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The AIB1/NCOA3/SRC-3 Oncogene

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Abstract

A member of the NCOA/SRC/p160 co-activator family, AIB1 is amplified and overexpressed in multiple cancer types, notably breast, ovarian, and pancreatic cancer. Common to all members of the NCOA/SRC/p160 family are bHLH-PAS, receptor interaction, and CBP/p300 interacting activation domains. The protein acts as a scaffold to support the transcriptional activity of many DNA binding transcription factors, such as the ER, AP-1, E2F, NFκB, and TEADs. In doing so, the multi-domain protein facilitates chromatin remodeling and oncogenic gene transcription. Further, the AIB1Δ4 isoform promotes tumorigenesis and metastasis through interaction with chromatin in the nucleus or at the periphery of the cell. Pathologically, AIB1 promotes the transformation of normal tissue to cancerous lesions in multiple diseases, and loss delays progression. AIB1 has also been implicated in cancer recurrence and pharmacological resistance. We will discuss the structure and isoforms of AIB1, the physiological consequences of its interaction with transcription factors and hormone receptors, and clinical significance of the protein.

Keywords: AIB1, NCOA3, SRC-3, nuclear coactivator, steroid receptor co-activator, oncogene, breast cancer, transcriptional co-activation, chromatin modification

1. Introduction

Amplified in breast cancer 1 (AIB1) is a transcriptional co-activator and a member of the nuclear co-activator (NCOA) family; the protein was discovered concurrently by many groups and given a variety of names, including AIB1 [1], ACTR [2], TRAM-1 [3], RAC-3 [4], CIP1 [5], and SRC-3 [6]. AIB1 is an oncogene that is amplified and overexpressed in cancer, and acts by recruiting and stabilizing chromatin remodeling complexes [1, 2, 7]. In its well-known capacity, AIB1 interacts with nuclear receptors such as the estrogen, progesterone, and androgen receptor, to promote hormone dependent transcription and repression. Less well-studied, AIB1 promotes disease progression and de-differentiation by potentiating oncogenic signaling through interaction with a diversity of transcription factor interactions in hormone-independent disease contexts [8–11]. Thus, AIB1 acts as an oncogene by stabilizing transcription complexes, recruiting chromatin modifying enzymes, and thereby amplifying oncogenic signals. Unsurprisingly, high AIB1 levels are a poor prognostic marker across many cancer types, and also the protein can facilitate resistance to therapeutics in patients. Herein, we describe the form and function of AIB1, and its role in cancer and the clinical setting.

2. Structure of the oncogene

2.1 The nuclear coactivator (NCOA) family

The NCOA/SRC/p160 co-activator family consists of NCOA1 (also called SRC-1), NCOA2 (also called SRC-2, GRIP1, or TIF2), and AIB1 (also called SRC-3, NCOA3, ACTR, TRAM-1, and RAC-3). The three family members share ~55% sequence homology, and common to all members is a bHLH-PAS domain, nuclear receptor interaction domains, and C-terminal activation domains (reviewed in [12]). As a result, the members of the NCOA family interact with many different transcription factors, nuclear receptors, and chromatin modifying enzymes. Apart from mediating transcription factor interactions (detailed in Section 6 of this review), the homologous domains and residues are critical to protein stability and turnover. The bHLH-PAS domain contains residues essential for proteasomal mediated protein turnover (K17 and R18) as well as a nuclear localization signal; site-directed mutagenesis of these residues promote protein hyperstability [13]. Though the bHLH-PAS domain shares homology to DNA recognizing motifs found in both *Drosophila* and Humans, the NCOA family members do not directly bind the DNA, but rather facilitate transcriptional activity of binding partners [14]. The bHLH-PAS domain mediates the NCOA members' interaction with transcription factors, repressors, and coactivators, but is lost in the clinically relevant $\Delta 4$ isoform (see Section 2.2). The middle region of the NCOA family proteins contains three α -helical LXXLL motifs (NR Boxes) that mediate nuclear receptor (NR) recognition and specificity [15, 16]. The affinity of the NCOA is both dependent on the individual nuclear receptors, as well as the specific bound ligands [17]. Interaction with nuclear receptors, including the well understood interaction with the Estrogen Receptor, is detailed in Section 4. The C-terminus contains two activation domains, as well as a glutamine-rich region. These activation domains are critical to the recruitment of chromatin remodeling enzymes CBP/p300, P/CAF, CARM1, and PRMT1 [2, 18]. Similar to the bHLH-PAS domain, the C-terminal activation domains are critical for a variety of transcription factor interactions. Furthermore, SRC-1 and AIB1 both have minimal acyltransferase activity, associated with their C-terminal activation domains, which function as histone acetyltransferases [2, 19]. **Figure 1** illustrates the structure of AIB1 and some of its known binding partners.

