REVIEW

BAG1L: a promising therapeutic target for androgen receptor-dependent prostate cancer

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Abstract

Androgens are important determinants of normal and malignant prostate growth. They function by binding to the C-terminal ligand-binding domain (LBD) of the androgen receptor (AR). All clinically approved AR-targeting antiandrogens for prostate cancer therapy function by competing with endogenous androgens. Despite initial robust responses to androgen deprivation therapy, nearly all patients with advanced prostate cancer relapse with lethal castration-resistant prostate cancer (CRPC). Progression to CRPC is associated with ongoing AR signaling, which in part, is due to the expression of constitutively active AR splice variants that contain the N-terminus of the receptor but lack the C-terminus. Currently, there are no approved therapies specifically targeting the AR N-terminus. Current pharmacologic targeting strategies for inhibiting the AR N-terminal region have proven difficult, due to its intrinsically unstructured nature and lack of enzymatic activity. An alternative approach is to target key molecules such as the cochaperone BAG1L that bind to and enhance the activity of the AR AF1. Here, we review recent literature that suggest Bag-1L is a promising target for AR-positive prostate cancer.

Introduction

BAG1L belongs to a family of evolutionary conserved proteins present in several species including yeast, plants and mammals. These proteins contain an approximately 110 amino acid sequence termed the BAG domain that defines the protein family. The first BAG protein gene, Bag1, was identified in a screen of interacting proteins of the anti-apoptotic protein, BCL2 (Takayama et al. 1995). Bag1-transfected 3T3 fibroblasts also exhibited prolonged cell survival in response to an apoptotic stimulus (Takayama et al. 1995). These properties contributed to the name BCL2-associated athanogene 1 (BAG1) (derived from the Greek for ‘against death’ (‘athánatos’)) member 1. BAG1 itself is made up of different isoforms translated from several stimuli, including staurosporine, anti-Fas antibody and cytolytic T cells, relative to cells that contained either BAG1 or BCL2 protein alone (Takayama et al. 1995). Bag1-transfected 3T3 fibroblasts also exhibited prolonged cell survival in response to an apoptotic stimulus (Takayama et al. 1995). These properties contributed to the name BCL2-associated athanogene 1 (BAG1) (derived from the Greek for ‘against death’ (‘athánatos’)) member 1. BAG1 itself is made up of different isoforms translated from a single mRNA by alternative translational initiation, resulting in similar carboxy-terminal sequences but different N-terminal sequences (Fig. 1). In humans, there

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- coehaperone
- signal transduction

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are four BAG1 isoforms (BAG1L, 1M, 1 and 1S), while only two isoforms are found in mouse (BAG1L and BAG1) (Takayama et al. 1998).

In addition to BAG1, there are numerous other BAG family members including BAG2, BAG3 (CAIR-1/Bis), BAG4(SODD) and BAG5 (Fig. 1). The characteristic feature of these proteins is the BAG domain that is made up of a three parallel alpha helical bundle (Takayama & Reed 2001). BAG5 contains five shorter BAG domains in tandem, but it is the 5th C-terminal BAG domain that is found to be functionally similar to the BAG domain of BAG1 (Arakawa et al. 2010). The BAG domain of BAG6 which was previously thought to be similar to the canonical BAG domain has recently been shown to be completely distinct (Kuwabara et al. 2015) and is therefore not included in Fig. 1. The BAG domain in the BAG family of proteins is highly conserved, while the remaining domains share no significant sequence homology. The BAG protein family affects diverse cellular behaviors including cell division, migration, differentiation and death (Takayama & Reed 2001, Kabbage & Dickman 2008).

In this review, we will focus on recent findings investigating the function of BAG1L and its critical interactions with molecular chaperones and nuclear receptors. For more detailed reviews of the other BAG family members, the reader is directed to the following reviews (Binici & Koch 2014, Behl 2016, De Marco et al. 2018).

