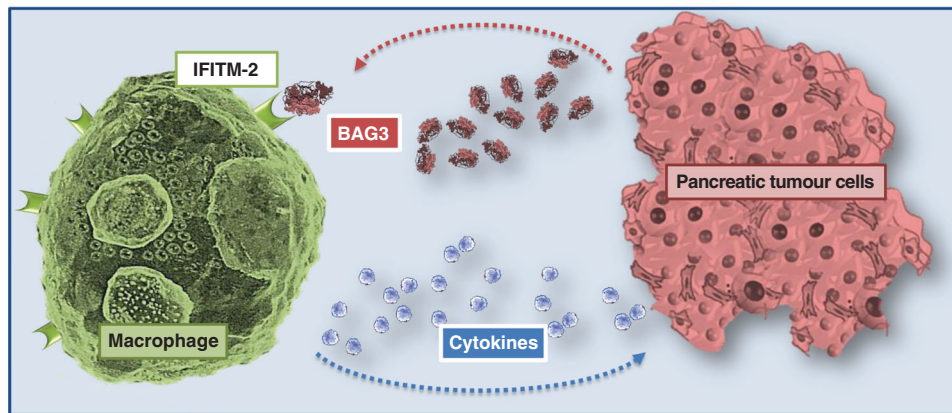
mitochondrial outer membrane permeabilisation and the release of pro-apoptotic factors, such as cytochrome c. In glioblastoma cells, which overexpress BAG3, BAX was detected in the cytosol in a complex with HSP70 and BAG3; BAG3 knockdown by siRNA facilitated the release of BAX and its translocation to mitochondria, thereby sensitising the cells to apoptosis, indicating that BAX retention in the cytosol prevented BAX-dependent apoptosis.<sup>6</sup> A further pro-survival effect of BAG3 relates to its role in the translocation of heat shock factor 1 (HSF1)<sup>43</sup> from the cytosol to the nucleus, where this transcription factor can induce the expression of Heat Shock Protein (HSP) genes involved in the homeostatic response to stress. Newly synthesised HSPs enable the refolding of damaged proteins, inhibit apoptosis by interacting with apoptotic regulators (such as BAX, AIF and APAF1) and prevent protein aggregation, thus having a cytoprotective effect.<sup>44</sup> For this reason, HSF1 has been considered a predictive biomarker and a therapeutic target in neoplastic malignancies<sup>45</sup> (Fig. 2).

#### TUMOUR PROGRESSION

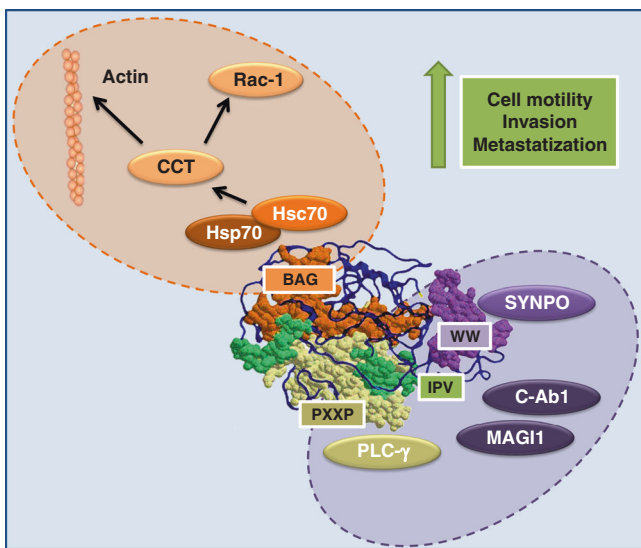
The pro-tumour effect of BAG3 depends not only on its cancer-cell-autonomous activities, but also on its ability to influence the interaction of the tumour with its microenvironment.

#### BAG3 and tumour growth

Some cell types, such as pancreatic cancer cells, are capable of releasing a soluble form of BAG3,<sup>46</sup> and secreted BAG3 can be detected in serum samples from patients with pancreatic ductal adenocarcinoma.<sup>47</sup> Soluble BAG3 can bind to a receptor—BAG3R, also known as IFITM2 (Interferon-Induced Transmembrane Protein 2), a member of the IFITM protein family—that is expressed by monocytes in the tumour microenvironment.<sup>48</sup> The BAG3–BAG3R interaction activates monocytes/macrophages, which release cytokines, such as interleukin (IL)-6 and interleukin (IL)-10, which sustain the proliferation of pancreatic cancer cells<sup>46</sup> (Fig. 3). This paracrine loop involving cancer cells and tumour-associated macrophages might also have a role in other types of tumour.



