The emerging role of Cables1 in cancer and other diseases

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Abbreviations: Cables1, Cdk5 and Abl enzyme substrate 1; Cdk5s, cyclin-dependent kinase; CDKN1A, cyclin-dependent kinase inhibitor 1A; DCC, deleted in colorectal carcinoma; MEFs, mouse embryonic fibroblasts; PSMA3, proteasome subunit alpha type 3; Trap, tudor repeat associator with Pctaire 2.
Abstract

Cdk5 and Abl enzyme substrate (Cables) 1 is adaptor protein to link cyclin-dependent kinase (Cdks) with nonreceptor tyrosine kinases and regulate the activity of Cdks by enhancing their Y15 phosphorylation. The emerging evidences also show Cables1 can interact with p53 family proteins, 14-3-3, β-catenin and so on, suggesting Cables1 may be a signaling hub to regulate cell growth. Abnormal expression of Cables1 has been observed in multiple types of cancers and other disease. In this review, we summarize the characteristics of Cables1, and highlight the molecular mechanisms through which Cables1 regulates the development of cancer and other disease. Finally, we discuss the future challenges to demonstrate the role and potential application of Cables1 in cancer and other disease.
Introduction

Cables1 (Cdk5 and Abl enzyme substrate 1), also named as ik3-1, is initially identified as a novel cyclin-dependent kinase (Cdk)-binding protein to connect Cdks (Cdk2, Cdk3 and Cdk5) and nonreceptor tyrosine kinases (Abl and Wee1) and regulate the activity of Cdks by enhancing their Y15 phosphorylation. In neurons, Cables1 promotes Abl to phosphorylate Cdk5 at Y15, leading to an increase of Cdk5 kinase activity and outgrowth of neurite (Zukerberg et al., 2000). In the proliferating cells, Cables1 enhances Wee1 to phosphorylate Cdk5 at Y15, resulting in a decrease of Cdk2 kinase activity and inhibition of cell proliferation (Wu et al., 2001). The mRNA expression of Cables1 among adult mouse tissues is highest in brain, and also present in lung liver and kidney, but almost undetectable in heart, spleen, smooth muscle and testis (Zukerberg et al., 2000). It is now increasingly apparent that Cables1 has crucial roles in the development of both tumor and neuron. The differential interaction of Cables1 with p53 family proteins including p53, p63 and p73 makes Cables1 as multi-faceted regulator of cell death (Tsuji et al., 2002; Wang et al., 2010). The observation of high frequent Cables1 loss in multiple types of cancers and increased incidence of endometrial cancer in Cables1−/− mice intensively indicates that Cables1 may have a suppressive effect on tumorigenesis (Dong et al., 2003; Park do et al., 2007; Tan et al., 2003; Zukerberg et al., 2004). Recently, our work reveals a novel tumor suppressor function of Cables1 by antagonizing proteasome subunit alpha type 3 (PSMA3) to increase the stability of cell cycle regulator p21 proteins (Shi et al., 2015a). Besides, we have showed a dynamic regulatory system by which activated Akt is able to phosphorylate Cables1 and recruit 14-3-3 to neutralize the tumor suppressor function of Cables1, suggesting the Akt/Cables1 interface may represent a novel anticancer target for
potential therapeutic interventions (Shi et al., 2015b). As shown in the history of Cables1 (Fig. 1), much work has been done to explore the biological function of Cables1 in the physiological and pathological conditions over the past decade. Here, we reviewed a comprehensive summary of the role of Cables1 in cancer and other diseases.