Interaction with molecular chaperones

Structural biological approaches have further elucidated the functional domains and interactions of BAG proteins. Multidimensional NMR methods have been implemented to demonstrate the antiparallel three-helix bundle of the BAG domain. NMR chemical shift experiments marked surface residues on the second (alpha 2) and third (alpha 3) helices in the BAG domain that are involved in binding to the molecular chaperone Hsc70 (Briknarova et al. 2001). These structural predictions were confirmed by site-directed mutagenesis which resulted in loss of binding of the BAG domain to the Hsp70 cognate protein, Hsc70, in vitro, in yeast two-hybrid assay as well as in transfection experiments in Cos-7 cells (Briknarova et al. 2001).

Molecular docking of the BAG domain to Hsc70 and subsequent mutagenesis studies of Hsc70 have indicated that the molecular surface of the ATPase domain of Hsc70 is necessary for interaction with the BAG domain (Briknarova et al. 2001). The crystal structure of the BAG domain with Hsc70 has since been solved at a resolution of 1.9 angstroms and the three-helix bundle of the BAG domain was found to induce a conformational switch in the ATPase domain that is incompatible with nucleotide binding (Sondermann et al. 2001). The interaction of the BAG domain with the nucleotide-binding domain of Hsp70 is therefore thought to facilitate the release of ADP, accelerate the ATP cycle and activate the refolding activity of Hsp70. The same switch is observed in the bacterial Hsp70 homolog, DnaK, upon binding the nucleotide exchange factor, GrpE; as a result, the BAG1 protein was classified as a eukaryotic nucleotide exchange factor of Hsp70.

It is well documented that the BAG proteins regulate Hsp70 ATPase activity through their BAG domains. However, little is known about their other functions in Hsp70 activity or whether they contribute to Hsp70 client specificity. A mass spectrometry combined with quantitative high-throughput luminescence-based mammalian interactome (LUMIER) assay has been implemented to systematically characterize the chaperone–cochaperone–client interaction network in humans cells (Taipale et al. 2014). These results suggest diverse roles for the BAG proteins in cellular processes. BAG1 interacted with the E3 ligase listerin (LTN1) that is involved in ribosomal quality control of stalled polypeptides, a result that points to a role of BAG1 in ribosomal quality control (Fig. 2). These results have been supported by further interactome analysis of BAG1L that

Figure 1

Schematic diagram of the structure of the human BAG proteins. Shown are the BAG domain, the ubiquitin-like domain (UBL), the nuclear localization sequence (NLS), the WW domain, the IPV (Ile-Pro-Val) motifs and the PXXP sequence. The domains of the proteins are derived from UniProt. BAG6 which has recently been shown not to possess a canonical BAG domain (Kuwabara et al. 2015) has not been included in this figure.
showed that the BAG1 proteins have a role in proteome homeostasis and in protein folding (Cato et al. 2017). The LUMIER studies further showed association of BAG3 with heat shock proteins Hsp22/HSPB8, Hsp27/HAPB1, and with the master regulator of the heat shock response, HSF1 (Taipale et al. 2014). BAG4 interacted with three central components of the mRNA capping complex DCP1A, EDC3 and DDX6, while BAG5 interacted with the spindle checkpoint components Mad1/MAD1L1 and Mad2/MAD2L1 (Taipale et al. 2014). In contrast, BAG2 appeared to be a general cofactor of Hsp70 with little client protein preference. With the exception of BAG2, these studies suggest unique roles for each of the BAG proteins beyond just supportive function for Hsp70 (Taipale et al. 2014).

**Interaction with nuclear receptors**

In addition to the large-scale non-biased screens that identified substrates for BAG1, prior studies have hinted at the interaction of this protein family with nuclear receptors. The first report was the identification of a cDNA that coded for a protein with a molecular weight of 46 kD that interacted with the glucocorticoid receptor (GR) in a bacteriophage lambda expression library screen (Zeiner & Gehring 1995). This protein was shown to interact with both non-activated and ligand-activated nuclear receptors and was denoted as receptor-associated protein 46 (RAP46). RAP46 was later identified by sequence comparison to be BAG1M and was shown to inhibit the transactivation function of the GR (Kullmann et al. 1998). Several follow-up studies showed that members of the BAG1 family interacted with nuclear receptors but differentially regulated their activities (Table 1).

