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A Functional Proteomics Perspective of DBC1 as a Regulator of Transcription

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Abstract

The past few years have seen significant advances in the use of modern proteomics approaches for biological discoveries. Among the fields impacted by proteomics is that of epigenetics, as mass spectrometry-based approaches have allowed the identification and characterization of transcriptional regulators, epigenetic marks, and the constantly evolving epigenetic landscape of a cell in health and disease states. These studies have substantially expanded our understanding of critical genes that mediate cell processes, such as differentiation, cell cycle regulation, and apoptosis. Not surprisingly, a great emphasis has been placed on defining factors that are de-regulated in cancers, in an attempt to define new and specific targets for therapeutic design. Differential gene expression observed during carcinogenesis can be induced by aberrant activities of transcription factors and chromatin remodeling enzymes. Through a series of recent mass spectrometry studies of histone deacetylases and nuclear receptors, Deleted in Breast Cancer 1 (DBC1) has emerged as a master regulator of transcriptional processes. DBC1 acts as a modulator of cellular epigenetic mechanisms and is frequently associated with human metastasis. Through its negative regulation of SIRT1 and HDAC3 deacetylation activities, DBC1 has a broad impact on gene expression, downstream cellular pathways, and associated human diseases. Here, we review the identified roles of DBC1, highlighting the critical contribution of mass spectrometry to these findings. Additionally, we provide a perspective of integrative proteomics approaches that can continue to shed light on the interplay between DBC1 and its protein targets, helping to further define its role in epigenetic modifications and to identify novel targets for cancer therapy.

Keywords: Deleted in breast cancer 1; DBC1; Histone deacetylase; Protein interactions; Sirtuin; Transcription; Cancer prognosis

Introduction

Gene expression is the result of a finely tuned balance between transcription factors and epigenetic mechanisms. Transcription factors are either directly recruited to genomic loci or their targeting is mediated by bridging proteins that specifically bind DNA. On the other hand, the concerted action of chromatin remodeling enzymes dynamically alters the physical state of chromatin, thereby allowing or preventing access to transcription factors and RNA polymerases. The critical roles of the resulting epigenetic landscape of the cell are clearly illustrated by the impact that misregulation of chromatin remodeling enzymes or transcription factors have on progression of numerous human diseases.

Recent years have seen a significant expansion in the use of modern proteomics methods within multidisciplinary studies, integrating techniques from other 'omic' fields (i.e., genomics, metabolomics), molecular biology, and specialized fields (e.g., immunology, virology, developmental biology). These hybrid studies have established mass spectrometry as a critical component of biological discovery. The broad field of epigenetics is a powerful illustration of the impact of mass spectrometry and its promise for future significant discoveries. Mass spectrometry-based approaches have provided the core technologies through which protein interactions and post-translational modifications have been studied in the context of epigenetic profiles and gene expression. Both targeted and high-throughput affinity purification-mass spectrometry (AP-MS) studies [1] have enabled the identification of functional protein interactions and the construction of interaction networks for chromatin remodeling enzymes and transcription factors [2-7]. These studies have significantly increased the current knowledge of the function of these complexes, providing insights into the precise cellular pathways employed in regulating gene expression. Mass spectrometry-based approaches have also provided accurate means for determining histone post-translational modifications (PTMs) that modulate gene expression [8]. Additionally, proteomics-based approaches have helped establish that chromatin remodeling enzymes and transcription factors are also regulated by PTMs. For example, modifications of histone deacetylases (HDAC) have been linked to direct alteration of enzymatic activity, and temporal-spatial control of HDAC functions, such as protein re-localization and cell cycle-dependent transcriptional repressive functions [9-11]. The misregulation of the activity, interactions, or PTMs of chromatin remodeling enzymes can cause large-scale alterations in gene expression that have been functionally linked to numerous diseases, including cancers, neurological disorders, and heart disorders [12-17]. It is, therefore, not surprising that chromatin remodeling enzymes have been a main focus of drug design studies to restore gene expression during disease progression. Additionally, the identification of cellular factors that can regulate the activity of chromatin remodeling enzymes has been of particular interest.