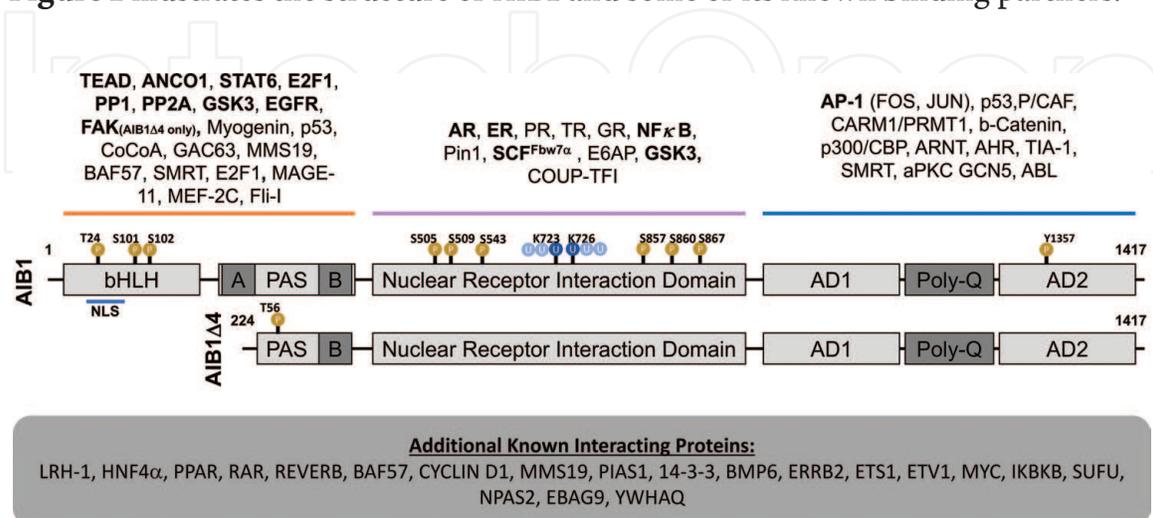


Figure 1.

AIB1 interacts with many proteins through its independent domains. Line diagram showing some of the proteins that *AIB1* and *AIB1 $\Delta 4$ interact with in a cell. Bolded proteins are described in the text of this chapter. Critical residues for phosphorylation (yellow) and ubiquitination (blue) are shown. bHLH = basic helix-loop-helix domain; PAS = PER-ARNT-SIM domain. AD = activation domain.*

It is the independent functions of the multiple domains of AIB1 that allow it to perform a variety of different cellular activity. Of note, the capacity of AIB1 to act as a co-activator is dependent not on mutually exclusive interactions, but rather on many simultaneous binding events in which it acts as a scaffold to support multi-protein complex formation.

2.2 The AIB1 Δ 4 isoform

We have identified an isoform of the AIB1 oncogene that lacks the N-terminal bHLH-PAS A domain, originally designated Δ 3, now called AIB1 Δ 4. Alternative splicing results in the loss of exon 4, thereby shifting the start codon to exon 7 and forming a N-terminal truncated, yet functional protein, missing 224 amino acids [20, 21]. As the isoform retains its nuclear receptor interacting domain, poly-Q region, and its C-terminal activation domains, the isoform can localize to estrogen response elements in the nucleus and recruits CBP/p300 to activate hormone dependent transcription [22]. Moreover, Δ 4 isoform can also utilize its C-terminal activation domains, meaning that it can still co-activate many of hormone receptors and transcription factors on which it usually acts (see Sections 4 and 6). While retaining the ability to perform a significant portion of its normal activity, AIB1 Δ 4 cannot bind an endogenous repressor, ANCO1, and this may contribute to a significant portion of its hyperactivity [23, 24].

AIB1 Δ 4 has been shown to be highly responsive to growth factor and estrogen signaling. While much of this chapter will be devoted to the role of AIB1 on promoting transcription, it can also act as a key player in repression complexes. The ANCO1 repressor binds and represses the AIB1-ER complex on an estrogen response element in the Her2 gene body. This mechanism allows for estrogen mediated repression of the Her2 gene, as AIB1 in turn recruits histone deacetylases to the site to suppress transcription. The AIB1 Δ 4 isoform is not repressed by this mechanism, as it lacks the bHLH-PAS domain that ANCO1 requires as a binding interface, and thus maintains transcription of the Her2 growth factor receptor. As a result, the AIB1 Δ 4 isoform enhances growth factor signaling input via increasing the mRNA levels of the receptor [24].

Interestingly, by lacking the N-terminal bHLH-PAS domain containing the nuclear localization signal, the Δ 4 isoform localizes both to the nucleus and cytoplasm. In the cytoplasm, the isoform interacts with both EGFR and FAK to promote cell migration through promotion of Src phosphorylation. The bHLH-PAS domain generally acts as an inhibitor of this interaction [25]. Knockdown of AIB1 in ovarian cells also results in a mislocalization of FAK, which may be due to loss of the isoform [26].

The role of AIB1 Δ 4 has been validated in vivo. AIB1 Δ 4 has been shown to increase mammary cell proliferation in tissue specific genetically engineered mouse models, potentially by increasing levels of IGF-1R levels on the cell surface [27]. Overexpression of the isoform resulted in similar hyperplasia as compared to the overexpression of the full-length protein, however it further presented with an increase in progesterone receptor signaling and cell cycle promoting cyclin levels. Compared to the full-length protein, AIB1 Δ 4 acted synergistically with ER α to promote mammary gland stromal and epithelial hyperplasia [28]. The isoform is not only seen in estrogen dependent breast cancer, as AIB1 Δ 4 expression levels are increased in metastatic triple negative (hormone-independent) and pancreatic cancer cell lines, compared to their isogenic parental lines [22]. More data is needed to explain the complete function of AIB1 Δ 4, which clearly plays a role in normal physiology, and may contribute to disease progression and invasion.

3. Modifications and PTMs regulate AIB1 activity

Post-translational modifications (PTM) are critical to modulating the stability and activity of AIB1. Within the 165 kDa protein, there are multiple phosphorylation and ubiquitination sites, some of which have been well described by recent experimentation.