The human BAG1 proteins (BAG1L, 1M, 1 and 1S) are translated from a single mRNA by alternative translation initiation codons (Takayama et al. 1998, Yang et al. 1998). Synthesis of BAG1L occurs from an upstream CUG, whereas the other BAG1 proteins are initiated from the AUG codon (Takayama et al. 1998, Yang et al. 1998). The biological significance of non-AUG alternative initiation is thought to promote different subcellular localization and/or distinct biological functions of the isoforms translated from the single mRNA (Touriol et al. 2003). It is therefore not surprising that BAG1L, the largest member of the BAG1 protein family, possesses an N-terminal nuclear localization sequence (NLS), is localized to the nucleus and has functions that are different from the other isoforms (Takayama et al. 1998, Shatkina et al. 2003). The other BAG1 members are mainly cytoplasmic, although under stress conditions, BAG1M can also migrate to the nucleus (Zeiner et al. 1999). Translation of BAG1 is reported to be initiated by cap-dependent and cap-independent mechanisms leading to a different level of expression of this protein compared to the other isoforms (Coldwell et al. 2001).

Most of the findings on the effect of the BAG1 proteins on nuclear receptor action were generated in reporter gene assays where these proteins were overexpressed. Although this type of assay has been used to demonstrate the action of several transcription factors in gene regulatory networks, the results from such assays with cochaperones or chaperones should be viewed critically. Molecular
chaperones in general bind transiently to a wide range of substrate proteins (clients) and promote their folding, trafficking or degradation (Saibil 2013). Cochaperones bind to molecular chaperones and regulate the rate of their client release or recruitment of specific clients. Overexpressing cochaperones may trigger effects that do not reflect the true function of these proteins through indirect mechanisms that affect the activity of the chaperones. This complicates in vivo experiments using cell lines that overproduce the cochaperone proteins, as too much of the cochaperone may disturb its cellular distribution and normal functions. For example, the in vivo concentration of BAG1M varies in different tissues considerably but is generally sub-stoichiometric to the constitutively expressed Hsc70, which implies a positive regulatory effect on this chaperone (Takeyama et al. 1998, Kanelakis et al. 1999). Overexpression of BAG1M produced inhibitory effects on the transactivation function of the GR at higher levels but stimulatory effects at low levels (Knapp et al. 2014).

It is important to note that most of the functions assigned to the BAG1 proteins in regulation of nuclear receptor action could be traced to the BAG domain that regulates the chaperone activity of Hsp70 (Briknarova et al. 2001). As molecular chaperones, Hsp90 and Hsp70 play important roles in the maturation cycle of nuclear receptors (Cato et al. 2014); concordantly, most of the reported actions of BAG1 proteins on nuclear receptors may involve Hsc70. However, increasing evidence suggests that cochaperones may play more than a supportive role for the action of molecular chaperones. Some cochaperones possess intrinsic chaperone activity themselves, and others independently regulate cellular processes distinct from those of their chaperones (Taipale et al. 2014). It is therefore important, in the future, to distinguish between the chaperone-dependent and -independent functions of the BAG1 proteins in nuclear receptor action.

**BAG1L regulation of AR action**

The effect of the BAG1 family of cochaperones on nuclear receptor action has focused mainly on BAG1L. Initial studies showed the BAG domain to be important, although the unique N-terminal region of the BAG1L protein was also identified to be necessary (Froesch et al. 1998, Knee et al. 2001, Shatkina et al. 2003). These studies showed that the BAG domain contributed to the enhancement of the transactivation function of the AR possibly through the chaperoning action of Hsp70 at the AR LBD. Support for this came from experiments where the exchange of amino acid residues at the sites of interaction with the nucleotide-binding site of Hsp70 inhibited the BAG1L-mediated enhancement of the transactivation function of the AR (Briknarova et al. 2001).

Contrasting reports were made by Shatkina and colleagues who demonstrated that BAG1L bound and enhanced AR transactivation through the interaction of the BAG domain with the AR N-terminal tau 5 region (360–528 amino acids) (Shatkina et al. 2003). Solution NMR experiments showed that this interaction resulted in the reduction of resonance intensities within the C-terminal part of AR AF1, indicating that the interaction is transient (Cato et al. 2017). The residues in the AR tau 5 most affected by the BAG1L BAG domain interaction correspond to a region previously identified as partially folded in the intrinsically disordered AR N-terminus (De Mol et al. 2016). Moderate decreases in resonance

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**Table 1** Regulation of nuclear receptor action by BAG1 family of proteins.