**Fig. 3 Paracrine loop operated by cancer cell-secreted BAG3 on macrophages surrounding the tumour.** BAG3 binding through its receptor IFITM2 stimulate macrophages to produce cytokines such as IL-6 and IL-10 that promote tumour development.



**Fig. 4 BAG3 interactors in cancer.** BAG3 interactors and downstream effectors in cellular processes involved in the promotion of cell motility, invasion and metastasis of cancer cells.

#### BAG3 and cell motility

The soluble factors secreted by activated macrophages not only sustain tumour cell growth but also promote their ability to spread and metastasise. The extravasation of circulating tumour cells involves their cross talk with endothelial cells, basement membrane and macrophages. It has been shown that bone marrow derived macrophages (BBMs) located on the basal-lateral side of endothelial cells are able to enhance the trans-endothelial migration of tumour cells in a CCL2 dependent manner, a chemokine expressed by all three types of cells and acting through the receptor CCR2 present only on macrophages.<sup>49</sup> The same CCL2-CCR2 axis stimulates the production of CCL3 in metastasis associated macrophages (MAMs), which in turn regulates the MAM's retention in the metastatic site through the CCR1-CCL3 axis.<sup>50,51</sup> Moreover, other macrophage derived factors like VEGF and endothelin-1 (ET-1) play a role in metastatic colonisation (see Doak GR et al., 2018 and references therein).<sup>52</sup>

It has also been reported that BAG3 might regulate some components of the actin cytoskeleton that are involved in mediating cell motility at the leading edge of migrating epithelial cancer cell lines.<sup>53</sup> BAG3 could then play a role during the protrusion—and indirectly on the adhesion—of the moving cells, by driving the remodelling of the actin cytoskeleton through the

interaction with other molecular partners involved into the folding processes. In this respect, although the molecular mechanisms are not yet fully elucidated, experimental pieces of evidence empirically support this hypothesis. In fact, other than the well-known interaction with Hsp70/Hsc70 chaperones, BAG3 interacts with CCT, a chaperonin having the actin as a substrate,<sup>54</sup> and it is able to regulate Rac1 activity.<sup>53</sup> Furthermore, other proteins involved into the process of cell motility have been proposed as BAG3 partners, like PLC $\gamma$ , c-Abl, synaptopodin, MAGI-1, given their ability to bind to its PXXP motifs (SH3 proteins) or WW domain.<sup>55,56</sup> Several pieces of evidence let thus infer that the expression of BAG3 in many tumours could enhance cell motility, and consequently be relevant for their invasiveness and metastasis (Fig. 4).

#### BAG3 IN GYNAECOLOGICAL CANCERS

Recent results in ovarian, endometrial and cervical cancers indicate a role for BAG3 in these tumours. We will here analyse these pieces of evidence, trying to outline, where possible, the underlying mechanisms (Fig. 5).