3.1 Transcriptional regulation of AIB1

AIB1 protein level is regulated by multiple processes, with levels primarily peaking during the cell cycle. AIB1 autoregulates its own expression and is recruited to its own promoter in complex with E2F1 (see Section 6.3). AIB1 transcription is responsive to cell cycle cues mediated by Rb hypophosphorylation and resulting activation of E2F; thus, AIB1 levels increase during G1, and attenuate during S phase (when comparing relative levels during the cell cycle) [29]. Downregulation of AIB1 protein levels is, in part, mediated by the FoxG1 tumor suppressor, which acts by interacting with AIB1 and disrupting the interaction with E2F1 on its own promoter (FoxG1 additionally interrupts AIB1's activity with other transcription factors, such as NF κ B, AP-1, and the Estrogen Receptor) [30]. Tight control of the AIB1 activity is regulated by its own positive feedback and tempered by inhibitory protein interactions.

AIB1 is also targeted by microRNA that regulates its expression. miR-17-5p targets at least two sites on the AIB1 mRNA, and miR-20b also binds to AIB1 mRNA. The two miRs are negatively correlated with AIB1 expression and loss of miR expression is associated with taxol resistance in breast cancer [31, 32]. These miRs, in addition to down regulating AIB1, interact with multiple other proteins to differentially regulate their gene expression. As a result, they are implicated in the progression or suppression of several cancers, in a context dependent manner.

3.2 The AIB1 life cycle

The stability and half-life of the AIB1 protein, once translated, is regulated by a series of phosphorylation and ubiquitination events (see **Figure 1**). There is a balance achieved between multi-mono-ubiquitination and long chain poly-ubiquitination that creates a phosphorylation dependent 'time clock' for the stability of AIB1. GSK3 phosphorylates AIB1 at S505 and S509 in the absence of AKT signaling to promote its multi-mono- or poly-ubiquitination by SCF^{Fbw7 α} at K723 and K726. Amplified AKT signaling inactivates GSK3. Multi-(mono)ubiquitination of AIB1 then promotes hormone dependent activity through interaction with the estrogen receptor, whereas poly-ubiquitination promotes degradation of AIB1 [33].

An alternative variety of phosphatases regulate proteasome mediated turnover by binding and dephosphorylating AIB1. PDXP, PP1, and PP2A dephosphorylate AIB1 at S101 and S102, and negatively regulate Estrogen Receptor binding and cooperation. PP1 specifically stabilizes AIB1 by preventing proteasomal turnover by dephosphorylating the N-terminal C-region degron at the S101 and S102 residues. Though more stable, decreased affinity for the estrogen receptor significantly reduced AIB1 mediated cell growth in estrogen dependent cell lines [34]. Regulation of AIB1 protein levels is thus regulated by a complex series of phosphorylation and ubiquitination events in response to cell signaling and stimulation. The phosphorylation and ubiquitination sites may be unique to the pathways promoting turnover, and thus further study is warranted to better understand the signaling cascades that control AIB1 levels.

3.3 AIB1 post-translational modifications modulate activity

AIB1 is phosphorylated at different serine and threonine residues throughout the different domain structures by a variety of kinases. As a result, AIB1 is responsive to many different upstream signaling cascades, contributing to its oncogenic nature. JNK, p38, ERK, IKK, and PKA can all phosphorylate AIB1 at different residues to promote interaction with CBP and subsequently activate transcription (see **Figure 1**). Phosphorylation sites within AIB1 have been well profiled as mediators of certain protein-protein interactions. Six phosphorylation sites were originally described as essential for interaction with the estrogen and androgen receptors (T24, S505, S543, S857, S860, and S867). Conversely, phosphorylation at only T24 and S867 was required for TNF mediated NF κ B interaction and activity [35]. These phosphorylation events in response to estradiol, TNF α , and upstream IGF signaling thus activate AIB1 to interact with partners and potentiate transcription [35–37]. Additionally, we have found that Abl kinase phosphorylates AIB1 at Y1357 in response to IGF, EGF and estradiol stimulation, which results in AIB1 interaction with essential chromatin modifying enzymes. This phosphorylation event is critical for AIB1's coactivator function [38]. Phosphorylation of AIB1 is thus a critical step in activation of the protein and is mediated by a variety of upstream signals that converge on the oncogenic coactivator. Some phosphorylation sites are required for all described activity, yet much work needs to be done to better understand what regulates the selectivity of AIB1 to bind with specific transcription factors and nuclear receptors.

4. AIB1 potentiates hormone receptor signaling

Estrogen, progesterone and androgen receptors (ER, PR, AR) are nuclear steroid receptors, which play a major role in sexual development and reproduction. Upon binding to their ligands, these receptors dimerize and translocate to the nucleus. They act as transcription factors by binding directly to unique DNA sequences termed response elements. Subsequently, histone modifying enzymes and transcriptional co-regulators are recruited to activate target gene transcription. AIB1 has been shown to directly interact with ER, PR and AR via its LXXLL motifs [39, 40]. There are two classes of estrogen receptors; ER α and ER β . AIB1 binds and enhances ER α receptor-stimulated gene transcription in a ligand-dependent manner [6]. Upon binding to ER α , AIB1 recruits chromatin-remodeling histone acetyl-transferases enzymes and thus increases ER α transcriptional activity. On the other hand, AIB1 can regulate ER α protein levels when bound to estradiol (E2). AIB1 recruits ubiquitin-proteasome complex to the ligand bound ER α leading to its degradation. Studies have shown that when AIB1 levels are reduced, ER α levels are stabilized [41]. In AIB1 $-/-$ mice, delays in puberty and mammary gland development as well as aberrant reproductive functions have been reported [5]. Similar observations are seen in both PR $-/-$ and ER $-/-$ mice emphasizing the essential role of AIB1 in ER and PR dependent functions. In breast cancer, AIB1 potentiates the development of hormone-dependent tumors and contributes to antiestrogen resistance [42, 43]. Lacking the inhibitory domain, AIB1 Δ 4 isoform has been shown to potentiate ER and PR transcription activity to a much greater extent than AIB1 [20]. In breast tumor samples, the association between AIB1 level and both ER and PR levels has not been clearly determined. One study showed that amplification of AIB1 in breast cancer correlates with high expression of ER and PR [44]. Yet another study showed that overexpression of AIB1 in breast cancer samples was associated