<table>
<thead>
<tr>
<th>Cochaperone</th>
<th>Nuclear receptor</th>
<th>Hormone binding</th>
<th>DNA binding</th>
<th>Transactivation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAG1</td>
<td>RAR/RXR</td>
<td>Repressed</td>
<td>No effect</td>
<td>Repressed</td>
<td>Liu et al. (1998)</td>
</tr>
<tr>
<td>RAP46/</td>
<td>BAG1M</td>
<td>No effect</td>
<td>n.d.</td>
<td>No effect</td>
<td>Kullmann et al. (1998)</td>
</tr>
<tr>
<td>BAG1L</td>
<td>AR, ERα, ERβ, VDR</td>
<td>n.d.</td>
<td>Repressed</td>
<td>Repressed</td>
<td></td>
</tr>
<tr>
<td>BAG1L</td>
<td>GR</td>
<td>No effect</td>
<td></td>
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intensity were also observed in AR tau 1 (100–360 amino acids), in a region centered around residue 275, which has the propensity to adopt an extended conformation (Cato et al. 2017). This describes a protein structure defined by crystallographic, isotopic labeling NMR and transferred nuclear overhauser effect studies as an important element of secondary structure at the binding site of many peptide/protein complexes (Siligardi & Drake 1995). Thus, the binding of the BAG1L BAG domain to the AR tau 5 may affect protein–protein interactions such as the recruitment of cofactors to the AR AF1. The changes in resonance intensities observed in the NMR experiments with the BAG domain and the AR tau 5 protein were greatly reduced when a mutant Bag-1L with triple alanine substitutions in the BAG domain was used. This triple mutation, introduced into helices 1 and 2 of the BAG domain, impaired the binding of the BAG domain to the AR tau 5 and its transactivation function, but did not affect binding of the BAG domain to Hsp70/Hsc70 (Cato et al. 2017). The triple mutation differed from the previously described mutations in helices 2 and 3 that were identified as interaction sites of the BAG domain with Hsp70 that impaired AR activity (Briknarova et al. 2001). Combined, these results suggest BAG1L may use both Hsp70-dependent and -independent activities to regulate AR activity. Evidence that Hsp70 may be involved in BAG1L action at the AR N-terminus comes from surface plasmon resonance and functional studies that show that the BAG domain/AR tau 5 interaction is enhanced by the addition of Hsp70 (Shatkina et al. 2003). Recent studies have also provided corroborating evidence that Hsp70 can bind to a region encompassing the AR tau 5 to enhance AR action (Dong et al. 2019). Furthermore, small-molecule Hsp70 inhibitors, either alone or in synergy with enzalutamide, have been shown to inhibit the proliferation of 22Rv:1, a CRPC cell model (Dong et al. 2019).

**N- and C-terminal interaction**

Although the C-terminal BAG domain of BAG1L has been shown to be important for the regulation of AR action, BAG1M was found to be a weaker regulator of AR transactivation even though it possesses the conserved C-terminal BAG domain but lacks BAG1L N-terminal sequences. It was therefore thought that the absence of an NLS in BAG1M and the cytoplasmic localization of this protein was the cause for the weak regulatory effect on AR transactivation. Tagging BAG1M at its N-terminus with an NLS sequence from SV40 to generate a nuclear NLS–BAG1M fusion protein increased its ability to enhance AR activity but not quite to the level achieved by BAG1L (Shatkina et al. 2003). This suggested that N-terminal sequences of BAG1L other than the NLS somehow contributed to the action of this protein on the AR. These sequences were later found to be a duplication of the motif (GARRPR) that interacted with an allosteric region in the AR LBD termed binding factor 3 (BF3) (Jehle et al. 2014) (Fig. 3). The BF-3 is a surface site in the AR LBD identified by Estébanez-Perpiñá et al. (2007) and suggested to be important for co-regulator recruitment, AR transactivation and N-terminal/C-terminal interactions (Estébanez-Perpiñá et al. 2007, Grosdidier et al. 2012). The GARRPR motif is unique to BAG1L as it is not found on other AR coactivators such as the p160/SRC family of proteins or MED1 that binds to the N-terminus of the AR (Alen et al. 1999, Bevan et al. 1999, Christiaens et al. 2002, Jin et al. 2012). BAG1L therefore appears to bridge the N and C-terminal domains of the AR, interacting with the N-terminus of the AR through its BAG domain and with the C-terminal BF-3 of the AR through its N-terminal GARRPR motifs (Fig. 3). So far there is no link between BAG1L and other factors such as MAGE-A11 that modulate AR N/C Interaction and it appears BAG1L functions independently of such proteins (Liu et al. 2011, Minges et al. 2013).