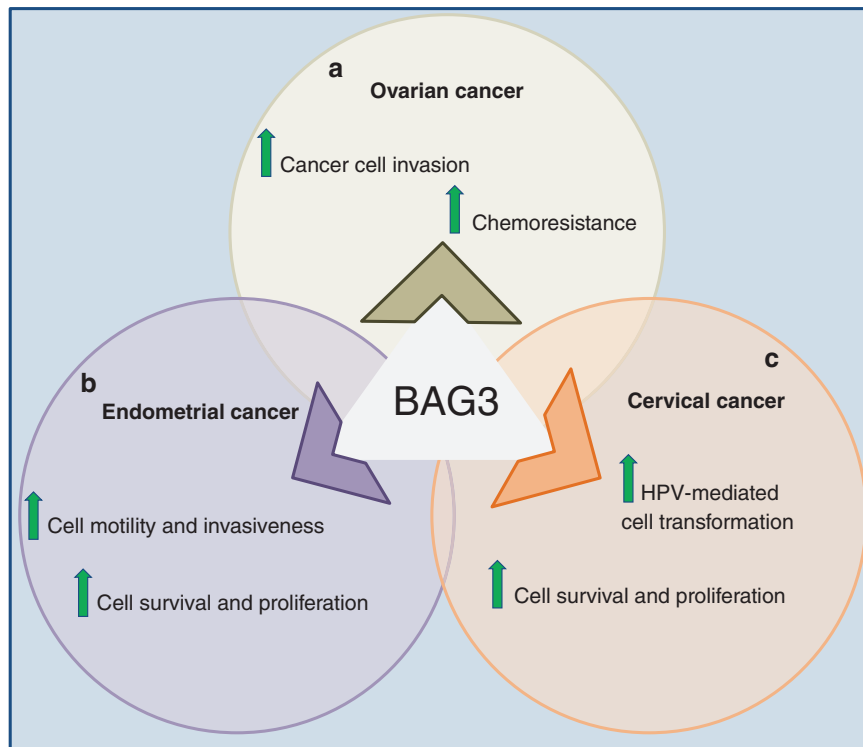
#### BAG3 IN OVARIAN CANCER

##### BAG3 in invasion

In ovarian cancer, BAG3 has been shown to interact with matrix metalloproteinase-2 (MMP2), a calcium-dependent endopeptidase that is involved in remodelling the extracellular matrix and, therefore, in cancer cell invasion.<sup>57</sup> The BAG3–MMP2 interaction in ovarian cancer suggests that BAG3 might positively regulate the invasion of ovarian cancer cells via MMP2. The overexpression of MMP2 in ovarian carcinoma cells has been reported to be associated with poor prognosis;<sup>58</sup> consequently, mechanisms that maintain low levels or decrease MMP2 levels are of interest in the investigation of potential therapies for ovarian cancer. BAG3 has been demonstrated to participate in sustaining MMP2 levels accordingly, BAG3 silencing resulted in a reduction of MMP2 mRNA levels and of the intracellular levels of this enzyme in ovarian cells. Furthermore, it was also demonstrated that the reduction in MMP2 levels in BAG3 knockout ovarian cells was not caused by increased proteasome-mediated proteolysis, thus inferring that BAG3 regulates MMP2 expression at the mRNA level.<sup>57</sup>

##### BAG3 in therapy resistance

In addition to *apc*, *mapk3* and *s100a10*, *bag3* was among a panel of four potential biomarkers that was found to be indicative of a poor chemotherapy response and/or poor outcome in ovarian



**Fig. 5 Cellular processes upregulated by BAG3 expression in gynaecological malignancies.** Summary of the reported evidence in BAG3-dependent upregulation of key cellular processes in **a** ovarian, **b** endometrial, **c** cervical cancer.

cancer.<sup>21</sup> Other evidence also indicates a potential correlation between BAG3 expression and the response of ovarian cancer cells to therapy. Indeed, downregulation of BAG3 was found to block cisplatin-induced autophagy, thereby increasing cell sensitivity to this agent,<sup>36,37</sup> and to reduce levels of the anti-apoptotic protein MCL-1, thereby increasing the response to paclitaxel.<sup>59,60</sup> Cytoprotective autophagy and apoptosis suppression are increasingly recognised in BAG3-mediated resistance to therapy, as demonstrated in doxorubicin- or 5-fluorouracil-treated triple-negative breast cancer and ST80/bortezomib-treated rhabdomyosarcoma, among other tumour types.<sup>61</sup> Furthermore, BAG3 knockdown has also been shown to sensitise ovarian cancer cells to treatment with olaparib, a poly ADP-ribose polymerase (PARP) inhibitor, reducing cellular viability and promoting apoptosis.<sup>62</sup>

#### BAG3 in cell viability and proliferation

In another study, for the first time, BAG3 has been linked to the phosphatidylinositol 3-kinase (PI3K)–AKT pathway. Overexpression of miR-340 inhibits the proliferation of ovarian cancer cell lines and promotes apoptosis through the downregulation of BAG3. Consistent with this observation, BAG3 silencing significantly induces cell apoptosis, and abolishes the increase in cell viability induced by the suppression of miR-340 that was accompanied by the activation of PI3K/AKT.<sup>63</sup> BAG3 has also been described to promote the proliferation of ovarian cancer cells via upregulation of S-phase kinase associated protein 2 (SKP2), a cell-cycle regulator. By binding to the SKP2 3'-untranslated region (UTR), BAG3 antagonises the suppressive action of miR-21-5p.<sup>64</sup>

