Mini-review

Unveiling the potential of prohibitin in cancer

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ABSTRACT

Recently, research has shed new light on the role of Prohibitin (PHB) in cancer pathogenesis across an array of cancer types. Important mechanisms for PHB have been unveiled in several cancers, especially with regard to the androgen independent state of prostate cancer (PC) and oestrogen dependent breast cancer. However, PHB is often overlooked due to its complex but subtle roles within the cell. Having gathered both historical and current research exploring PHB's role in different cancer types including prostate and breast, here we aim to pair this information with its molecular properties in the hope of translating this information into a clinical perspective, thus discussing its possible use in future cancer therapy.

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Introduction

Ubiquitously expressed prohibitin (PHB), also known as B-cell-receptor-associated protein 32 (BAP 32), along with its homologue PHB2 (BAP 37) are evolutionarily conserved proteins expressed in an array of eukaryotic organisms [1]. Indeed the PHB sequence is highly conserved across species as evidenced by mouse and rat *PHB* protein-coding sequences only differing from the human sequence by one amino acid [2].

The *PHB* gene, first identified in the 1980s, is located on chromosome 17q21 and consists of two alleles. One allele expresses an exon 6 associated single strand conformation polymorphism (SSCP) identified by cleavage at a polymorphic intronic E*co*RI site. The second allele however, is not cleaved in a similar manner [3]. *PHB* mRNA transcribes for the PHB protein that has a molecular weight of 32 kDa and comprises of 272 amino acid residues. PHB belongs to a family of proteins which have an evolutionary conserved prohibitin-like domain, otherwise known as band-7 family of proteins, along with a transmembrane domain at the N-terminus and a coiled-coil domain at the C terminus [3] (Fig. 1). The structure of the PHB protein lacks motifs characteristic for signal transduction, nuclear localisation, ATP binding sites or transcriptional factors [3]. McClung's group initially observed PHB exhibiting an anti-proliferative function and thus acknowledged it as a protein with tumour suppressor characteristics [3]. The cellular functions of PHB have since been recognised in aging, inflammation and obesity,

however, the molecular functions of PHB in carcinogenesis are still to be fully clarified [5].

Cellular location

Mitochondrial PHB

In mammalian cells PHB is considered a mitochondrial marker as this is where staining is most prominent. The PHB protein complex found within the mitochondria embodies two subunits, namely PHB1 and PHB2, which physically associate with one another and share more than 50% identical amino acid homology [1,5,6]. The C-terminal coiled co-domain is responsible for the interaction of the two subunits, PHB1 and PHB2, which together form heterodimers [7]. These heterodimers organise into ring like structures which establishes the integrity of the mitochondrial structure and regulates mitochondrial function [3]. PHB1 and PHB2 affix into the inner membrane of the mitochondria via hydrophobic stretches at the N-terminal [5]. Upon deletion of the *PHB2* gene, *PHB1* is also reduced suggesting that they are mutually dependent on one another. The
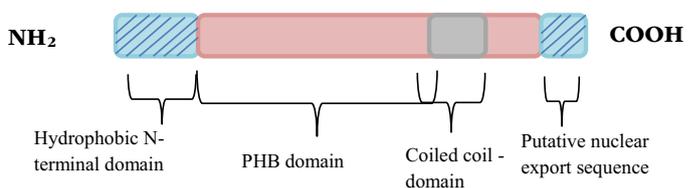

Fig. 1. Image displaying schematic representation of the human PHB gene. NH2 = N terminal, COOH = C terminal, adapted from [4].

mitochondrial role of PHB was first seen in a *C. elegans* model, where loss of PHB resulted in fragmentation and disorganisation of the mitochondria in comparison to control mitochondria, which appeared to be elongated and well structured. The discovery that it was loss of PHB causing the disruption of the mitochondrial structure, was confirmed by the observation that loss of PHB2 resulted in deterioration of optic atrophy 1 (OPA1) [4]. OPA1 has a specific function in governing the formation of mitochondrial cristae and resides with the inner mitochondrial membrane. The interaction between PHB and OPA1 was exhibited by PHB depleted mouse embryonic fibroblasts (MEF) cells showing a highly similar fragmentation pattern which was also seen in OPA1 down-regulated MEF cells [4].

PHB can also partake within the dynamics of the mitochondria. For example, stomatin-like protein (SLP-2) can form a subunit with PHB within the inner mitochondrial membrane to form a transmembrane protein [8,9]. Upon depletion of SLP-2 in HeLa cells, proteolysis of PHB1 and PHB2 ensued, suggesting PHB stability is dependent upon SLP-2 during mitochondrial stress [8], which is of particular relevance as SLP-2 holds importance in the biogenesis and activity of the mitochondria [10].