The AR N to C-terminal link brought about by BAG1L occurs in addition to the ligand-induced interdomain interaction between the N-terminal 22FFXXLL27 motif and the C-terminal AF2 of the receptor (He et al. 1999, 2000).

**Figure 3**

A model depicting the putative mode of action of BAG1L in the regulation of AR activity. BAG1L contributes to the N/C interaction of the AR. It uses its N-terminal GARRPR motifs to bind to the BF3 pocket at the AR LBD and its BAG domain (BD) to bind to the tau 5 region at the N-terminus of the AR. The interaction with AR tau 5 and the ensuing conformational changes brought to the AR N-terminus by BAG1L may involve the chaperoning activity of Hsp70/Hsc70. Thus, BAG1L functions as an adaptor protein for the N/C-terminal interaction of the receptor. Its interaction with BF3 possibly impacts the allosteric regulation of the action of the AF2 pocket by BF3. The AF2 pocket is bound by the FXXLF motif at the N-terminus in an intramolecular interaction that is needed for the recruitment of LXXLL bearing coactivators and other coactivators to the AR to potentiate its activity.

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**References**

- Alen et al. 1999
- Bevan et al. 1999
- Christiaens et al. 2002
- Jin et al. 2012
- Liu et al. 2011
- Minges et al. 2013
- He et al. 1999, 2000
- Shatkina et al. 2003
- Cato et al. 2017
- Siligardi & Drake 1995
- Dong et al. 2019
- Briknarova et al. 2001
- BAG1L and prostate cancer
- Estébanez-Perpiñá et al. 2007
- Grosdidier et al. 2012
- Bag-1L
This interdomain interaction is thought to provide the AR with the correct conformation for the recruitment of coactivators for its activity but Fluorescence Resonance Energy Transfer (FRET) experiments in cells lacking BAG1L have shown that BAG1L also contributes to the conformation of the receptor (Cato et al. 2017). It therefore appears that the AR interdomain interaction and the N/C interaction mediated by BAG1L are both required for the optimal activity of the AR. Future experiments will be needed to determine the extent to which BAG1L contributes to the conformation of the AR and recruitment of coactivators as opposed to the AR interdomain interaction.

The AR interdomain interaction requires the FXXLF motif at the N-terminal amino acids 23–27 and the C-terminal AF-2, while the AR N/C-terminal interaction mediated by BAG1L requires the N-terminal tau 5 sequence (amino acids 360–528) and the BF-3 region at the C-terminus of the AR (Shatkina et al. 2003, Jehle et al. 2014, Cato et al. 2017). Intriguingly, the AR tau 5 region contains WXXLF a homolog of FXXLF that has been implicated in the N/C-terminal interaction of the AR (He et al. 2000). A possibility exists that BAG1L binding to the AR tau 5 somehow affects the folding of this sequence to generate an additional anchor for the N/C-interaction. Although the binding of the BAG domain of BAG1L to the AR N-terminus and the proposed AR N/C interaction brought about by BAG1L (Fig. 3) is an attractive model, other actions of the BAG domain cannot be ruled out. The regulation of the chaperone activity of Hsc70 by the BAG domain and the involvement of Hsc70 and Hsp90 in maintaining the AR ligand-binding pocket suggests additional action of the BAG domain in regulating AR AF2 activity. This additional potential regulatory action of BAG1L has not been included in the model (Fig. 3) as it has not yet been experimentally tested.