Deleted in breast cancer 1 (DBC1) has recently emerged as a master regulator of transcriptional processes through its regulation of both chromatin remodeling enzymes and transcription factors. It has a unique position in the signaling cascade that impacts gene expression and epigenetic mechanisms, and has been identified as a potential therapeutic target during cancer progression. This review describes

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the emerging cellular functions of DBC1, highlighting the critical contribution of mass spectrometry to these findings. We also provide a perspective of the impact that integrative proteomics approaches can continue to have on defining the full range of DBC1 functions in epigenetics, and its establishment as a potential drug target in cancer treatment.

DBC1 as a Regulator of Chromatin Remodeling Enzymes

Deleted in Breast Cancer 1 (DBC1), also known as p30 DBC or KIAA1967, was first identified over a decade ago by cloning the human chromosomal region 8pq21 that was observed to be homozygously deleted in breast cancer [18]. Its precise role in the cell remained relatively uncharacterized until 2008, when DBC1 started to be established as a negative regulator of selected deacetylases, which are prominent chromatin remodeling enzymes that modulate the epigenetic landscape of the cell. Two parallel studies used AP-MS [1] approaches and identified DBC1 as an interactor and specific inhibitor of the NAD^+-dependent histone deacetylase SIRT1 [19,20]. These reports showed that DBC1 blocked substrate access by directly interacting with the catalytic domain of SIRT1 through its N-terminus, containing a leucine zipper domain (Figure 1) [19]. More recent studies investigating the mechanism of inhibition showed that DBC1 competed with a 25 amino acid peptide in the C terminus of SIRT1 for binding to the deacetylase core [21]. The negative regulation of SIRT1 by DBC1 is an important finding with broad impact, as SIRT1 is known to have a multitude of targets and interactions through which it modulates housekeeping cellular pathways. For example, DBC1-dependent inhibition of SIRT1 activity and genotoxic stress led to the hyperacetylation of the SIRT1 target p53 and activation of the apoptotic pathway [20]. Inhibition of SIRT1 also caused an increase in the acetylation of FOXO transcription factors, leading to the down-regulation of stress survival genes [19]. Overall, these results showed that DBC1, as a potent inhibitor of SIRT1, acted to promote apoptosis, operating as a tumor suppressor to prevent cell survival under stress conditions.

The role of DBC1 as an inhibitor of deacetylases was further strengthened by the later discovery that DBC1 also negatively regulates the enzymatic activity of HDAC3, another member of the histone deacetylase family [22]. The N terminal region of DBC1 directly interacts with the C terminal unique region of HDAC3 to inhibit its catalytic activity (Figure 1). Overexpression of DBC1 prevented HDAC3 mediated deacetylation of the transcription factor MEF2D, by competing with MEF2D for binding to HDAC3 [22]. Additionally, our mass spectrometry-based global interactome studies of human histone deacetylases have identified DBC1 as an interaction partner for other deacetylases, including SIRT7 [3], HDAC5 [9] and HDAC9. Altogether, these recent studies indicate that DBC1 may act as a global regulator of deacetylase activity in the cell, thereby controlling the expression of numerous genes and their downstream pathways.

The connection between DBC1 and chromatin remodeling was also expanded to other enzymes, as DBC1 was shown to inhibit the activity of the histone methyltransferase Suv39H1, which is responsible for heterochromatin formation [23]. The N-terminal domain of DBC1 was implicated in binding to the pre-SET and catalytic SET domains of Suv39H1 (Figure 1). Interestingly, SIRT1 also interacts with Suv39H1: it binds to and deacetylates the N-terminal chromodomain, increasing Suv39H1 methyltransferase activity [24]. The binding of DBC1 to Suv39H1 disrupted the SIRT1-Suv39H1 activated complex [23], and relieved the suppression of the E2F1 gene via Rb-recruited Suv39H1, suggesting that DBC1 can also play a role in cell proliferation [25].