In a yeast model, PHB1 transports into the mitochondria, combines with Tim8/13 complexes allowing for the biogenesis of transmembrane proteins within the intermembrane space of the mitochondria [8]. There has also been evidence that PHB functions as a chaperone for newly made proteins which form parts of the mitochondrial complex 1 [11]. PHB can also act as a scaffold, enlisting membrane proteins into a lipid environment, essential for mitochondrial morphogenesis [4].

The localisation of PHB within the mitochondrial membrane may hold importance in preventing apoptosis in yeast and mammalian cells, against metabolic stress [12]. Accumulating evidence suggests that PHB1 also plays a role in preventing oxidative stress in an array of cell lines. For example, oxidative stress occurring in the intestinal epithelial cells results in a drop in PHB1 levels a trend also observed in *ex vivo* lung tissue undergoing hypoxia [13]. Furthermore, knockdown of PHB1 in endothelial cells resulted in mitochondria produced reactive oxidative species (ROS) due to a blockade of the electron transport chain [14]. Conversely, over-expression of PHB1 in cardiomyocytes served as a protective mechanism against hydrogen peroxide induced injury and maintained the structure mitochondrial membrane [2].

Nuclear localisation of PHB

The roles and location of PHB have been extensively defined in the mitochondria, however, recent evidence dependent on cell types, also exhibits a role for PHB located within the nucleus [2].

A study showed that PHB was localised to the nucleus and cytoplasm of LNCaP prostate cancer cells [8]. Moreover, PHB was consistently expressed in the nucleus of these cultured human prostate cancer cells, interacting with E2F transcription factors – suppressing their function and causing the recruitment of HDAC-1 and N-CoR [8]. Further, data from this study suggested that in transformed cells PHB translocated from the nucleus towards the mitochondria in response to apoptotic signalling [8]. Export of PHB out of the nucleus is complex as it is usually bound to other proteins such as retinoblastoma protein (Rb) and is therefore unable to undergo passive diffusion through the nuclear membrane. Instead PHB exports from the nucleus via short amino acid stretches known as nuclear export signals [15]. This well-defined nuclear export signal, similar to the most common type of export sequence seen in the HIV virus and type 1 rev protein, has a core of large hydrophobic amino acids such as leucine which are recognised by the CRM-1 export receptor [15,16]. This receptor aids in the nuclear transport of PHB, as the removal of nuclear export signals inhibits the transport of PHB out of the nucleus. PHB was also shown to localise in the nucleus of breast cancer cells but re-localises to the cytoplasm in camptothecin-treated cancer cells [17]. This also indicates that PHB undergoes transport out of the nucleus upon receiving apoptotic or stress signalling. This transportation of PHB out of the nucleus in response to camptothecin appeared to eliminate its interaction with the E2F1 transcription factor in the nucleus [17].

The *PHB* gene is differentially expressed in the testes and ovaries. Within the rat ovary, PHB is localised to the granulosa cells and oocyte cells. Interestingly, PHB studies in rat ovaries revealed that PHB translocates from the cytoplasm into the nucleus in atretic follicles, germinal vesicle-stage oocytes, zygotes and blastocytes [18] suggesting PHB has a regulatory role in the nucleus of theca-interstitial cells in the ovum throughout follicular maturation. Observed PHB expression within these cell types was ample when compared to proliferating-cell nuclear antigen (PCNA) expression during follicular maturation, suggesting a positive correlation with anti-proliferating cells and PHB expression [19]. Similarly, within rat testes, expression of PHB was not noted in actively dividing spermocytes [19]. This could be linked to PHB interacting with steroid receptors such as the androgen and oestrogen receptor.

The ability of PHB to translocate to various subcellular locations in response to different signals, along with regulating E2Fs and P53 raises the idea that depending on the environment it is placed in, it can either promote apoptosis or proliferation, a property that is rather unique.

Membrane bound PHB

Enrichment of PHB has been observed within the lipid rafts of the plasma membrane. Further evidence has also demonstrated the shedding of PHB into the circulation by cancerous colon cells [20]. In addition, PHB has also been shown to be involved in the Raf signalling pathway altering epithelial cell adhesion and migration. The direct interaction of PHB with C-Raf is needed for the localisation and phosphorylation of C-Raf at serine 338 at the plasma membrane [20]. The localisation of PHB to the plasma membrane has also been shown to have an immunology link as evidence suggests it associates with the IgM receptor [21].