BAG1L and prostate cancer

The regulation of AR activity by BAG1L and the driver role of the AR in prostate cancer progression has generated an interest in the action of BAG1L in prostate cancer. A link between BAG1L and prostate cancer was first documented by Shatkina and colleagues. They reported that while BAG1L was expressed in the basal cells of benign prostate tissue and in the secretory epithelium of tumor samples, the AR was expressed in the epithelium of both benign and tumor cells. They therefore postulated that BAG1L and the AR would be expressed in the same cells only in prostate tumors where the enhancing effect of BAG1L on AR action would occur (Shatkina et al. 2003). Later immunohistochemical studies reported an increased expression of nuclear BAG1 (BAG1L) in hormone refractory (n = 34) compared to localized untreated tumors (n = 58) (P < 0.0001) (Krajewska et al. 2006). In 64 early-stage patients treated with external-beam irradiation, cytosolic BAG1 (BAG1M, BAG1 and BAG1S) levels correlated with higher pretreatment levels of serum prostate specific antigen and a shorter time to disease progression (Krajewska et al. 2006). Moreover, elevated cytosolic BAG1L protein levels were found to be more common in men who subsequently developed metastatic disease in a study where the patients were uniformly treated with external-beam irradiation (Krajewska et al. 2006). Altogether, these studies show that increases in both the levels of cytosolic and nuclear BAG1 correlate with aggressive variants of prostate cancer. Although the cytosolic BAG1 proteins do not play a prominent role in androgen-dependent prostate cancer, they nonetheless regulate prostate cancer progression via other signaling pathways. Prior mechanistic studies have shown that the cytoplasmic BAG1 binds RAF1 and activates the MAP kinase pathway independent of the AR (Fig. 4) (Wang et al. 1996, Song et al. 2001). It is therefore likely that this represents a mechanism whereby the cytoplasmic BAG1 proteins contribute to prostate cancer cell proliferation.

Figure 4

BAG1 actions in prostate cancer. (A) AR and HSPs are bound in the cytosol and upon androgen binding, AR dimerizes and translocates to the nucleus. Upon AR nuclear translocation, AR/BAG1L complexes form, binding chromatin and recruiting additional cofactors to activate transcription of AR target genes that ultimately lead to increased PSA, prostate cancer cell proliferation and survival. AR synthesis inhibitors, AR antagonists and cochaperone inhibitors may be used to inhibit this essential pathway in prostate cancer cells. (B) Cytoplasmic BAG1 binds RAF1 and enhances RAF1 activity, leading to increased activation of MAPK/ERK pathways and stimulating prostate cancer cell proliferation.
In other immunohistochemical studies, Maki and collaborators came to a similar conclusion as Krajewksa et al. (2006) on the contribution of nuclear BAG1 (BAG1L) to prostate cancer. They reported that BAG1L protein expression was significantly higher in hormone refractory than in primary tumors (Maki et al. 2007). Their work on BAG1 mRNA expression analyzed by qRT-PCR did not show any significant increase in BAG1L expression in hormone refractory prostate tumors compared to benign prostate hyperplasia or untreated primary tumors. Since the BAG1 isoforms are translated from the same transcript (Maki et al. 2007), there was no way of designing assays that would measure the contribution of the different BAG1 isoforms at the mRNA level. The qRT-PCR results therefore support the conclusion that the increased expression of the BAG1 proteins in prostate cancer progression occurs at the translational level. Future studies on the translational control of BAG1L expression from the non-cannonical CUG codon would provide further insight into BAG1L-mediated regulation of AR action in prostate cancer.

In the same study, Maki and colleagues also reported BAG1 gene amplification in 6 of 81 (7.4%) hormone refractory clinical tumors, whereas no amplification was found in 130 untreated tumors (Maki et al. 2007). More recent studies in matched hormone-sensitive and metastatic CRPC confirmed earlier studies and showed that nuclear BAG1 (BAG1L) expression correlated with prostate cancer progression. It was additionally reported that nuclear BAG1 levels in hormone-sensitive prostate cancer was associated with a reduced clinical benefit from abiraterone (Cato et al. 2017).