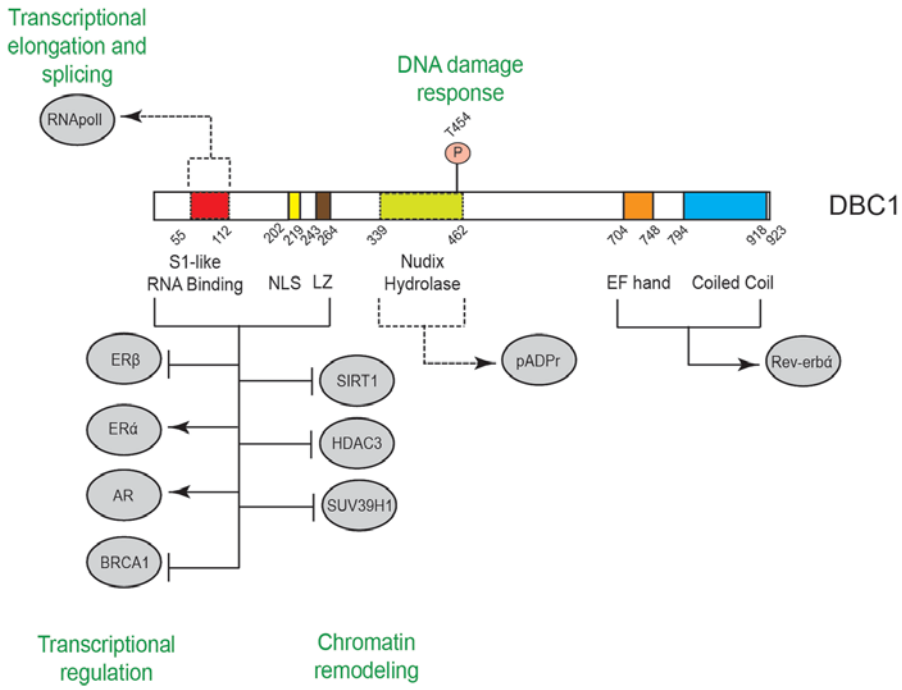

Figure 1: Domain structure, interacting partners, and downstream functions of DBC1. Boundaries for the nuclear localization signal (NLS, yellow), leucine zipper (LZ, brown), EF hand (orange), and coiled coil (blue) regions are indicated. Dotted lines represent predicted domains for RNA binding (red) and Nudix hydrolase (green) regions. Numbers refer to amino acid positions. The domain-dependent interactions and their biological effect are represented, pointing to DBC1 functions as a transcriptional co-factor via its activation or inhibition of chromatin remodeling enzymes and transcription factors. The phosphorylation at T454 residue (pink) in response to DNA damage is indicated.

Taken together, these studies have highlighted DBC1 as an inhibitor of selected chromatin remodeling enzymes (Table 1), thereby indirectly controlling epigenetic histone modifications, gene expression, and downstream cellular pathways.

Mass Spectrometry-Based Approaches Led to the Identification of DBC1-Nuclear Receptor Complexes