PHB and cell cycle machinery components

There is a complex combination of factors and proteins which regulate the progression of the mammalian cell cycle. Members of the Rb family and their downstream targets such as members of the E2F family are examples of proteins which are heavily involved in the progression of the cell cycle [22]. Mis-regulation of these proteins has been influenced in the onset of multiple cancer types [23] and therefore it is interesting to note how PHB interacts with members of the E2F family to suppress their activity. Rb and its family members; p107 and p130 repress the G1/S phase transition, however cyclin D phosphorylates Rb, allowing the transition to the S phase to take place [22]. Studies showed that PHB can bind to all three family members of the Rb family with robust efficiency [24] even though PHB lacks the canonical LXCXE motif which most Rb binding proteins have. However, unlike other Rb binding proteins such as cyclin D, PHB does not have a negative effect on Rb's function, instead it heightens its function much like Brg1 protein. Wang et al. also found that PHB can bind to the E2F1-5 family members unlike Rb which can only bind to E2F1-,3 [24] thus suggesting that PHB binds to a domain which is common amongst all the family members of the E2Fs. It was also shown that PHB could suppress the transcriptional activity of E2F1 in T47D cells [24]. There is also evidence to suggest that there are potential mechanisms independent of Rb which allows the suppression of E2Fs by PHB. Interestingly, the suppression of E2Fs via PHB cannot be reversed when the adenovirus E1A protein is added, however this is the case
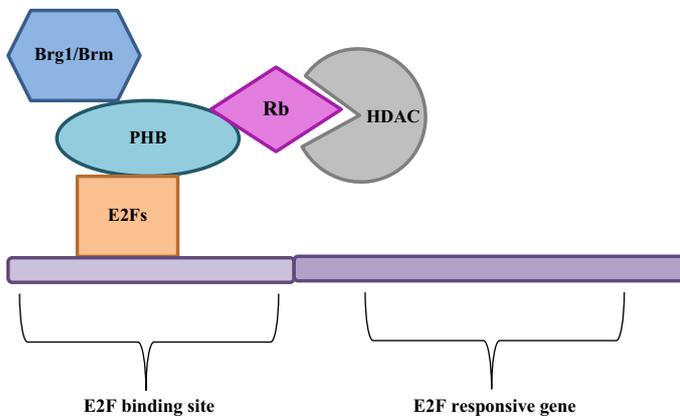
Fig. 2. Schematic representation of where PHB binds to E2Fs and Rb, causing the recruitment of HDACs. Image adapted from [12].

with regard to Rb's suppression of E2Fs [24]. E1A can disrupt the interaction of Rb and E2Fs in the activation domain, allowing E2F to be uninhibited. It is likely that PHB prevents E1A from carrying out this function and thus suppression of E2Fs is enabled even in the presence of E1A. Overall, there is an established relationship between PHB, Rb, E2Fs and cell proliferation [24] based upon the evidence that PHB lacking a Rb binding domain cannot suppress E2Fs nor cellular proliferation. A statement which can be considered accurate as E2Fs heavily influences proliferation [24]. Specifically, E2F1 inhibition via PHB occurs through a putative coiled-coil domain, and it is this region alone that could hinder the transcriptional activity of E2F1.

Further studies on PHB could unveil mechanisms associating PHB and the regulation of the cell cycle (Fig. 2) which is vital in uncovering information regarding the initiation of cancer.

Multiple roles and associations of the PHB protein in diseases and cancer

PHB is known to interact with proteins that regulate cell cycle progression as well as hinder DNA replication in numerous cell types. PHB also interacts with several transcription factors that have integral roles in diseases and carcinogenesis. The remainder of this review will predominantly focus on the role PHB has in prostate cancer (PC) and breast cancer.

PHB, the androgen receptor and PC

PC is a heterogeneous disease initially driven by the androgen receptor (AR). Circulating androgens cause the activation of the AR leading to the transcription of androgen responsive genes [25]. Therapies targeted against the AR e.g. anti-androgens are initially successful. However, as the disease progresses it becomes refractory to hormonal interventions and is therefore classified as androgen independent which currently remains an incurable stage of the disease. Often however, PC is still driven by the AR even in the apparent absence of androgens, leading to 'apparent androgen independent disease'. Hence, there is a definite need to establish mechanisms underpinning androgen independent PC (AIPC), and why treatment options often fail [26,27].