**The characteristics of Cables1**

Human Cables1 gene is located on 18q11.2-12.1 (Matsuoka et al., 2000; Wu et al., 2001; Zukerberg et al., 2000). The loss or deletion of the chromosome 18q, where previously characterized putative tumor suppressor genes DCC, Smad2, and Smad4 are located, is one of the common chromosomal abnormalities in various types of cancers including colorectal cancer, ovarian cancer, lung cancer and endometrial cancer, etc. (Bonifant and Waldman, 2005; Morin, 2008; Park et al., 2007; Tan et al., 2003). Human Cables1 gene encodes a 633 amino acid protein, predominantly localizing in the nucleus (Wu et al., 2001). There are three classic nuclear localization signals composed of three basic amino acids and either histidine or proline at amino acid positions 48, 54, and 406 of Cables1 protein (Christie et al., 2016; Hicks and Raikhel, 1995; Wu et al., 2001). Cables1 protein also has six minimal SH3 domain binding motifs, and two of which contain additional proline residues (PPXP and PXPP) in the N-terminal of the protein with good potential for binding to the SH3 domain of the c-Abl and c-Src (Fig 2. A) (Cicchetti et al., 1992; Dai and Pendergast, 1995; Ren et al., 1993; Shi et al., 1995; Zukerberg et al., 2000). In addition, Cables1 contains two tyrosine-based sorting motifs (YXXLE), which are important in axonal growth cone sorting (Zukerberg et al., 2000). The N-terminal 98 amino acid region of Cables1 is necessary for Cables1-Trap interaction (Yamochi et al., 2001a). The C-terminal of Cables1 has a cyclin-like domain, which is a key
element of the interaction of Cables1 with Cdk s. The 3D structure of Cables1 cyclin-like
domain has previewed through SWISS-MODEL according to 3D structures of Cyclin-A2 and
Cyclin-T2 (Arnold et al., 2006; Benkert et al., 2011; Biasini et al., 2014), and their
homologous sequence identities are 20.28% and 20.51%, respectively (Fig2. B, C, D and E).

The regulation of Cables1 at the transcriptional and post-transcriptional level has still largely
unknown. Progesterone can induce Cables1 mRNA expression in low-grade endometrial cancer
cells but not in high-grade endometrial cancer cells, and the progesterone receptor antagonist is
able to abrogate the progesterone-induced increase of Cables1 (DeBernardo et al., 2005). Additionally, knockdown of the Argonaute protein PiwiL2 reduces the expression of Cables 1
in adult mouse mesenchymal stem cells (Wu et al., 2010). Protein phosphorylation is a common
post-translational modification of proteins, causing the structural conformation change to
modify the function of proteins (Ciesla et al., 2011). Cables1 can be phosphorylated by Cdk3 at
S274 of mouse Cables1 (S313 of human Cables1) (Fig 2. A) (Yamochi et al., 2001b). Akt also
phosphorylates Cables1 at T309 and T415 sites, which recruits 14-3-3 binding (Shi et al.,
2015b).

The role of Cables1 in cancer

Loss of Cables1 expression is detected with high frequency in human lung, colon, ovarian and
endometrial cancers, but not in breast and pancreas cancers (Dong et al., 2003; Park do et al.,
2007; Tan et al., 2003; Zukerberg et al., 2004) (Tables 1). In colorectal and ovarian cancers,
promoter methylation and loss of heterozygosity are two main reasons of Cables gene
inactivation, and overexpression of Cables1 significantly inhibits the growth of cancer cells.
(Park et al., 2007; Sakamoto et al., 2008). In addition, there are aberrant splicing of Cables1
genes in endometrial and colon cancers (Zhang et al., 2005). Knockout Cables1 in mice leads to an increased incidence of endometrial cancer and a reduced survival rate after unopposed estrogen treatment and an increased incidence of colorectal cancer caused by 1,2-dimethylhydrazine (Kirley et al., 2005a; Zukerberg et al., 2004). MEFs isolated from these Cables1−/− mice show an increased rate of growth, delayed onset of senescence and decreased serum dependence (Kirley et al., 2005b). In the ApcMin/+ mouse model, loss of Cables1 promotes tumor progression and activates the Wnt/β-catenin signaling pathway (Arnason et al., 2013). Together, these evidences strongly indicate that Cables1 functions as a tumor suppressor.