with loss of both ER and PR expression [45]. Moreover, AIB1 strongly binds AR and co-activates its target gene transcription. In prostate cancer, AIB1 is shown to be overexpressed and its levels correlate with higher tumor grade and increased disease recurrence but did not correlate with serum PSA levels [46]. Several studies have established AIB1 as a preferred coactivator for hormone-activated AR. Mutations in AR that alter its binding potency to AIB1 has been found in prostate cancer patients, suggesting an oncogenic role of AIB1 in prostate cancer [40].

5. AIB1 potentiates membrane receptor signaling

Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase (RTK). It is activated by EGF which mediates several functions including cell proliferation, cell survival, and development. AIB1 knockdown abrogated EGF growth response in lung, breast, and pancreatic cancer cell lines. This was a result of reduced tyrosine phosphorylation of EGFR at multiple residues both at autophosphorylation and Src kinase phosphorylation sites via less recruitment of Src homology 2 domain-containing proteins to the EGFR. EGF-dependent phosphorylation of HER2 was also decreased yet no effect was seen on phosphorylation of platelet-derived growth factor receptor (PDGFR), HER3 or other RTKs. This suggests that the oncogenic effect of AIB1 may be mediated by EGFR and HER2 signaling pathways [47]. In a MMTV-Neu mouse model, homozygous deletion of AIB1 completely inhibits Neu-induced mammary tumor formation. The role of AIB1 in HER2/Neu oncogenic activity was elucidated in the Neu/AIB1+/- tumors showing decreased phosphorylated Neu, cyclin D1, and cyclin E [48]. In addition to its role as a transcription coactivator, AIB1 isoform, AIB1 Δ 4, can act in the periphery of the cell mediating EGFR and FAK direct interaction. Overexpression of AIB1 Δ 4 increased cell migration and MDA-MB-231-induced breast tumor metastasis [25].

The insulin-like growth factor (IGF)-I regulates protein turnover and has a role in cell proliferation and differentiation. IGF-I binds to its receptor activating a cascade of intracellular tyrosine kinases which phosphorylate downstream substrates including IRS and Shc [49]. AIB1 is rate-limiting for IGF-I signaling and functions in human breast cancer cells. Knockdown of AIB1 in MCF7 cells reduced IGF-1-stimulated anchorage-independent proliferation and IGF-I-dependent anti-anoikis [37]. In AIB1-/- mice, impaired insulin-like growth factor I pathway reduced mammary tumorigenesis and metastasis with no change in ER or PR regulated genes [50]. In addition, AIB1 regulates the expression of proteins involved in the IGF-1 signaling pathway. For example, inhibition of mTOR prevented mammary hyperplasia and hypertrophy that was caused by AIB1 overexpression in the mouse mammary gland. In mice, mTOR inhibition prevented the growth of xenografts from AIB1-induced mammary tumors [51].

6. AIB1 interacts with transcription factors

Though AIB1 has been primarily studied for its interaction with the Estrogen receptor (see Section 4), AIB1 interacts with a diverse set of transcription factors which may explain its oncogenic role in cancer. We will focus on the interaction of AIB1 with known oncogenic transcription factors to highlight the importance of AIB1 as a transcriptional co-activator across a variety of signaling pathways. As a general pattern, AIB1 acts to potentiate transcription of signaling pathways; when it binds to a transcription factor, it tends to increase the expression of target genes synergistically. Additionally, many pathways simultaneously activate their effectors and act on AIB1 in

the cytosol to increase nuclear translocation. Thus, many growth factors signaling cascades converge on transcription factors and AIB1. We have selected a few of the most well studied oncogenic transcription factors whose activity is increased by AIB1 in cancer. **Figure 2** illustrates these interactions and their phenotypic consequences.

6.1 AP-1

The activator protein transcription factor is a heteromeric complex consisting of Fos, Jun, ATF, and MAF family members, and can act as an oncogene that drives proliferative signaling. The complexes regulate a large swath of human gene expression and can contribute to both pro- and anti-tumorigenic gene expression (reviewed in [52]). AIB1 uses its C-terminal activation domains to interact with the Fos and Jun family members to activate and potentiate signaling, as measured by synthetic luciferase reporter assays and target gene expression [10]. Clinically, this interaction has been identified to be relevant in driving many pro-proliferative cancer genes. In hormone independent prostate cancer, responsiveness to IGF-Akt signaling by AIB1-AP-1 cooperation synergizes their effects on the transcription of target genes, thereby promoting cell growth and division [53]. In both hormone-dependent and -independent breast cancer (in which AIB1 is amplified), AIB1 interaction with and co-activation of AP-1 specifically promotes the transcription of matrix metalloproteinases, contributing to invasive progression [54]. Such invasive behavior has also been linked to turnover of Focal Adhesions by AIB1 through an AP-1 dependent interaction [55]. Of note, the AIB1 Δ 4 isoform contains the domains necessary to interact with AP-1 and FAK, which may explain some of its endogenous role, as well as a dual functionality of this oncogenic isoform [25, 55].