PHB overexpression represses AR induced gene activation, and suppresses tumour growth of AR-dependent LNCaP xenografts. In the absence of PHB, or in cells with lowered PHB expression levels, prostate cancer cells have been shown to become sensitive to low levels of androgens and indeed to weaker adrenal androgens [28]. PHB loss may therefore contribute to AIPC by reducing the threshold
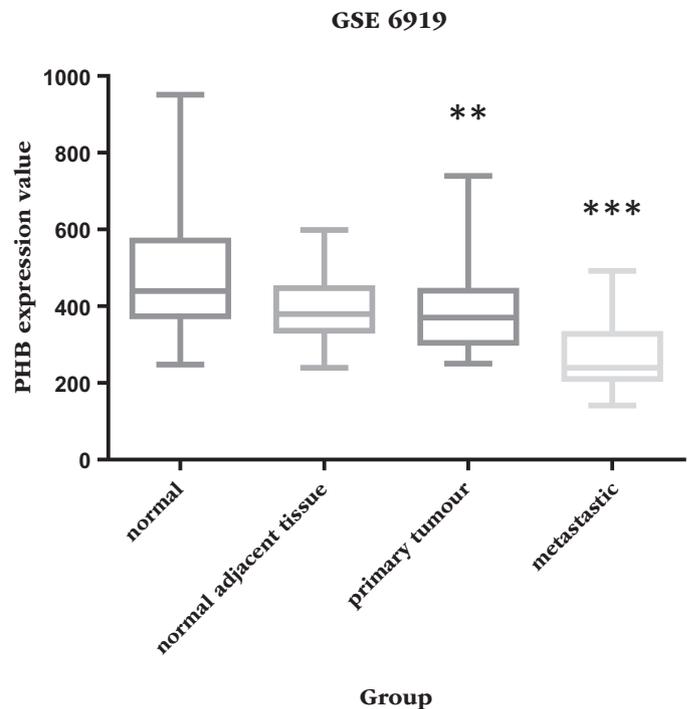

Fig. 3. Geodatabase analysis. Expression analysis of PHB levels between normal tissue, PCa tissue, primary tumour and metastatic tissue. **p value <0.001, ***p value <0.0001.

in which PC cells may respond to low levels of androgens or weak androgen-like hormones [28].

A study carried out a comprehensive gene expression analysis of 152 human samples divided into normal tissue, PCa tissue, normal adjacent tissue and metastatic tissue. Analysis was carried out using the Affymetrix U95a, U95b and U95c chip sets. Results are freely published as a Geodatabase (GSE6919) [28,29]. Fig. 3 above ** represents analysis of the PHB gene across PC tissue samples. A one-way ANOVA test was carried out and significance was measured against the normal tissue.

The interactions between AR and PHB are complex. Firstly, it was noted that PHB protein levels were diminished by 50% in LNCaP lysates following androgen stimulation for 16 hours [12]. A similar trend (30% decrease) was seen in the metastatic and androgen independent cell line PC-3 which had been stably transfected with the androgen receptor (AR) [12]. To further demonstrate the interaction of the AR signalling pathway and PHB, a study highlighted that a decrease in PHB levels either via androgen stimulation or siRNA mediated knock down, triggered enhanced growth of xenografts in mice, due to increased AR activity [30]. An alternative study [31] used proteomic analysis to identify significant changes in protein expression of 23 needle biopsies from patients considered high risk for PC. This confirmed that PHB was highly elevated in PC, as opposed to benign prostatic hyperplasia (BPH). The elevated PHB expression was also noted at an mRNA level [26].

The relationship between androgen stimulation and PHB was further established when increasing concentrations of Dihydrotestosterone (DHT), up to 100 nM, caused complete repression of PHB protein expression on a Western Blot [12]. Stimulation of PHB overexpressing LNCaP cells with DHT failed to enter the cell cycle with 97% of the cell population remaining in the G1 state, compared to 80% of the non-transfected cells entering the cell cycle [12]. Furthermore, the importance of PHB down-regulation has also been assessed. Small inhibitory RNA (si-RNA) oligos complementary to either exon 1 of PHB or to the 3'UTR of PHB were designed and stably transfected into LNCaP cells. FACs analysis of cell cycle entry
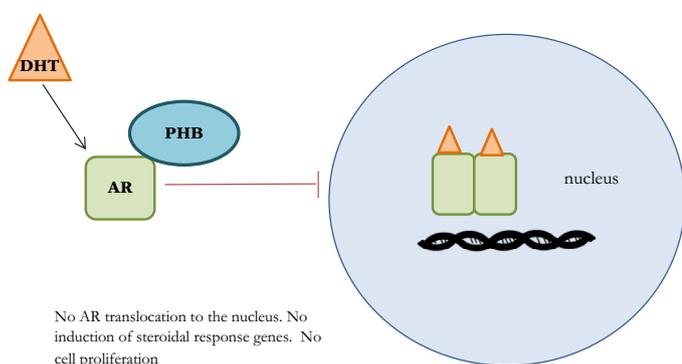
Fig. 4. Schematic representation of PHB repressing the translocation of the AR-DHT complex towards the nucleus, thus transcription of androgen responsive genes is halted. Image adapted from [1].

highlighted that cells containing PHB si-RNA had an increase in cell population entering the S/G$_2$ M phase. Specifically 2% of these cells were seen in this phase when compared to control cells, which increased to 10% after DHT stimulation [27].