Mechanistically, Cables1 can promote the binding and phosphorylation of Wee1 to Cdk2 (Wu et al., 2001). Cdk2 is a small serine/threonine kinase that regulates cell cycle progression, and its activity is tightly controlled by phosphorylation (Elledge et al., 1992; Hirai et al., 1992; Pagano et al., 1993). Wee1 phosphorylates Cdk2 at Y15, leading to inhibition of Cdk2 activity (Parsons, 1998; Wroble et al., 2007). Therefore, Cables1 can inhibit the proliferating cell growth via enhancing Cdk2 Y15 phosphorylation by Wee1 (Wu et al., 2001), which contributes to its tumor suppressor function. Cables1 also binds with p53 family proteins, including p53, p63 and p73, which plays critical role in tumorigenesis ([Botchkarev and Flores, 2014; Irwin and Kaelin, 2001; Orzol et al., 2015]. In osteosarcoma cells U2OS, ectopically expressed Cables1 potentiates p53-induced cell death but not p73-induced cell death, and coexpression of Cables1 deletion mutant lacking cyclin-like domain inhibits p73-induced cell death but not p53-induced cell death (Tsuji et al., 2002). Cables1 interacts with p63 to protect it from proteasomal degradation, promoting cell death after genotoxic stress (Wang et al., 2010). Recently, we identified a novel function of Cables1 as tumor suppressor by controlling the
protein stability of cyclin-dependent kinase inhibitor 1A (CDKN1A, also known as p21/Cip1) (Shi et al., 2015a). Our data show that Cables1 competes with PSMA3 to bind p21 and protects it from PSMA3-mediated proteasomal degradation, and the expression level of Cables1 is correlated with that of p21 in human lung cancer tissues (Shi et al., 2015a). Additionally, we found a phosphorylation-dependent and 14-3-3-mediated mechanism to regulate the tumor suppressor function of Cables1 (Shi et al., 2015b). Akt directly interacts with and phosphorylates Cables1 to recruit 14-3-3 to the complex, and activated Akt is able to prevent cell apoptosis induced by Cables1 (Shi et al., 2015b).

The role of Cables1 in the other diseases

In the nervous system, Cdk5 and c-Abl play pivotal role during development of neuron (Cancino et al., 2011; Lee et al., 2008). Cables1 is initially identified as an adaptor protein of Cdk5 and c-Abl, and enhances the c-Abl mediated phosphorylation of Y15 of Cdk5, which increases the kinase activity of Cdk5 and promotes neurite outgrowth (Zukerberg et al., 2000). On binding of the secreted axon guidance cue Slit to its Robo receptor, Cables1 is recruited to Robo-associated Abl to form a multimolecular complex through directly binding to N-cadherin-associated β-catenin, which leads to Abl-mediated phosphorylation of β-catenin on Y489 and decreases the affinity of β-catenin with N-cadherin, resulting in loss of N-cadherin function and activation of transcription mediated by β-catenin and the transcription factor Tcf/Lef (Rhee et al., 2007). In a transgenic mouse of agenesis of the corpus callosum, the genomic region containing exon 4 of Cables1 is deleted by transgene insertion, and the truncated Cables1 only containing exons 1-3 indeed impairs callosal formation through dominant negative effect (Mizuno et al., 2014). Additionally, it is reported that Cables1 is
required for embryonic neural development of zebrafish in a mechanism of likely involving interactions with Cdk5 (Groeneweg et al., 2011).

Recently, loss of Cables1 expression is found in around 55% of corticotrope adenomas which finally results in Cushing disease and glucocorticoids resistance, and Cables1 expression can be induced by glucocorticoids in the mouse corticotrope tumor cells AtT-20 (Roussel-Gervais et al., 2016). Knockout Cables1 gene in mice has minimal to no effect on hematopoietic stem cell dynamics, but female Cables1−/− mice show a significant expansion of germline at the expense of oocyte quality throughout adulthood (Lee et al., 2007). Interestingly, Cables1 expression is significantly decreased in both paediatric and adult samples of male genital lichen sclerosus (Edmonds et al., 2011).