Protein members of the nuclear receptor superfamily are frequently misregulated during cancer progression due to their involvement in the transcriptional control of a variety of genes responsible for development, metabolism and proliferation. A large emphasis has hence been placed on identifying co-factors that impact the activity of these proteins. Several concurrent studies aimed at characterizing nuclear receptors led to the co-isolation of DBC1, hence establishing it as an important regulator of transcription activity (Table 1). Through a proteomics-based screen of ligand-dependent Androgen Receptor (AR) binding partners, Fu et al. [26] showed that AR specifically interacted with the N-terminal region of DBC1 (Figure 1). DBC1 binding was required to stimulate chromatin binding and transcriptional activity of AR in both *Xenopus* oocytes and LnCAP prostate cancer cells. Surprisingly, a change in DBC1 levels was not observed in prostate cancer specimens, suggesting that it may not correlate with the AR activity in the context of oncogenesis [26]. DBC1 was also shown to directly interact with the estrogen receptors ERα and ERβ [27-29]. Using an AP-MS approach, Trauernicht et al. [28] identified DBC1 as a ligand-independent binding partner of ERα. The N-terminal domain of DBC1 was again found as the point of contact for direct binding to the receptor, acting to stabilize the protein levels of ERα (Figure 1). In contrast to the apoptosis-promoting DBC1-SIRT1 interaction, DBC1 bound to ERα promoted cell survival and proliferation of breast cancer cells [28]. These observations were further expanded as another proteomic study showed that, in addition to binding to ERα, DBC1 concomitantly binds to CCAR1 (the cell cycle and apoptosis regulator 1), which functions as a co-activator of ERα to stimulate estrogen-dependent proliferation in breast cancer cells [29,30]. DBC1 promoted the estrogen-dependent recruitment of ERα to a subset of target genes, resulting in breast cancer cell survival and growth [29]. The above studies showed that DBC1 was required for ERα dependent cell survival, although it is not yet clear if the DBC1-ERα interaction is ligand dependent or not. DBC1 was also reported to repress ERβ mediated transcription of anti-apoptotic genes, again leading to cell survival [27]. This function was mediated through a direct binding between the N-terminus of DBC1 and the AF2 domain of ERβ (Figure 1), and was dependent on estrogen binding to ERβ [27]. Altogether, these studies suggest that DBC1 acts to promote cell survival through two independent pathways-ERα activation and ERβ suppression.

Intriguingly, the identified DBC1 interactions with nuclear receptors may also be connected to tumor suppression. An AP-MS-based approach led to the identification of DBC1 as a component of the nuclear receptor co-regulator (NRC) interacting factor 1 (NIF-1) complex [31]. Within this complex, DBC1 was required for the activation of retinoic acid receptor (RARα), triggering the transcriptional up-regulation of RARα target genes, such as *Sox9* and *HoxA1*, in MCF-7 breast cancer cells [31]. As *Sox9* is required for RARα-mediated inhibition of cancer cell growth, these results point to a role for DBC1 as a tumor suppressor.

DBC1 was also shown to interact with the chicken ovalbumin upstream promoter transcription factor 1 (COUP-TF1) nuclear receptor and NCoR1 [30]. Both the N- and C-termini were necessary for binding to NCoR1; however, only the N-terminus of DBC1 was required for the COUP-TF1 interaction (Figure 1). Interaction with DBC1 was required for COUP-TF1 mediated repression of *TNFAI8*. Upon TNFα treatment, COUP-TF1 protein levels are down-regulated, leading to an increase in TNFAIP8 and an inhibition of apoptosis. In an independent study, DBC1 was found to trigger apoptosis through mislocalization to the cytoplasm following the cleavage of its N-terminal NLS bearing domain [32]. These findings suggest opposing roles for DBC1 in TNFα-mediated apoptosis, whether acting in a pro-apoptotic manner through mislocalization or in an anti-apoptotic pathway through COUP-TF1 interaction. There are likely other still unidentified factors that interact with DBC1 to modulate its functions in the cytoplasm or in *TNFAIP8* transcription.

The role of DBC1 in regulating the functions of nuclear receptors was recently further expanded, as DBC1 was shown to regulate the protein levels of the Rev-erbα nuclear receptor, impacting on downstream transcription of circadian genes [33]. Rev-erbα is responsible for integrating circadian rhythm with metabolism and differentiation. DBC1 binding to Rev-erbα acted to protect the receptor from ubiquitination and subsequent degradation [33]. In response to serum shock, a reduction in DBC1 levels caused Rev-erbα degradation and transcriptional up-regulation of downstream targets, including *BmalI* [33]. This was the first example of an interaction mediated through the C-terminus of DBC1 (Figure 1), although its N-terminal helped to stabilize Rev-erbα. As the Rev-erbα receptor is known to rely on binding to HDAC3 and its associated repressor complex NCoR for targeting to genomic loci, these findings raise the question of whether these proteins together form a regulatory complex.