6.2 The TEADs

Many recent publications have implicated YAP and TAZ, effectors negatively regulated by Hippo Signaling pathway, as potent oncogenes critical to the

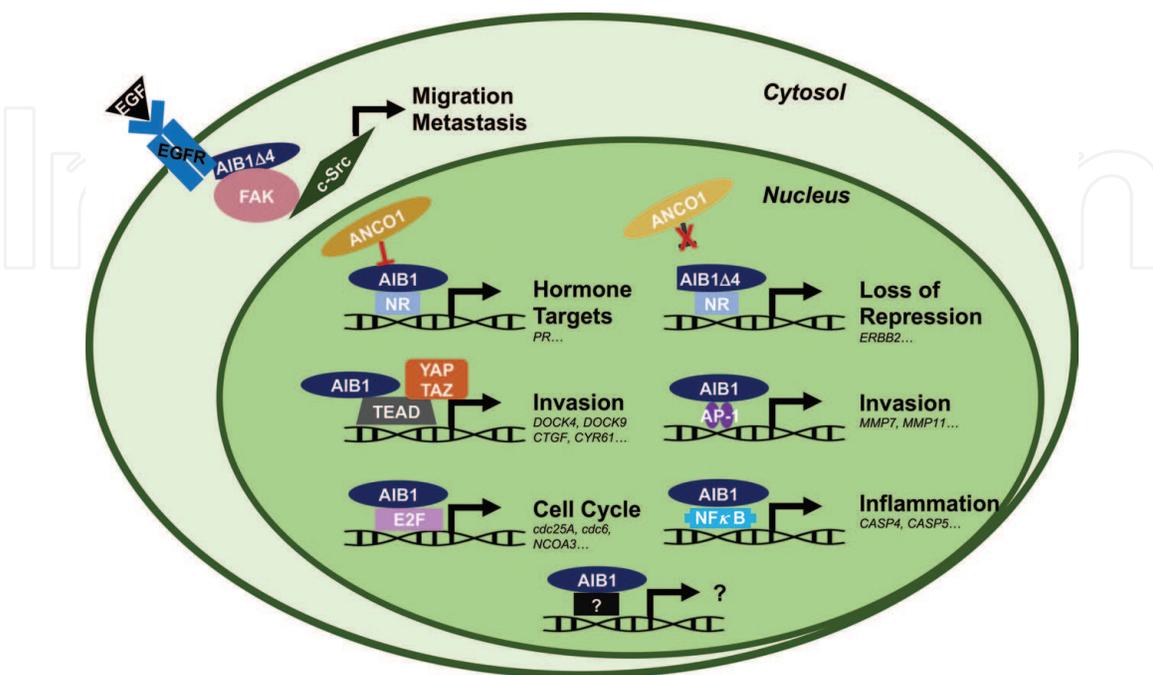


Figure 2. Oncogenic effects and gene expression changes involving direct AIB1 interaction with transcription factors and membrane proteins. Graphical depiction of AIB1 interaction with binding partners, resulting phenotypic behavior, and select transcriptional targets (*italicized*).

transformation of normal tissue and the progression of disease. The effectors bind with the TEAD family of proteins to exert their oncogenic potential as a transcription factor complex [56–58]. Before paralogs YAP and TAZ, were implicated as transactivators of the TEAD family of transcription factors, the TEAD family was shown to interact with all the members of Nuclear Coactivator Family [8]. Specifically, the bHLH-PAS domain of AIB1 interacts with TEADs, likely in a larger complex with YAP or TAZ, and may then recruit histone modifying proteins to propagate transcription as measured by target genes [59]. Similarly, knockdown of AIB1 in cell lines significantly reduces TEAD target genes CTGF and CYR61 [60]. Currently, it is unclear to what extent the oncogenic YAP/TAZ-TEAD complex requires AIB1 or other members of the NCOA family to act as oncogenes. Knockdown of AIB1 in multiple studies has resulted in a modest, but significant reduction of TEAD transcriptional targets [60]. Interestingly, the *Drosophila* homolog of AIB1, called Taiman, contains PPxY motifs that are known to interact with the YAP homolog at its conserved WW domain. These PPxY motifs, however, are neither conserved in human AIB1, nor any member of the NCOA/SRC/p160 family [61].

Importantly, AIB1 may be the critical mediator of TEAD cooperation with AP-1. Early reports show an oncogenic signature associated with AP-1 and TEAD co-occupancy in triple negative breast cancer that promotes more aggressive disease [62]; this interaction was recently shown to be mediated by AIB1 acting as a bridge between AP-1 and TEAD [59]. However, a clear panel of genes co-regulated by AIB1-TEAD interaction has yet to be elucidated. Further, while reports have shown AIB1 to be part of the TEAD-SRF (Serum Response Factor) complex, the data is unclear as to whether AIB1 is required for complex formation, or whether YAP can recruit SRF in the absence of AIB1 [60, 63]. It is apparent that TEAD, AP-1, and SRF all have coordinated responses to external growth stimuli [64], but the extent to which AIB1 and other co-activators are required to propagate and potentiate oncogenic signaling remains an exciting and unanswered question.