#### BAG3 IN ENDOMETRIAL CANCER

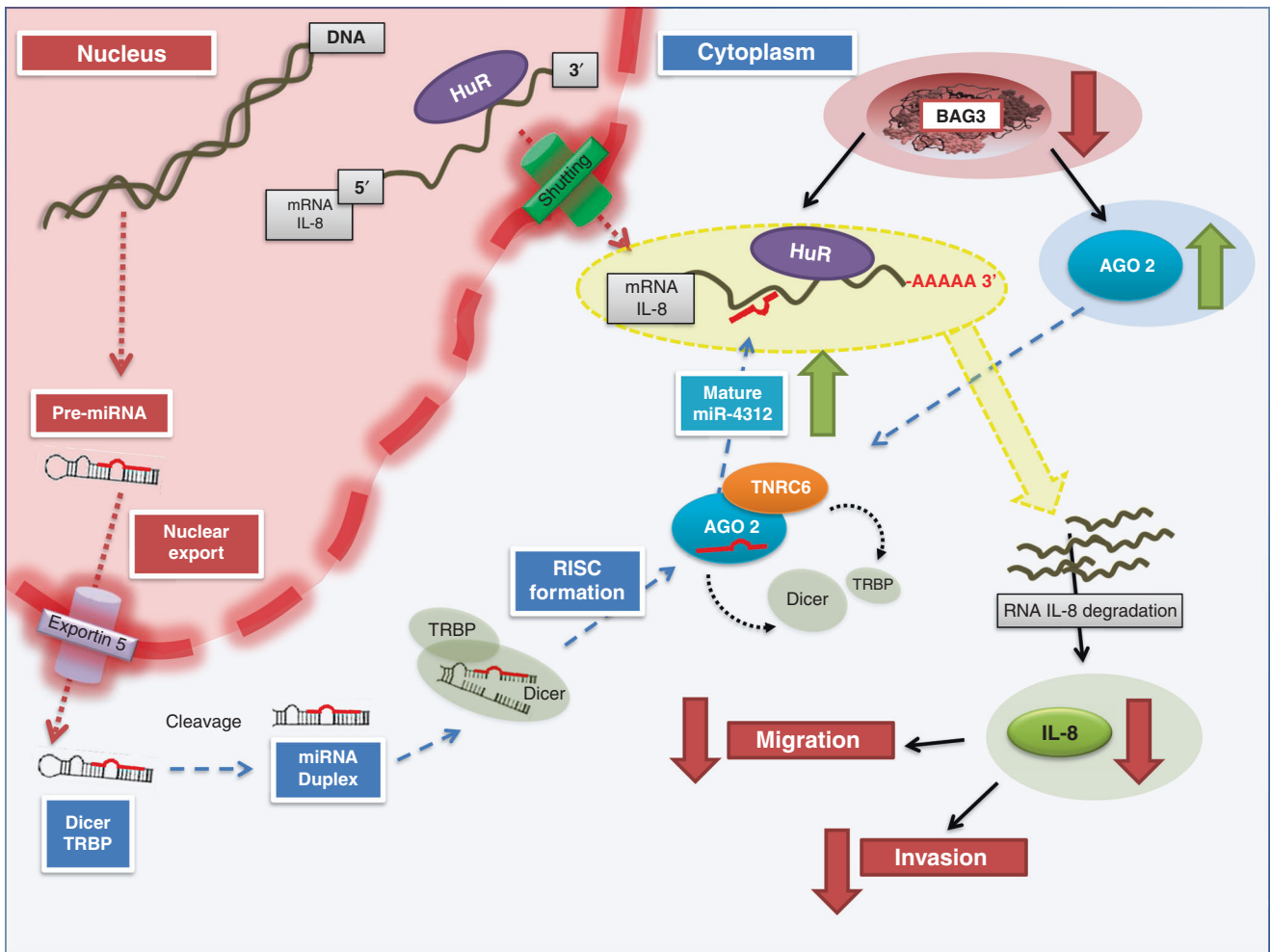
##### BAG3 and MMP2

The BAG3 protein is known to be overexpressed in endometrioid endometrial cancers.<sup>24</sup> Furthermore, BAG3 has been reported—by the same authors who described the binding of BAG3 to MMP2 in ovarian cancer cells<sup>57</sup>—to influence MMP2 protein levels in two