It is clear that the integration of mass spectrometry-based approaches for studying protein interactions with biochemistry and molecular biology functional analyses has expanded our understanding of the diverse roles of protein interactions. Importantly, such studies have helped establish that a protein can carry out a multitude of functions, sometimes opposing functions, through interactions with different functional protein complexes. As described above, DBC1 is an excellent example, where the increasing knowledge of its interactions has helped elucidate its distinct roles in promoting or repressing tumorigenesis through a direct regulation of transcription (Table 1).

DBC1 is a Mediator of Cellular Responses to Stress

The inability of a cell to mount an appropriate response to cellular stresses, such as heat shock or DNA damage, is a well-established trigger of apoptosis. DBC1 has a prime role in regulating cell survival and proliferation through the regulation of transcription factors and the direct inhibition of SIRT1 activity. As discussed above, SIRT1, through its inhibition of p53, acts as a master regulator to maintain an anti-apoptotic response to stress stimuli. But until recently, it remained elusive if DBC1 can function in alternate pathways to regulate stress response. The first insight into this came from a study that investigated the HSF1-mediated transcriptional response to heat shock stress. Increased HSF1 acetylation is accompanied by a decreased ability to bind to *hsp70* promoter sites, therefore leading to a decreased *hsp70* expression. In this case, SIRT1-mediated deacetylated HSF1 enhanced its DNA binding ability. Increased DBC1 levels were correlated with HSF1 acetylation, ultimately leading to a decrease in heat shock response [34]. It remains to be determined if DBC1 directly interacts with HSF1, or whether its impact on heat shock response is through inhibition of SIRT1.

In response to a different type of cellular stress, UV-induced DNA damage, DBC1 was shown to interact with BRCA1 (Figure 1)

	DBC1 Interactions	Type of Study	Effect of DBC1	Reference
Functional Relevance Demonstrated	AR	Targeted	Activator	[26]
	BRCA1	Targeted	Inhibitor	[35]
	COUP-TF1	Targeted	Activator	[31]
	ERα	Targeted	Activator	[28,29]
	ERβ	Targeted	Inhibitor	[27]
	HDAC3	Targeted	Inhibitor	[22]
	MYC	Targeted	Activator	[59]
	RARα	Targeted	Activator	[30]
	Rev-erbα	Targeted	Activator	[33]
	RNA polII	Targeted	Activator	[44,60]
	SIRT1	Targeted	Inhibitor	[19,20]
	SUV39H1	Targeted	Inhibitor	[23]
Function Unknown	ACTR3B	High Throughput*	Unknown	[61]
	AIMP1	High Throughput*	Unknown	[62]
	AIMP2	High Throughput*	Unknown	[62]
	ASH2L	Targeted	NIF-1 Complex	[30]
	BAG2	High Throughput	Unknown	[63]
	C11ORF30/EMSY	Targeted	NIF-1 Complex	[30]
	CCNA1	High Throughput*	Unknown	[61]
	CDK7	High Throughput*	Unknown	[64]
	CDK9	High Throughput*	Unknown	[62,64]
	CEP170	High Throughput*	Unknown	[65]
	CIAO1	High Throughput*	Unknown	[61]
	CNOT1	High Throughput*	Unknown	[62]
	COPS5	High Throughput*	Unknown	[66]
	CUL3	High Throughput*	Unknown	[66]
	DCTN3	High Throughput*	Unknown	[62]
	E1F2S3	High Throughput*	Unknown	[62]
	EEF1A1	High Throughput*	Unknown	[67]
	EEF1E1	High Throughput*	Unknown	[62]
	FN1	High Throughput*	Unknown	[68]
	GBF1	High Throughput*	Unknown	[62]
	GSTK1	High Throughput*	Unknown	[61]
	HDAC5	Targeted	Unknown	[8]
	HNRNPA1	High Throughput	Unknown	[62]
	HSPA8	High Throughput	Unknown	[63]
	HTRA2	Targeted	Unknown	[69]
	IQGAP1	High Throughput	Unknown	[62]
	ITGA4	Targeted	Unknown	[70]
	KBTBD7	High Throughput*	Unknown	[71]
	KIAA0101	High Throughput*	Unknown	[72]
	MAGEA6	High Throughput*	Unknown	[61]
	MAGED1	High Throughput*	Unknown	[61]
	MEPCE	High Throughput*	Unknown	[64]
	METTL21B	Targeted	Unknown	[73]