6.3 E2F family

The E2F family of transcription factors are direct targets of the hypo-phosphorylated Rb cell cycle regulation machinery, so many E2F members promote the transcription of pro-proliferative genes and controls the entry into S phase (reviewed in [65, 66]). AIB1 interacts with E2F family members through its N-terminal bHLH-PAS domain to promote the transcription of *cdc25A*, *cdc6*, MCMs, cyclins and Cdk. Depletion of AIB1 prevents cells from entering S-phase and undergoing mitosis. Furthermore, AIB1 controls its own expression through binding to E2F1 on its own promoter. As a result, AIB1 levels increase during G1 [9, 29]. Not only does E2F interact with AIB1 at its own promoter, but it also acts on other transcription factors, such as SP1, to further augment AIB1 expression [67]. This shows that direct and indirect binding of cell cycle effectors promote transcription of AIB1. Recently, the importance of an AIB1-E2F1 axis was highlighted while studying the efficacy of CDK4/6 inhibitor Palbociclib across all subtypes of breast cancer; AIB1 loss partially phenocopied Her2 inhibition and correlated with the CDK4/6 inhibitor treatment [68]. Thus, AIB1 contributes to cell cycle progression through E2F interaction, which is commonly dysregulated in cancer. This directly links AIB1 to regulation of cell cycle progression, implicating AIB1 further in pro-proliferative activities separate from external stimuli and nuclear receptor interaction.

6.4 ETS factors

The Ets1, Ets2, and Pea3 members of the Ets family of transcription factors bind to DNA in response to upstream Her2 activation and resulting kinase cascades, mediated by ERK and JNK [69]. The Ets family members have been shown to be co-expressed with AIB1 and both independently serve as a negative prognostic marker in breast and lung cancer [70, 71]. AIB1 was later shown to interact directly with the Ets family members to potentiate transcription of matrix metalloproteinases such as MMP2 to promote cell invasion and metastasis in vitro and in patients [71–73]. Once phosphorylated by ERK3 at S857, AIB1 specifically localized to the promoters of MMP2 and MMP9 in complex with Pea3 to promote invasive behavior [74]. Interestingly, these MMP targets seem highly dependent on AIB1 levels, as knock-down of AIB1 abrogated most of their expression [71].

6.5 NFκB pathway

Though many binding partners are shared between members of the NCOA family, mostly between SRC-1 and AIB1, cooperation with IKK is unique to AIB1. IKK mediates the degradation of IκB, the inhibitor of NFκB, in response to TNFα stimulation. Interestingly, in parallel to this, AIB1 is phosphorylated by IKK, increasing its nuclear localization and then can act on NFκB bound to DNA through its C-terminal activation domain [11, 75]. This emphasizes the ability of AIB1 to be to play a multi-faceted role within a signaling pathway, and the importance of its regulation to control its potency.

6.6 STAT6

Opposed to its role in the NFκB pathway, where AIB1 is the unique family member interacting with a kinase, STAT6 solely interacts with SRC-1. However, this does not mean AIB1 does not play a critical role. While SRC-1 directly interacts with STAT6 on the chromatin via its bHLH domain, AIB1 cannot. Recruited p300 bound to STAT6 can then recruit AIB1, which potentiates STAT6 signaling. This represents a unique cooperation between SRC-1 and AIB1, as SRC-1 is required for the co-activation of STAT6, and AIB1 then potentiates the transcription complex's activity. This was found to be an IL-4 dependent interaction, which acts in a dose-dependent manner [76–79]. Such interactions are controlled by PP2A, as described in Section 3.2 [80].

We attempted to highlight some of the most categorized interactions between AIB1 and transcription factors that are well studied oncogenes. See **Figure 1** for a more complete list of proteins that interact with AIB1.

7. AIB1 as an oncogene

7.1 Genetically engineered mouse models

Genetic models have also clearly established AIB1 as an oncogene in multiple cancer types. Genetically engineered mouse models that overexpress AIB1 have been shown to increase incidence and growth of tumors, as well as significantly increase hyperplasia in the breast [7]. This is not solely due to its interaction with the estrogen receptor, the same mice presented an increase in tumors in hormone

independent tissues, such as lung, skin, and bone, suggesting the oncogenic role of AIB1 may be mediated by a variety of different tissue specific transcription factor interactions [46, 81]. Removal of the NCOA3 gene that encodes AIB1 in v-Ha-Ras driven mouse model of breast cancer also delayed tumor formation by negatively impacting growth factor signaling [50]. In breast cancer studies, it is clear that AIB1 exerts its oncogenic potential through hormone receptor signaling and by positively affecting many pro-proliferative pathways.

There have also been genetic models implicating AIB1 as a critical mediator of the development and maintenance of hormone responsive and castration resistant prostate cancer. In hormone sensitive prostate cancer, AIB1 mediates its effects through androgen receptor activity, eventual castration resistant/hormone insensitive disease was marked by AIB1 potentiation of Akt-mTOR signaling (similar to studies in the breast) [81–83]. Taken together, these data suggest that AIB1 is critical for the formation and progression of many cancer types, in both hormone dependent and independent settings. Especially in the hormone-independent diseases, it is critical to study the binding partners of AIB1 in order to better understand how AIB1 is acting as an oncogene.

7.2 AIB1 levels and clinical outcome

Patient data has shown that the levels of AIB1 correlate with the severity and stage of disease. In each tissue type of origin, it is likely that AIB1 is acting as an oncogene in a different capacity or selectively amplifying a variety of oncogenic signals. We have described a few specific cancer types below that detail the predictive capacity of AIB1 in disease progression.