endometrioid carcinoma cell lines, Ishikawa and HEC108,<sup>65</sup> albeit through a different mechanism to that operating in ovarian cancer cells. In these endometrioid carcinoma cell lines, BAG3 enhances MMP2 levels by inhibiting the expression of miR-29b, a miRNA that can reduce the levels of the metalloproteinase, thereby increasing the cell motility and invasiveness of endometrioid adenocarcinomas.<sup>65</sup> In a recent study the same researchers demonstrated that the ER $\alpha$  overexpression upregulates BAG3, whose in turn downregulates miR-29b leading to overexpression of Mcl-1 and, most likely, MMP2.<sup>66</sup> How BAG3 and miR-29b interact warrants further investigation. In human pancreatic cancer cell lines, a post-transcriptional mechanism controlling IL-8 expression by BAG3 through the interaction of the RNA-binding protein HuR or miR-4312 with the IL-8 transcript has been reported. HuR binding to *cis* elements located in the 3'-translational region (UTR) of the IL-8 transcript stabilises it, whereas recruitment of miR-4312-containing miRNA-induced silencing complex (miRISC) to the adjacent seed region destabilises it. BAG3 knockdown in pancreatic adenocarcinoma cells results in the retention of HuR in the nucleus and its increased phosphorylation at Ser202, thereby preventing its recruitment to the cytoplasmic IL-8 transcript; phosphorylation of Argonaute 2 (Ago 2) at Ser387 is increased by BAG3 knockdown, which further promotes the recruitment of miR-4312 to the IL-8 transcript, thus destabilising the IL-8 transcript and inhibiting its translation.<sup>67</sup> BAG3 is therefore likely to promote invasion in pancreatic adenocarcinoma cells by stabilising the IL-8 transcript through HuR (Fig. 6). Whether or not the BAG3–HuR interaction is also involved, either directly or indirectly, in modulating the levels of MMP2 in endometrial cancer through a similar mechanism remains an open question that would be interesting to answer.

##### BAG3 and p53

A 2020 publication reported that BAG3 interacted with p53 in Ishikawa cells and prevented the translocation of this tumour suppressor to the nucleus,<sup>68</sup> extending our knowledge of the



**Fig. 6** Mature miRNAs derive from longer double-stranded primary transcripts (pri-miRNA), processed in the nucleus and folded in a hairpin loop structure (pre-miRNA). Pre-miRNAs are then exported to the cytoplasm (through Exportin 5) where they are first cleaved by Dicer and later processed by RISC (RNA-induced silencing complex) to form the mature miRNA (miR-4312). RISC, that includes Ago 2 (Protein argonaute 2), also participates in the identification of miRNAs' targets. Interfering with BAG3 levels it was simultaneously observed an upregulation of miR-4312 and a disruption of Hur factor binding to IL-8 mRNA. This produces IL-8 mRNA degradation by lowering migration and invasion process of cancer cells.

mechanisms that govern the activity of p53 and the expression of genes responsible for cell-cycle arrest and/or apoptosis.

### BAG3 IN CERVICAL CANCER

The evidence for a role for BAG3 in the pathogenesis of cervical cancer is supported by the report of a direct correlation between the levels and localisation of BAG3 expression and the grade of dysplasia in cervical intraepithelial neoplasia (CIN)/squamous intraepithelial lesions (SILs).<sup>69</sup> An adequate definition of presence and degree of dysplasia is critical for the patient management, but among the parameters currently used in the clinical practice there are no helpful molecular markers to this aim. The results from this study suggest that BAG3 might prove useful in the diagnosis and prognosis of premalignant lesions.

### BAG3 in proliferation and survival

Studies in the human cervical squamous carcinoma HeLa cell line have contributed to our knowledge of the role of BAG3 in this cancer, with BAG3 knockout HeLa cells enabling the regulatory activity of this protein in the expression of genes involved in cell proliferation and survival to be investigated. Using DNA microarray-based transcriptome analysis and bioinformatics tools,

two genetic networks associated with cellular growth/proliferation and cell death/survival were found to be regulated by BAG3. Particularly, Matrix MetalloProteinase-2 (MMP2) that contributes to tumour cell apoptosis probably through the degradation of poly (ADP-ribose) polymerase; Platelet Derived Growth Factor C (PDGFC) that has an anti-apoptotic effect and promotes cell proliferation, and the transcription factors RUNX2 and PPARG involved in modulating cell survival/proliferation, are transcriptionally upregulated by BAG3 deletion. On the other hand, ERBB4 encoding the tyrosine kinase receptor HER4 belonging to the epidermal growth factor receptor family; the tissue inhibitor of MMP2, TIMP3; the transcription factor linked to tumour cell growth, KLF4, and BMP2 that inhibits apoptosis through the activation of BMP receptor 2, were downregulated by BAG3 deletion.<sup>70</sup>