	METTL23	Targeted	Unknown	[73]
	NCAPD2	High Throughput*	Unknown	[62]
	NCAPG	High Throughput*	Unknown	[62]
	NEK6	High Throughput*	Unknown	[61]
	pADPr	Targeted	Unknown	[41]
	PLEC	High Throughput*	Unknown	[62]
	PNMA1	Targeted	Unknown	[74]
	PRMT5	High Throughput	Unknown	[63]
	QARS	High Throughput*	Unknown	[62]
	RAD21	Targeted	Unknown	[75]
	RARS	High Throughput*	Unknown	[62]
	RbBP5	Targeted	NIF-1 Complex	[30]
	RPL27A	High Throughput*	Unknown	[62]
	RUVBL2	High Throughput*	Unknown	[64]
	SIRT7	High Throughput*	Unknown	[2]
	SF3A2	Targeted	Unknown	[76]
	SMC2	High Throughput*	Unknown	[62]
	SMC4	High Throughput*	Unknown	[62]
	TH1L	High Throughput*	Unknown	[61]
	WRAP73	High Throughput*	Unknown	[61]

Table 1: DBC1 protein interactions. Validated and putative DBC1 interactions identified via targeted or high-throughput mass spectrometry-based studies are indicated. *refers to large-scale mass spectrometry-based interactome studies.

to block its activation of SIRT1 [35]. Through this opposing control of SIRT1 activity, the DBC1-BRCA1 complex triggered an increase in the acetylation levels of the SIRT1 substrate p53, suggesting a shift to an apoptotic response [35]. The DBC1-dependent modulation of the SIRT1 target p53 was also shown to be regulated by post-translational modification (PTM). DBC1 phosphorylation at T454 was first predicted computationally and via a phospho-proteomic screen [36,37], and then observed in cell culture following DNA damage [38]. This phosphorylation acted as a toggle of DBC1 functions, leading to an increased association with SIRT1, releasing p53, and activating the apoptotic response to DNA damage. While this was the only functionally characterized DBC1 modification to date [38], these studies emphasize the importance of identifying DBC1 PTMs and their possible impact on modulating its diverse roles. A different study examined the role of DBC1 upon DNA damage response in cells lacking SIRT1. This study reported that a loss of DBC1 led to a failure in activating the DNA damage checkpoint and JNK pathway for cell survival, following UV-induced DNA damage. These findings suggest that DBC1 promotes genome stability in response to DNA damage, albeit in a SIRT1-independent manner [39].

The above studies implicate DBC1 as a regulator in maintaining cellular homeostasis by modulating stress response pathways. In agreement with its multi-faceted interactions with various transcription factors, DBC1 can act to either block or promote cell survival during stress conditions.

DBC1 Structure Provides an Insight into its Non-Transcriptional Functions

Further insights into the function of DBC1 can be derived from careful examination of its structure. DBC1 contains an N-terminus NLS, a Leucine Zipper (LZ) domain, and a C-terminus inactive EF hand domain and coiled coil region (Figure 1) [19,20]. Further structural predictions by the Hidden Markov Model, which used DBC1 paralogs from other organisms, revealed an inactive Nudix hydrolase domain, probably involved in binding NAD or ADP ribose sugars [40]. This may be relevant in the context of the identification of DBC1 as a member of a poly (ADP-ribose) (pADPr) complex (Figure 1, Table 1) [41]. Although this proteomic study did not verify if the interaction between PARP and DBC1 was direct, it may suggest that the Nudix domain of DBC1 is involved in the binding. PARPs enzymatically polymerize the ADP-ribosyl moiety of NAD into pADPr polymers; pADRr turnover is important for cell cycle progression, DNA repair, and apoptosis, as these polymers are important signals of DNA strand breaks, helping to recruit additional DNA repair machinery [42,43]. This DBC1-PARP interaction could provide yet another link to damage-response pathways and tumorigenesis.