Thus, there is a wealth of evidence to say that PHB has a major role in the onset of PC, especially in terms of the initiation of androgen independent tumours [32]. However, how the tumour acquires a decrease in PHB levels via androgen stimulation still needs to be further elucidated. Moreover, there may also be other mechanisms that completely bypass the AR signalling pathway, causing a down-regulation of PHB, that also needs to be fully understood. Fig. 4 describes the current known mechanism involving the AR and PHB.

Interactions of PHB, anti-androgens and the AR

As the AR signalling pathway is central to the initiation and development of PC, the main therapy available is the administration of androgen antagonists [33]. Interestingly, PHB was shown to enhance the efficiency of androgen antagonists. Androgen antagonists were shown to induce the recruitment of PHB and the ATP dependent helicase BRG1 to androgen responsive promoters, inhibiting the translocation of the AR, and thus increasing the efficiency of antagonist-mediated growth inhibition of prostate cancer cells [34]. Moreover, the recruitment of BRG1 to the *PSA* promoter was dependent on PHB, suggesting that prohibitin suppresses AR dependent transcription via a mechanism involving BRG-1 mediated chromatin remodelling in the presence of androgen antagonists [34].

PHB, the ER and breast cancer

More than 70% of primary breast cancers are initiated by the oestrogen receptor (ERα). The activation of the ERα by oestrogen intensely increases the proliferation and metastatic potential of breast cancer cells [35]. With breast cancer remaining the most common cancer in women worldwide [36], and with countries which previously had low breast cancer incidence showing increases, it is essential to note the role PHB has in this cancer.

Tamoxifen inhibits the interaction between oestrogen and ERα and there is evidence demonstrating that PHB improves the efficacy of standard treatment for ERα positive patients. This could be through the PHB homologue PHB2, which can bind to ERα repressing its transactivation to the nucleus, thus enhancing the effectiveness of tamoxifen bound ERα [37]. Within the same study, PHB was also identified as an ERα co-repressor in several other cell lines including the monkey kidney CV1 and cervical cancer HeLa cells suggesting PHB's role is not cell type specific [37]. A regulator of the oestrogen/ERα signalling pathway known as BIG3 was thought to co-localise

with PHB2 in the cytoplasm rendering PHB2 unable to repress ERα transcriptional ability, thus potentially contributing to tamoxifen resistance in ERα positive breast cancer [35]. To overcome BIG3 repressing PHB2, a dominant negative peptide named ERAP has been identified to competitively bind to PHB2, preventing its interaction with BIG3 thus allowing PHB2 to suppress ERα activity, leading to complete suppression of ERα positive breast cancer cell proliferation both *in vivo* and *in vitro* and thus perhaps offering a solution to inhibiting the development of tamoxifen resistance [35].

Additionally, both PHB and PHB2 were shown to repress SRC-3 activation of ERα. This is extremely important as SRC-3 is a known co-activator of ERα and is frequently amplified in breast cancer [38]. To further confirm this, anti-sense PHB2 RNA causes a heightened fold increase in ERα transactivation, highlighting that endogenous PHB2 dulls the stimulatory effect oestrogen has on ERα. Although the mechanism to how PHB2 counteracts SRC-3's function still needs to be defined, it is clear that PHB and PHB2 compete with SRC-3 for the ERα receptor, causing the recruitment of HDACs, suppressing ERα activation [37]. To further highlight PHB's role in oestrogen signalling co-repression, knock out of PHB in a murine model resulted in a hypoplastic uterus and impaired U2-induced uterus proliferation [39]. Moreover, during early pregnancy, mRNA levels of PHB found in the uterus of mice were heightened by 2-fold 2 days post-coitum. As blastocyst implantation occurred 4.5 days post-coitum, this high expression of PHB prior to the implantation suggests its role in maintaining the implantation status of the blastocyst [39]. Furthermore, in the same study, treatment of ovariectomized mice with oestrogen resulted in an increase in PHB mRNA transcripts in 6 hours [39]. This suggests that PHB is a probable target of oestrogen in the uterus as well as mammary tissue. Overall, there are clear results indicating that PHB is an oestrogen-regulated gene both *in vivo* and *in vitro*.