Localised thermal therapy/hyperthermia therapy has been proven to be effective in the treatment of cancer, especially in combination with radio- or chemotherapy, but the molecular mechanisms that underlie the cellular responses to heat stress have not been fully elucidated. A study aimed at identifying gene expression patterns responsive to mild hyperthermia in HeLa and human tongue squamous carcinoma cells (HSC-3) led to the inclusion of *bag3* in a gene network associated with normal

cellular functions and maintenance.<sup>71</sup> These results suggest that BAG3 is involved in cell homeostatic response to hyperthermia. In a further study, BAG3 was reported to be targeted by miR-206: by negatively affecting the stability of BAG3 mRNA, miR-206 down-regulates BAG3 protein levels and can attenuate the positive effect on the proliferation and migration of cervical cancer cells of this protein, thereby inhibiting its pro-tumour activity.<sup>72</sup> If such miR-206 activity is detectable also in other tumours, is then worthy of deeper investigation.

#### BAG3 and HPV

A 2014 publication reported on the interaction of BAG3 with the human papillomavirus (HPV) oncoprotein E6 in HeLa cells, which are HPV18<sup>+</sup> and therefore likely to undergo malignant progression. Such an interaction appeared to maintain levels of the E6 oncoprotein, which targets the tumour suppressor p53 for proteasomal degradation: indeed, BAG3 downregulation resulted in decreasing E6 levels, concomitant with an increase in p53 levels.<sup>73</sup> Therefore, BAG3 appeared to be part of the mechanism of HPV-mediated cell transformation. The interaction of BAG3 with papillomavirus has been more extensively investigated in cattle urothelial cancer cells infected by bovine papillomavirus (BPV): in these cells, BAG3 colocalised with E5, the main oncoprotein of BPV, suggesting a possible role for BAG3 in regulating its levels, localisation and/or activity;<sup>74–77</sup> potentially, this mechanism could also be relevant in the regulation of E6 in humans.

#### CONCLUSIONS AND DISCUSSION

To date, the diagnosis, surgical staging, risk assessment and adjuvant treatment of gynaecological cancers are still based on The International Federation of Gynaecology and Obstetrics (FIGO) clinical stage and histological examination with classic prognostic histological factors (i.e. histotype, FIGO grade, lymph node involvement, lymphovascular space invasion, organ infiltration).<sup>78</sup> Advances in cancer treatment have been made, but the number of deaths from gynaecological cancers remain substantial worldwide. Cervical cancer is preventable through a combination of HPV vaccination and systematic screening but, nevertheless, it remains the leading cause of cancer death for women in resource-poor countries.<sup>79</sup> With regard to ovarian cancer, even though the combination of cytoreductive surgery and standard chemotherapy with paclitaxel and carboplatin is effective, 70% of patients relapse, and long-term survival remains poor. Therefore, a new treatment strategy needs to be developed. The last edition of the FIGO Cancer Report outlined how advances in molecular biology of cancer have led to the development of targeted agents—in particular, monoclonal antibodies and small molecule compounds. Unlike traditional drugs that inhibit DNA synthesis and mitosis, these agents target the signalling pathways of cancer cells, stroma and vasculature in tumour tissues. Drugs such as PARP inhibitors (olaparib, rucaparib and niraparib), which exploit homologous recombination deficiency, have emerged as a key targeted therapeutic agent for maintenance after chemotherapy in platinum-sensitive ovarian cancer.<sup>80</sup> In the era of precision medicine, further understanding of cancer genomics and identification of biomarkers that can potentially predict a therapeutic response are essential to ensure better health for women with gynaecological cancer.<sup>81</sup> Rather than categorising cancers on the basis of the organs in which they originated, cancers can be categorised based on their molecular signature.<sup>82</sup> Considerable advances have already been made using this approach, with the additional involvement of The Cancer Genome Atlas Research Network (TCGA).<sup>81</sup> Nevertheless, there is still much to be investigated, and novel biomarkers are crucial to further tailor the diagnosis, risk assessment and treatment of patients. In this context, preliminary studies of BAG3 in gynaecologic cancers have shown interesting results. In particular, its targeted