The structure prediction analysis also indicated the presence of an S1-like RNA binding domain in DBC1 (Figure 1) [40]. Interestingly, a recent proteomic study, aimed at identifying factors affecting hnRNP proteins, identified DBIRD, a complex containing DBC1 and ZIRD (ZNF326) [44]. Both subunits of DBIRD bind RNApolII in an RNA-independent manner, suggesting that this interaction may be direct. Also, DBIRD interacts with the core mRNP in an RNA-dependent manner. Taken together, these data, and the observation that a depletion of DBIRD causes a higher rate of exon inclusion, suggest that DBIRD functions at the interface between transcriptional elongation via RNApolII and assembly of mRNA particles on the pre-mRNA [44]. It is hence possible that DBC1 indeed does use its RNA binding domain to recognize and bind to these sites. Further insights into the structure of DBC1 will help to provide the molecular details of its diverse cellular functions.

Emerging Role for DBC1 as a Biomarker

Several studies show that, despite its identification as a homozygous deletion in breast carcinomas [18], DBC1 expression is up-regulated in breast cancer cells [45]. Along with elevated SIRT1 expression, this can serve as a poor survival indicator for breast carcinoma [45]. However, Sung et al. [46] reported that, although both DBC1 and SIRT1 protein levels were increased in breast cancer specimens, their correlation was absent. This lack of correlation was suggested as a possible prognostic marker for tumor metastasis. In support of this observation, other studies have reported that the DBC1-SIRT1 interaction may be reduced in breast cancer [47]. Further support for this argument comes from research on breast core-needle biopsy specimens, where DBC1 and SIRT1 proteins were both found to be positively expressed, as examined by immune histochemistry; over-expression of DBC1 was observed to be significantly associated with tumor nuclear grade and a decreased HER2 expression. However, a significant correlation between SIRT1 and DBC1 was again absent [48].

Interestingly, several other studies also reported that, despite the role of DBC1 as a tumor suppressor, increased DBC1 levels were associated with tumor metastasis. These findings indicated that DBC1 can function as an oncogene, thus making it an attractive target for therapeutic design. Specifically, DBC1 was found to be up-regulated in esophageal squamous cell carcinoma (ESCC) tissue samples and cell lines. A reduction in DBC1 levels by siRNA reduced the migration and invasive properties of ESCC cell lines, suggesting that DBC1 acted as a tumor promoter [49]. DBC1 was also overexpressed in colorectal cancer, as indicated by immunohistochemical staining and tissue microarray analysis, and was associated with tumor metastasis, thus serving as a poor prognostic factor for survival [50]. In liver, human hepatocellular carcinoma (HCC), DBC1 and SIRT1 proteins were also significantly up-regulated, though, in this case, their levels were correlated; surprisingly, DBC1 did not affect SIRT1 activity. However, inactivation of DBC1 or SIRT1 reduced HCC cell viability, again indicating their role in tumor progression [51].

While recent studies have further strengthened the role of DBC1 in cancers, its impact as a tumor suppressor or promoter seems to be controversial and likely cancer-type or cell-type specific. For example, Pancreatic Ductal Adenocarcinoma (PDAC) cell lines were marked by a decrease in DBC1 levels by both microarray and immunohistochemical staining, although SIRT1 levels were unchanged [52]. An immunohistological examination of gastric adenocarcinoma tissues from patients showed that both SIRT1 and DBC1 associated with a lower histological grade of cancer, as well as an absence of lymphatic invasion [53]. However, a previous study indicated that these proteins were associated with a poor prognosis for gastric carcinoma [54], once again hindering the determination of the precise correlation of DBC1 levels with tumor progression in this tissue. In another example, patients with lung squamous cell carcinoma (lung SCC) displayed lower levels of DBC1 expression, whereas in lung adenocarcinoma (Lung AD) patients and cell lines, DBC1 levels were high and accompanied by an increase in p53 acetylation. In both cases, however, the levels of hypermethylation in cancer 1 (HIC1) were maintained low, suggesting that two different mechanisms control HIC1 expression, despite the differences in p53 acetylation [55].