PHB, Stat3 and irritable bowel syndrome

PHB has also been seen to exhibit its function in inflammatory diseases which often lead to cancer development [40], especially as it is associated with oxidative stress. A known transcriptional factor Stat3 (Signal transducer and activator of transcription 3) is activated via various ligands in an array of tissues. Interestingly, data [41] suggests that there is a link between mitochondrial Stat3 and PHB in the intestinal epithelium. It is thought that this interaction prevents mitochondrial dysfunction and it is this process that is disrupted in the pathogenesis of Irritable bowel syndrome (IBS). Phosphorylation of Stat3 occurs at serine 727 (S727) which is necessary for the mitochondrial function of Stat3 and PHB interaction [42]. Co-immunoprecipitation determined that PHB interacts with pS727-Stat3 in the mitochondria of cultured intestinal epithelial cell lines and *in vivo* in mouse colonic epithelium [41]. This PHB-pS727-Stat3 interaction is lowered throughout mitochondrial stress in response to TNF-α by the means of lowered PHB expression. However, upon PHB overexpression, PHB-pS727-Stat3 interaction is preserved during TNF-α induced stress [43]. It has been recognised that PHB expression levels are lower in inflamed epithelia of IBS patients when compared to healthy controls, signifying that down-regulation of PHB may be an event in the early onset of pathogenesis rather than a downstream effect of the disease [41]. A transgenic model over-expressing PHB in intestinal epithelial cells demonstrated an up-regulation of protection against experimental colitis and there was notably less oxidative stress present in the colon [41]. As previously mentioned, mitochondrial dysfunction is a familiar feature seen in cancers [44] due to reactive oxidative species (ROS) and loss of mitochondrial chaperones such as PHB. A recent report believed that loss of PHB led to the onset of dysplasia during ulcerative colitis [41]. Moreover, PHB protein expression found in colonic mucosa was decreased after mice were induced with
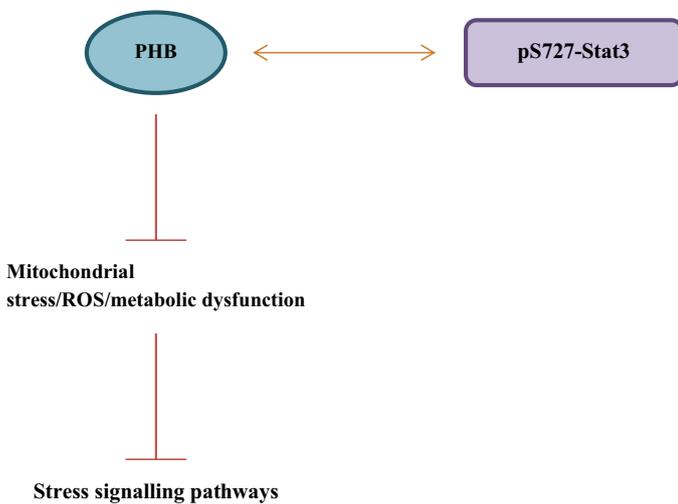
Fig. 5. Schematic representation of the interaction between PHB and pS727-Stat3, stopping stress signalling pathway activation. Image adapted from [2].

Table 1
Similarities of PHB-UTR sequence across mammalians.

| Name	Name	Similarity (%)
Human	Chimp	98.93
Human	Mouse	71.44
Human	Rat	68.66
Human	Dog	75.05
Human	Horse	80.36
Human	Cow	78.52
Human	Chicken	49.17

Fig. 6. Phylogram of PHB-UTR genetic differences between mammalian species. (NCBI, aligned in ALGGEN).

experimental colitis-associated cancer and vice versa [41]. Thus it is theorised that PHB could potentially prevent the onset of tumours by aiding mitochondrial stability. By targeting Stat3, one could enhance PHB's function to regulate mitochondria function that could theoretically aid intestinal epithelial cells homeostasis during colitis where PHB is lost. Mechanism is shown in Fig. 5.

The role PHB has within IBS only offers more evidence to help evaluate approaches on the incorporation of PHB with cancer therapies.