**BAG1L and CRPC**

Although early-stage prostate cancer is often curable by surgery or radiation therapy, androgen deprivation or antiandrogen treatment with or without docetaxel chemotherapy remains a therapy option for patients with metastatic prostate cancer or with prostate cancer recurrence after surgery or radiation therapy. Most patients receiving androgen deprivation therapy progress to metastatic castration-resistant prostate cancer (mCRPC) (Galletti et al. 2017). The mechanisms involved in the transition to mCRPC are many, but there is considerable evidence that activation of the AR is the main contributor at least early in this stage of the disease (Taplin et al. 2003) and that AR continues to be expressed in over 80% of locally advanced CRPC (van der Kwast et al. 1991, Hobisch et al. 1995, Visakorpi et al. 1995).

Progression to CRPC with ongoing AR signaling, is due, in part, to the expression of constitutively active splice variants (AR-SVs). Over the past decade, AR-SV that lack the LBD to which the androgen ablation therapies are targeted have been discovered in prostate cancer cell lines, human xenografts and clinical prostate cancer specimens. All the AR-SVs share in common an N-terminal and DNA-binding domain but lack the C-terminal LBD of the receptor (Ware et al. 2014). High AR-SV expression in bone metastasis has been associated with a shorter time to death in men with mCRPC (Hörnberg et al. 2011) and a high AR-SV, on the whole, is associated with disease recurrence (Guo et al. 2009). Thus, AR-SVs are important at the aggressive stage of the tumor especially during androgen deprivation. Of the different AR-SV identified, AR-V7 is the best studied. The conclusion that AR-V7 plays a major role in CRPC biology is supported by the following findings: (1) AR-V7 increases as patients progress from untreated hormone-sensitive prostate cancer (HSPC) to CRPC; (2) AR-V7 increases with emerging treatment resistance to abiraterone and enzalutamide; (3) AR-V7 is associated with poor overall survival of patients with CRPC (Ware et al. 2014). Currently, there are no approved therapies targeting AR-V7 specifically, and this remains a critically important, unmet medical need. Recent findings show that ARV7 heterodimerizes with full-length AR (ARfl) and contributes to CRPC by repressing the transcription of genes with tumor suppressive activity (Cato et al. 2019).

Against this background, recent attempts have been made to directly target AR AF1 and ARV7. Drugs such as EPI-001 and its derivatives bind to the AR N-terminus to inhibit prostate cancer growth in xenograft mouse models (Andersen et al. 2010, Myung et al. 2013, De Mol et al. 2016). However, a phase 1/2 clinical trial (NCT02606123) investigating the use of EPI in men with mCRPC who had progressed on enzalutamide or abiraterone was terminated early due to high pill burden. Another class of drugs that directly target the AR N-terminus are niphatenones. These drugs were shown to inhibit transactivation by ARfl and AR variants, but they also functioned as alkylating agents and are therefore unsuitable for prostate cancer therapy (Banuelos et al. 2014). An alternative approach to impede the activity of ARfl and the splice variants is to inhibit their interaction with coactivators, many of which are highly expressed in CRPC. Cofactors such as Vav3 and the p160 coactivators have been reported to bind the AR N-terminal AF1 and to control ARfl and ARV7 activities (Nakka et al. 2013, Magani et al. 2017). However, it is unclear what enables the interaction of these coactivators with the intrinsically disordered N-terminal...
Inhibitors of BAG1

In cell culture experiments, overexpression of BAG1L impaired the effect of the antiandrogen, cyproterone acetate, to inhibit the transactivation function of the AR, suggesting that reducing or inhibiting the level of this cochaperone may improve antiandrogen action (Froesch et al. 1998). Alternatively, blocking the action of BAG1L may be a way of targeting androgen-dependent prostate cancer.

Candidate inhibitors of AR activity targeting the BP-3 region of the receptor have been found to impair binding of the GARRPR motifs of BAG1L to the AR LBD, suggesting that the BAG1L–AR-binding interface in the LBD of the receptor may function as an important site for modulating AR action (Jehle et al. 2014, Munuganti et al. 2014). Thio-2, an N-ethyl-4-(6-methyl-1,3-benzothiazol-2-yl)aniline compound reported to inhibit the interaction of the BAG domain of BAG1 and Hsp70 (Enthammer et al. 2013), has recently been used as a tool compound to block BAG1L-mediated enhancement of AR tau 5 activity in a mammalian-1-hyrid assay (Cato et al. 2017). This compound also inhibited binding of BAG1L to the AR in a coimmunoprecipitation experiment and inhibited LNCap proliferation albeit at with an IC₅₀ 17.5µM (Cato et al. 2017). While encouraging, the high IC₅₀ value of Thio-2 and the lack of clear genetic evidence of its fully on-target activity suggest the need for caution in concluding that compounds interfering with the BAG1L/AR tau 5 interaction could be effective in inhibiting prostate cancer cell proliferation. Nonetheless, compounds that potently and specifically bind the BAG domain and inhibit AR function are worthy of further development and will allow this hypothesis to be rigorously tested.