Table 2 summarizes the current knowledge on DBC1 expression in various cancer tissues. While a consensus is being formed that the DBC1 expression level is an important consideration in cancer prognosis, additional investigations will be required to determine the exact roles of DBC1 in different types of cancers and cancer cell lines. Such studies will help determine if DBC1 can be used as a target for design of therapeutic context-specific inhibitors.

Future Perspectives for DBC1

As indicated above, DBC1 has recently emerged as a critical regulator of chromatin remodeling enzymes and transcription factors, and as a putative powerful target for therapeutic intervention. Its roles in regulating histone deacetylase activities are particularly important when thinking about clinical applications. As misregulation of histone deacetylases is a significant contributor to numerous human diseases, small molecules that inhibit Histone Deacetylase Activity (HDACi) are constantly being developed and already utilized for cancer treatment [15,56]. However, one major drawback of these inhibitors is that these compounds usually act as broad-range inhibitors, without ability to differentiate between various HDAC classes. Hence, the search for more specific and more effective inhibitors continues. Even in the rare cases when inhibitors specifically target only one enzyme, this inhibition can still impact numerous cellular pathways with diverse functions. HDACs and SIRTs carry out a broad range of biological functions through interactions with numerous protein complexes with distinct functions. This is clearly reflected in a recent study in which we built the first global interactome network for all eleven human histone deacetylases. To bring direct relevance to their roles in cancer, these HDAC interactions were studied in T cells. HDACi are currently used in treatment of cutaneous T-cell lymphomas (CTCL). A recent study showed that HDACi can target non-catalytic subunits of HDAC complexes, providing an alternate strategy for designing enzyme-specific inhibitors [56]. In particular, DBC1 acts not only as a specific deacetylase inhibitor, but also as a regulator of several nuclear receptors that have been correlated with the onset and progression of tumorigenesis. Consequently, DBC1 could potentially emerge as a therapeutic target for designing tissue-specific treatment.

To further establish DBC1 as a therapeutic target, it will be critical to fully understand its functions, as well as its means of regulation. So far, no study has been directed towards identifying DBC1 interactions and modifications in an unbiased fashion to determine the full range of DBC1 targets. It is likely that there are yet more unidentified factors that contribute to determining its function in transcriptional processes. Similarly, only one posttranslational modification has been identified for DBC1, i.e., its phosphorylation in response to genotoxic stress [38]. There may be other post-translational modifications that govern the stability, localization, or activity of DBC1. Therefore, future studies using mass spectrometry-based approaches will be valuable

Cancer Type	DBC1 Expression	References
Breast cancer	Increased	[39,45-48]
Colorectal cancer	Increased	[50]
Esophageal squamous cell carcinoma (ESCC)	Increased	[49]
Gastric carcinoma	Increased	[54]
Lung adenocarcinoma (Lung AD)	Increased	[55]
Liver human hepatocellular carcinoma (HCC)	Increased	[51]
Prostate cancer	No change	[26]
Breast cancer	Decreased	[18]
Lung squamous cell carcinoma (Lung SCC)	Decreased	[55]
Pancreatic Ductal Adenocarcinoma (PDAC)	Decreased	[52]

Table 2: Changes in DBC1 levels associated with tumor progression.

at defining the roles of DBC1. Such approaches may also involve chemoproteomic studies. Recent reports have shown a remarkable promise for chemoproteomics approaches for the characterization and identification of target-specific inhibitors [57,58]. The known functions of DBC1 place it in a unique position for the exploitation of these approaches. DBC1 as a specific deacetylase inhibitor can be used to improve the design and probe for novel and effective HDACi, while DBC1 itself can be used as a probe to screen a library, in an attempt to identify its inhibitors.

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