PHB in other cancer types

As PHB has been showed to have a huge implication in PC and breast cancer, a functional role has also been noted in ovarian and bladder cancer. Firstly, in line with PHB aiding apoptosis, it was noted that up-regulation of PHB was seen in normal ovarian cells undergoing apoptosis in the presence of gonadotropin releasing hormone (GnRH) [45]. As this was seen in the mitochondria, this emphasises that PHB is maintaining the mitochondrial integrity and loss of PHB may be associated with defective apoptosis in ovarian cancer. This may be the reason why PHB accumulation was seen in the perinuclear and cytoplasmic regions in the epithelial cells in papillary serous ovarian tumour cells [45]. On the other hand, in bladder cancer PHB up-regulation was shown to be associated with the proliferation of bladder cancer cells through PHB1's phosphorylation at thr258 by AKT [46]. In the same study it was also observed that up-regulation of PHB, at both a transcriptional and translational levels, in bladder cancer tissue were associated with poorer bladder cancer prognosis when compared to normal adjacent urothelial tissue [46]. PHB phosphorylation has also been observed in response to insulin signalling causing PHB to interact to a lesser extent with PIP3 [47]. This may disrupt PHB's ability to attenuate the PI3K pathway, which often is aberrant in cancers [47].

PHB and miRNAs

PHB-untranslated region (UTR) and miRNAs

Where the coding region is 95–100% similar in the *PHB* gene across species, the UTR of the highly conserved *PHB* gene shows high variability across mammals, as indicated in Table 1.

Alignment analyses of PHB's UTR across mammals indicate there is variation amongst mammals (Fig. 6). Table 1 demonstrates the
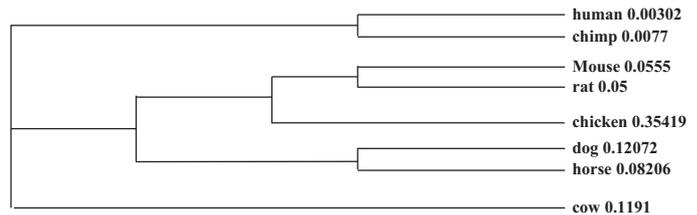
percentage similarities between species along with the genetic differences between them. The variations in the UTR region of PHB across species and the multiple miRNA target sites suggest it is widely and variably regulated, however this is not the case for the PHB protein, although the UTR could regulate 'fine tuning' of the PHB protein.

It is interesting to note some of these miRNA targets on PHB's UTR are classified as oncogenic such as miR-27a [26] (Fig. 7). Therefore, theoretically administration of the PHB-UTR absent of the PHB protein could act as a miRNA 'sponge' [48].

PHB interacts with MicroRNAs in PC

One mechanism which could potentially bypass the AR signalling pathway is PHB's interaction with miR-27a.

MicroRNAs are small noncoding strands of RNA, usually around 25 nucleotides long, and can control gene expression by mRNA degradation or inhibition of protein production [49]. Interestingly, microRNA gene locations are often found in cancer associated genes or in fragile sites, making them vulnerable to single nucleotide
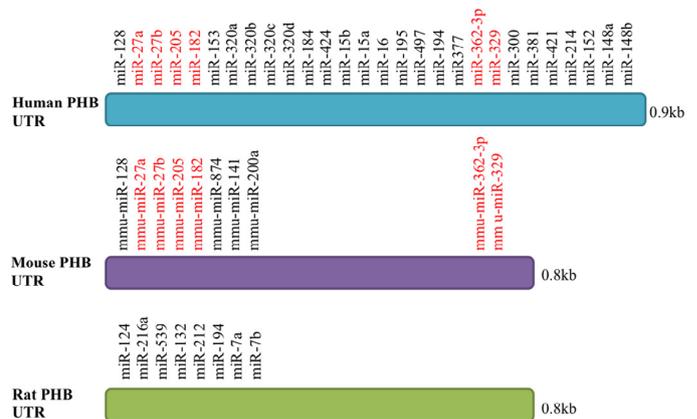