- Breast cancer: our group and others have shown that AIB1 is overexpressed and amplified in breast cancer compared to normal breast tissue [1, 20]. It is estimated that the mRNA expression in tumors is up between 10 and 60% in primary tumors, and increased 30% in metastatic sites [21, 84]. When stratified by grade of lesion, there is a clear positive correlation of AIB1 mRNA levels with worsening stage, with nearly a 65% increase in expression compared to normal tissue in grade 3 tumors [45]. We have also shown that increasing mRNA levels are associated with worse patient outcome [43]. These patients have dysregulated signaling pathways as previously described: augmented estrogen receptor signaling (in ER+ disease) and increased IGF/growth factor levels and enhanced in PI3K-Akt-mTOR pathway activity.
 - Early stage breast cancer: only 20–30% of women with stage 0 Ductal Carcinoma In Situ (DCIS) will progress to invasive disease. It is still unclear what factors promote the invasion, as genetic expression signatures of DCIS patients resemble those of invasive disease [85, 86]. We have shown that AIB1 is required for the formation of DCIS lesions in mice, and loss of AIB1 increases tumor necrosis, and decreases proliferation and tumor burden. Further, genetic ablation of AIB1 significantly reduces CD44+/CD24– breast cancer initiating cells, thus more closely resembling differentiated luminal epithelium. This is in part due to disruption and downregulation of the Notch and Her2 signaling pathways, where AIB1 was shown to regulate mRNA expression of Notch, Jag, and DLL family members [87]. Thus, AIB1 may be promoting a breast cancer initiating cell subpopulation that is required to promote the invasive transition.
- Ovarian cancer: AIB1 was also found to be overexpressed and amplified in ovarian cancer [1]. In high grade ovarian cancer samples, 64% of patients

stained positive for nuclear AIB1, whereas less than 10% of patients with cystomas or borderline cancer cases stained positive. [26] AIB1 polymorphs at the sequence level may also be predictive of ovarian cancer. CAG sequence polymorphisms within the glutamate track (poly-Q region) of AIB1 may also be predictive of ovarian cancer aggressiveness—codon lengths can vary between 24 and 30 repeats. The shorter the track, the shorter the time to disease recurrence compared to patients with longer sequences [88].

- Pancreatic cancer: AIB1 is rarely expressed in normal pancreas ducts (<6% of patients) yet is increased in pancreatitis and high-grade neoplasia between 15 and 20% of samples. Finally, upon progressing to pancreatic ductal adenocarcinoma (PDAC), nearly 65% of patients are positive for AIB1 mRNA and protein. Some patients also present with increased copy number, which may explain some, but not all of the overexpression of AIB1. Of note, the AIB1 Δ 4 isoform is present in pancreatic cancer cell lines, suggesting it acts not only through dysregulated hormone receptor signaling, but may also be playing unexplored roles [89]. AIB1 has also been shown to increase inflammatory conditions by upregulating CXCL1, CXCL2, and CXCL5 during disease development in a mouse model [90]. This may partially explain why the increase of mRNA and protein in pancreatitis and early stage disease is selected for as PDAC progresses in patients.
- Prostate cancer: levels of AIB1 are associated with severity/grade of prostate cancer. Higher levels across all stages are a negative prognostic marker in recurrence free survival. The Kaplan Meyer curves are similar for PSA and nuclear AIB1, suggesting it could serve as a biomarker for disease prognosis and progression [46, 81]. Of note, the same polymorphic CAG sequence that may be prognostically relevant to ovarian cancer may be relevant to prostate cancer—a case study of Chinese men suggested an optimal length of mid-quantity CAG repeats [91]. However, these data need to be repeated in a population with a higher incidence and risk of the disease.
- Colorectal cancer: overexpression of AIB1 was detected in 35% of samples, and amplification of the NCOA3 gene was detected in 10% of patients with colorectal cancer [92]. Levels not only varied significantly when compared to normal tissue, but also significantly increased by tumor grade [82]. One potential role of AIB1 in colorectal cancer is interaction with Estrogen Receptor Beta, which is expressed in CRC. In 110 patients, increasing grade of lesion showed significant upregulation in the levels of expression of AIB1, ER β , and SRC-2. Paradoxically, the same study noted both an increase in invasion associated with higher AIB1 levels, but an increase in survival outcome [93]. AIB1 $^{-/-}$ mice were also unsusceptible to colorectal cancer induction by azoxymethane/dextran sodium sulfate treatment [94]. Clearly, more data is needed to explain the role of AIB1 in colorectal cancer, and whether AIB1 may modulate pro- and anti-tumorigenic behavior.

7.3 AIB1 promotes metastasis

As AIB1 acts to potentiate a variety of signaling cascades, it contributes not only to the growth of the primary cancerous lesion, but also promotes metastasis to distant sites. AIB1 loss suppressed lung metastasis in MMTV-PyMT breast cancer models, significantly reducing the ability of the cells to metastasis. Following transplantation of the AIB1 $^{-/-}$ tumors from knockout mice to wild-type PyMT mice, metastasis was completely lost [74]. Pancreatic and breast tumors formed in AIB1 $^{-/-}$ also exhibit a more epithelial, E-cad high tumor phenotype, suggesting

a repression of epithelial-mesenchymal transition [74]. This is reflected in patient data, where more PDAC patients samples with lymph node metastasis (68%) stain positive for nuclear AIB1, as opposed to patients without metastasis, who also stain negative for AIB1 (42%) [95]. Similarly, in papillary thyroid cancer, levels of AIB1 positive increase significantly from high grade lymph node positive disease (73.2%), compared to non-metastatic disease (41.2%) [96]. Patient samples highlight the role of AIB1 in metastasis, as presence of nuclear staining not only correlates with increased disease grade, but metastasis rate.

In molecular studies, specific transcription factor interactions with AIB1 have been identified as integral to promoting metastasis. The ERK3 mediated interaction of AIB1 with the Ets family member PEA3 results in the oncogenic transcription of matrix metalloproteinases MMP2 and MMP9, which promote an EMT phenotype and destruction of the surrounding extracellular matrix, leading to invasion [74]. Reduction of ERK3 or AIB1 by shRNA attenuates metastasis in lung cancer models; unsurprisingly ERK3 is also upregulated in lung cancer clinical samples [71]. Similarly, AIB1 interaction with AP-1 upregulates MMP7 and MMP10, leading to increased metastasis in breast cancer, regardless of hormone receptor status. Alternatively, AIB1 binds to the promoters of Notch intracellular domain targets to enhance Notch proliferative signaling and effecting cell cycle progression in colorectal cancer. AIB1 reduction by genetic knockout reduced the Notch targets HES1 as well as Cyclins (likely controlled by E2F1-AIB1, however). Reduction of AIB1 levels yields significantly reduced tumor burden and lung metastasis [94].