downregulation might improve the response to cisplatin, paclitaxel<sup>36,37</sup> and olaparib<sup>62</sup> in ovarian cancer patients. On the other hand, given its ability to bind to p53 (and thus prevent p53 translocation to the nucleus),<sup>68</sup> BAG3 might be included in the TCGA group of endometrial carcinomas that have the worst prognosis (i.e. copy number high/p53-mutated group), which could therefore help in the management of patients with endometrial cancer.<sup>83,84</sup> Finally, in view of the biochemical interaction of BAG3 with the HPV oncoprotein E6, BAG3 might be involved in HPV-mediated cell transformation in cervical cancer pathogenesis, which raises the potential of future preventative strategies that target this protein, as well as its use in the prognostic assessment of precancerous cervical lesions. BAG3 might also have a role in the diagnosis of cervical SILs, as the levels and localisation of BAG3 expression appeared to correlate with the grade of dysplasia.<sup>69</sup>

The study of the BAG3 interactome in gynaecological cancer cells could lead to the identification of other proteins and mechanisms that have an as-yet-unrecognised role in these pathologies. Indeed, a study of the BAG3 interactome under proteostatic stress<sup>85</sup> has uncovered a broad spectrum of interacting proteins and biological processes in which BAG3 is involved, thus confirming the pivotal role of this protein in the cellular adaptation process in response to stress. Among such other interacting proteins identified is the SRC tyrosine kinase YES1. YES1 has been described as a prognostic factor in patients with epithelial ovarian cancer as its high cytoplasmic expression correlated significantly with a favourable prognosis and increased sensitivity to platinum-based chemotherapy.<sup>86</sup> Notably, in complex with HSP70, BAG3 has already been shown to interact with the Src-homology-3 (SH3) domain of SRC via its PxxP region, thus affecting the SRC signalling pathway as well as HSP70-mediated signalling in cancer cells.<sup>30</sup> On the other hand, it has been reported that in lung, esophageal and colorectal cancer cells, YES1 has an oncogenic role, and its gene amplification has been correlated to cancer resistance to EGFR or HER-2 inhibitors. Then, the possible BAG3-YES1 interaction let suppose a further interesting mechanism by which BAG3 overexpression can sustain cancer cell survival and resistance to therapy.<sup>87</sup>

Finally, although the involvement of intracellular BAG3 in the resistance of neoplastic cells to therapy has been investigated, this protein is now known also to be involved in promoting tumour progression through extracellular interactions with the tumour microenvironment. The extracellular role of BAG3 differs from its intracellular role and often involves the immune system. In this respect, BAG3 behaves like other proteins known as DAMPS (Damage-Associated Molecular Patterns) or alarmins, able to stimulate the immune response when released in the extracellular milieu by stressed, damaged or infected cells. For tumours, the response of immune cells, particularly Tumour-Associated Macrophages (TAMs), can paradoxically result advantageous for the tumour itself, as BAG3-activated TAMs release factors that support the survival and proliferation of cancer cells.

The BAG3-mediated circuitry is therefore likely to represent a mechanism by which tumours can stimulate the pro-tumour activity of cells of the tumour microenvironment. The ability of an anti-BAG3 antibody to sensitise an unresponsive tumour, such as PDAC, to therapy therefore indicates that blockade of BAG3 might be a successful in a combination approach with other anticancer treatments. Thus, BAG3 could constitute an innovative target for diagnostic, prognostic and therapeutic approaches, with research in the gynaecological field taking advantage of information and reagents that come from more advanced studies of BAG3 in pancreatic cancer,<sup>8,46,47,88–90</sup> melanomas<sup>9,10</sup> and other tumours.<sup>26,61</sup>

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## AUTHOR CONTRIBUTIONS

M.D.M., A.F., R.I., A.R., A.R., M.C.T. and L.M. acquired the literature and data, designed and wrote the paper. A.M., M.G., M.C. and V.N.U. contributed to the concept and design of the paper. M.D.M., G.G., F.D.C., M.C., M.C.T. and L.M. revised the paper and figures. All authors critically read and approved the manuscript being submitted.

## ADDITIONAL INFORMATION

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