One possible way of targeting the BAG domain AR interaction is to use small molecules that disrupt the Hsp70/BAG1 interaction. Studies on the crystal structure of the BAG domain and Hsc70 nucleotide-binding domain suggest that BAG1 has a large and significant effect on the conformation of the adenine-binding pocket, and would strongly affect the affinity of both nucleotide and non-nucleotide ligands that bind at that site (Sondermann et al. 2001). However, other experimental evidence suggests that the ability of the BAG domain to promote nucleotide exchange is rather through disruption of the phosphate-binding pocket of the nucleotide-binding domain (Gassler et al. 2001). Evans et al. (2017) demonstrated in experiments with high-affinity non-nucleotide fluorescence polarization probes that the BAG domain promotes nucleotide exchange through conformational changes of the phosphate rather than the adenosine-binding pocket. They suggested small-molecule non-nucleotide ligands as likely inhibitors of BAG domain-Hsc70 interaction. Whether such inhibitors will also disrupt the BAG domain AR tau 5 interaction is uncertain. The finding that the BAG domain uses non-Hsp70-binding sites to interact with the AR tau 5 (Cato et al. 2017) makes it questionable whether classical BAG domain-Hsp70 inhibitors would be efficacious in blocking BAG1L–AR tau 5 action. Furthermore, as sequences on BAG1L outside the BAG domain also play a role in the regulation of AR action, a putative BAG1L inhibitor would as well need to block the activity of those other interacting domains of BAG1L.

Targeting BAG1L

An important question that needs to be answered on the inhibition of BAG1L action is what the likely consequences would be giving its role as an Hsc70 cochaperone and its many substrates including several nuclear receptors. Knockout experiments in whole organism may provide answers to this question. Previous knockout of the two BAG1 isoforms (BAG1L and BAG1) in the mouse reported that Bag1 is an essential gene and that Bag1-knockout mice die between embryonic days 12.5 and 13.5 (E12.5 and E13.5) from massive apoptosis in cells of the fetal liver and developing nervous system (Götz et al. 2005). These studies need to be re-examined in light of recent findings that question the results of these knockout experiments. The Bag1 gene is separated by 414 bp from Chmp5, a gene that codes for charged multivesicular body protein 5 and the two genes are on different DNA strands with their 5’ ends positioned in a head-to-head orientation (Shim et al. 2006). The targeted disruption of Bag1 resulted in a double knockout, ablating the expression of both Bag1 and Chmp5 (Neeb A, Cato L and Cato ACB – unpublished data). The Chmp5 knockout, like the published Bag1 knockout, is embryonic lethal (Shim et al. 2006). It is therefore possible that the reported lethality of mouse BAG1 does not result only from the knockout of the Bag1 gene but of the two genes. A new strategy for Bag1-knockout mice (Bag1tm1R296Eucomm[Hmg1]lcs) has been generated by the
European conditional mouse mutagenesis (EUCOMM) program, and the knockout mice are not embryonic lethal. A full characterization of these mice is currently underway. Should these studies confirm that BAG1 is not essential, therapeutic approaches aimed at inhibiting the action on BAG1 may be less likely to exhibit toxicity.

Given the many proteins with a canonical BAG domain and with the ability to serve as nucleotide exchange factors of Hsc70, it is likely that other BAG proteins may interact with the AR and compete with BAG1L for binding and regulating the action of the receptor. Although BAG3 and BAG5 have been implicated in prostate cancer progression (Ammirante et al. 2011, Bruchmann et al. 2013), no interaction of these BAG proteins with the AR has been established. For now, the interaction and regulation of AR activity by the BAG protein family remains a unique feature of BAG1L.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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