Finally, an underexplored area of AIB1 activity may be its role in promoting invasion and migration by facilitating oncogenic transcription factor cooperation. Recently, AIB1 was shown to be recruited to larger TEAD and AP-1 transcription factor complexes, and promote cooperative transcription of DOCK4 and DOCK9, thereby increasing mobility [59]. Endogenous TEAD and AP-1 share a significant degree of genomic co-enriched ChIP-seq peaks, and it remains to be seen exactly how much of this is mediated by AIB1, as the two transcription factors are potent oncogenes [62]. Further, transcription factor cooperation has been suggested with TEAD, AP-1, SRF, and other stimuli responsive transcription factors—determining to what extent AIB1 or other NCOA family members are relevant to such signaling networks is an unanswered question [60, 63, 64].

7.4 The role of AIB1 Δ 4 in metastasis

The AIB1 Δ 4 isoform is an N-terminally truncated splice variant that lacks the bHLH-PAS domain. In vitro it localizes significantly less in the nucleus, and shuttles more often between the nucleus and cytoplasm. It may be more readily retained in the cytoplasm because of its interaction with FAK and its role in focal adhesions (see Section 2.2) [25]. Interestingly we have found that metastatic MDA-MB-231 triple negative cell lines and metastatic Colo357 pancreatic ductal adenocarcinoma lines have significantly higher AIB1 Δ 4/AIB1 ratio than their parental counterparts [22]. This aggressive phenotype may be due to loss of transcriptional repression by ANCO1, as we have shown previously [24].

In the cell periphery, AIB1 Δ 4 interacts with FAK in lamellipodia and filopodia in response to EGF stimulation in breast cancer cells. The isoform is unable to bind to the promoters of the MMPs previously described. Instead, it is phosphorylated by PAK1 which facilitates interaction with EGFR and FAK to promote transwell migration in vitro. These findings correlated with in vivo studies showing overexpression AIB1 Δ 4 significantly increased metastasis to the lymph node and lung [25]. These data match our earlier findings in isotypic cell lines and highlight the many ways this understudied isoform may be contributing to normal and disease biology.

7.5 Therapeutics and AIB1

Briefly, AIB1 can contribute to chemo- or hormonal therapeutic resistance, promoting disease relapse. Early indications of this 'escape route' came about when stratifying AIB1 levels and outcomes of ER+ patients treated with tamoxifen, a selective estrogen receptor modulator that is a first line treatment. Patients with high AIB1 levels after receiving treatment had significantly worse outcomes than those with low AIB1 (oddly, patients who never received tamoxifen and also had AIB1 did significantly better; this has yet to be explained). The same study showed conclusively that high levels of Her2/Neu and AIB1 significantly increase the chance of relapse, likely due to crosstalk between growth factor and hormone signaling pathways that thereby circumvent estrogen dependence [42, 97, 98]. Later, it was shown that direct competition between AIB1 and PAX2 occurs on the ERBB2 gene body encoding Her2. Loss of PAX2 reverses repression of Her2 transcription mediated by tamoxifen, and ectopic expression reverts AIB1 mediated tamoxifen resistance [99]. Finally, upregulation of AIB1 has also been shown to be a mechanism of taxol resistance in breast cancer [31].

8. Discussion and conclusion

AIB1 is an oncogene that contributes to disease progression in multiple cancers. It primarily acts to augment transcriptional activity, thereby amplifying pro-proliferative and pro-tumorigenic signaling cascades through binding to its many partners. Though primarily studied for its role in interacting with nuclear hormone receptors, AIB1 has been clearly implicated to play an oncogenic role in hormone independent cancers. Genetic manipulation or removal of the NCOA3 gene has almost universally slowed cancer progression wherever studied, likely by dampening all of the pathways it usually effects. From this role, its clinical importance is obvious, as its elevated levels is usually a negative prognostic marker.

Most clinical studies have underscored the importance of AIB1 in the progression of human disease. Overwhelmingly, expression of AIB1 is correlated with poor prognosis in breast, ovarian, pancreatic, prostate, and colon cancer, as well as increased metastasis [25, 100]. Heightened AIB1 levels have successfully been used in the clinic as a negative prognostic marker in post-menopausal breast cancer [101] and may mark tamoxifen resistance [102]. Further, preclinical investigation of compounds that promote the degradation of AIB1 have shown promising results in attenuating the effects of the oncogene [103, 104]. Interestingly, pharmacological hyperstimulation of AIB1 has also been shown to induce cell death by increasing cell stress [105].

Clarifying the extent to which AIB1 is critical in bridging cooperating transcription factors will further explain intracellular signaling biology and may also provide new targets for therapeutic development. Also, there remains a gap in knowledge surrounding the role of the AIB1 Δ 4 in the nucleus, especially in regard to global binding and transcriptional patterns of the isoform. Finally, elucidating the importance of AIB1 as an effector of growth factor and cytokine signaling may explain its potent oncogenic nature. It is clear that AIB1 may be both a clinically relevant prognostic marker and a promising therapeutic target, as evidenced by the promising preclinical data.

Conflict of interest

The authors declare no conflicts of interest.

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