Fig. 7. miRNA targets within PHB's UTR in humans, mice and rats. Similar targets across species are indicated in red. Dissimilar targets indicated in black. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polymorphisms (SNPs), thus allowing for the acquisition of either oncogenic or tumour suppressive characteristics [30]. It is therefore no surprise that microRNAs can become aberrantly activated during carcinogenesis, taking on oncogenic characteristics – named oncomiRs. In particular an oncomiR known as miR-27a located on chromosome 19 position p13.1, is encoded by a intergenic cluster along with both miR-23a and miR-24-2 [30]. The expression of this particular cluster has been modified in numerous cancers such as leukaemia and ovarian cancer [50]. Remarkably, its function can have opposing effects, for example in hepatocellular carcinoma, upregulation of this cluster can hinder TGFβ's ability to suppress tumour growth [51]. On the other hand, in human embryonic kidney cells, it can up-regulate apoptosis [52]. It is probable that posttranscriptional modification plays a role in these clusters alternative functions within different cellular environments [30]. With regard to miR-27a, it was proven by Fletcher et al. that upon androgen stimulation PHB expression was down-regulated, allowing target gene expression and thus PC cell growth. It was predicted that a binding site for miR-27a was found within the highly conserved 3' UTR of the PHB gene. This was confirmed by a luciferase reporter assay, which verified down-regulation of this UTR in response to androgen treatment, suggesting this region's susceptibility to androgen regulated miRNAs, hindering its activity. This mechanism was due to degradation of the PHB gene transcript rather than posttranscriptional modification/inhibition. To demonstrate that miR-27a possesses characteristics of an oncogene, manipulation of miR-27a caused an upsurge in androgen responsive genes such as *PSA* and *TMPRSS2* [30]. Several other targets of miR-27a have been identified such as the tumour suppressor gene *FOXO1*, *ZBTB10*, a repressor of the Sp family of transcriptional factors and *Wee-1* a vital regulator of cyclin B. Moreover, it is likely that miR-27a only weakens PHB protein expression rather than causing complete reduction, and it is likely that miR-27a targets other proteins associated with cell proliferation resulting in growth stimulation [30]. It is known that miRNAs based therapies for cancer intervention provides an exciting way to counteract tumorigenesis for integrated cancer treatment [53], therefore miR-27a could be an ideal candidate.

Avenues for cancer therapy

The lack of mutations within the PHB gene suggests its function is essential, as no clearly functional mutations were found in its chromosomal location in 32 men diagnosed with PC [54]. Therefore the lack of PHB mutations may hold the key for PHB targeted therapies such as microRNA based inhibitory oligos or drugs such as flavagline drugs.

Direct administration of PHB into cells can increase cellular stress and therefore other therapeutic avenues must be considered. miRNA therapy could provide a solution potentially using MiR-27a as a target. Especially as manipulation of certain miRNAs in cancers have minimal side effects [55]. More than 20 miRNA targets are undergoing clinical trials, emphasising the vast amount of research surrounding this category of treatment. However, the downfall with miRNA-targeted therapy comes with problems surrounding the delivery, stability and the potential of activating an immune response [55].

Interestingly novel potent anticancer agents have been discovered to also affect the activity of PHB. One such example is a natural product known as flavaglines that have been studied as PHB ligands [3]. Flavaglines display selective anticancer activities and have also been shown to enhance chemotherapies in several mouse models and display no signs of toxicity [56]. As PHB is known to interact with p53 in the nucleus of many cancer cells by increasing its transcriptionally activity, the use of flavaglines could enhance the exposure of p53 to PHB, attenuating the cell cycle and inducing apoptosis [3]. Moreover, flavaglines such as rocaglamide, increases

phosphorylation of p53 and increases levels of the p53 regulated pro-apoptotic protein Bax [57].

Moreover, as previously mentioned PHB has been identified to improve the efficacy of well-known licensed drugs such as Tamoxifen by working in synergy with the drug. This could potentially become another avenue for treatment, especially in terms of minimising chemotherapy resistance.

Concluding remarks

The ability of PHB to interact with such an array of receptors, proteins and miRNAs in a multitude of disease states, as well as being involved in oxidative stress and mitochondrial dysfunction makes it a very unique protein. Potentially, PHB could have high clinical relevance in the development of therapeutic agents. In particular PHB could hold huge importance in unveiling the mechanism to how androgen stimulation down-regulates PHB expression in PC, which is immensely significant in deciphering how prostate tumours transition from androgen dependence to an apparent 'androgen independent' state. This could also link to hormonally driven breast cancer, whereby PHB can repress SRC-3 activation of ERα, inhibiting both genomic and non-genomic ERα activation.

As cancers become increasingly aggressive and are rapidly proliferating, the levels of mitochondria are also increased. Yet analysis of cancers for PHB may have been misinterpreted as only protein levels of PHB in the mitochondria have been examined. However as demonstrated by this review nuclei and membranous PHB as well as its interactions with regulators of the cell cycle might also have a major influence.

Furthermore, the research revealing how the cell cycle is influenced by PHB and its interaction with E2Fs, P53 and Rb can be considered to have high relevance. These genes are frequently mutated in almost all human cancers thus PHB may be key in regulating their functions, especially in PC and breast cancer.

In summary, a growing body of evidence indicates that the subcellular localisation of PHB is altered between tumour and normal cells and that this alters PHB's function. These various functions that PHB carries out still requires further research, however it is clear that its role is heavily implicated across an array of cancers. In particular, better understanding of its function within PC is potentially making us one step closer to discovering therapies for androgen independent PC.

Conflict of interest

None.

Acknowledgements

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