Future prospects

The growing understanding of Cables1 functions has already led to the generation of signaling pathways of Cables1 (Fig. 3). The interaction network of Cables1 with Cdns, p53 family proteins, 14-3-3 and β-catenin, etc indicates Cables1 as a signaling hub to regulate cell cycle, cell growth and cell death (Fig. 4). However, there is much left to address. How is Cables1 regulated at the transcriptional level? The transcriptional factors mediated Cables1 expressions are still unknown. Whether any microRNAs or IncRNAs can affect Cables1 expressions? Besides phosphorylation, does Cables1 protein have other post-translational modifications such as acetylation, methylation, ubiquitylation and so on? And is the function of Cables1 affected by these modifications? Due to loss of Cables1 expression in certain types of cancers, it is possible to estimate the value of Cables1 as diagnostic or prognostic biomarker in some cancers. Development of small molecules or technologies, which can specifically induce Cables1
expression to suppress cancer cell proliferation, may provide a new strategy for cancer treatment. These challenging questions supply the exciting research avenues to explore the role and potential application of Cables1 in cancer and other disease in the future.
Authorship contributions

Wrote or contributed to the writing of the manuscript: Jia-Rong Huang, Guang-Mou Tan, Yong Li and Zhi Shi
Reference

Arnason T, Pino MS, Yilmaz O, Kirley SD, Rueda BR, Chung DC and Zukerberg LR (2013) Cables1 is a tumor suppressor gene that regulates intestinal tumor progression in Apc(Min) mice. Cancer Biol Ther 14(7): 672-678.


Cancino GI, Perez de Arce K, Castro PU, Toledo EM, von Bernhardi R and Alvarez AR (2011) c-Abl


Footnotes

Jia-Rong Huang and Guang-Mou Tan contributed equally to this work.

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Figure Legends

**Figure 1. The history of Cables1.** The important events and years of Cables1 have been shown.

**Figure 2. The prediction of 3D structure of Cables1 cyclin-like domain with SWISS-MODEL.** (A) A schematic representation of Human Cables1. (B) The prediction of 3D model of Cables1 cyclin-like domain is according to Cyclin-A2. (C) The alignment of amino acid sequences between cyclin-like domain of Cables1 and Cyclin-A2. (D) The prediction of 3D model of Cables1 cyclin-like domain is according to Cyclin-T2. (E) The alignment of amino acid sequences between cyclin-like domain of Cables1 and Cyclin-T2.

**Figure 3. The signaling pathways of Cables1.** PiwiL2 and progesterone can induce Cables1 expression. Cables1 enhances the Y15 phosphorylation of Cdk2 or Cdk5 by Wee1 or c-Abl respectively, resulting in inhibition of cell proliferation or outgrowth of neurite. Cell death mediated by p53 family proteins and gene transcription mediated by β-catenin can be strengthened by the direct interactions with Cables1. Additionally, Cables1 can be phosphorylated by Cdk3 at S311 and by Akt at T309 and T415 sites, which recruits 14-3-3 binding to promote cell proliferation.

**Figure 4. The interaction network of Cables1.** Black line respects the interactions of Cables1 and the other proteins. Orange line respects the interactions between the proteins that can interact with Cables1. The dot line means the interactions have been not confirmed.
Table 1. Summary of the published Cables1 expression in human tumors.

<table>
<thead>
<tr>
<th>Tumors (n)</th>
<th>Substyles (n)</th>
<th>Percentage of loss (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non–small cell lung cancer (113)</td>
<td></td>
<td>45</td>
<td>(Tan et al., 2003)</td>
</tr>
<tr>
<td>Squamous cell carcinoma (20)</td>
<td></td>
<td>60</td>
<td>(Wu et al., 2001)</td>
</tr>
<tr>
<td>Breast cancer (20)</td>
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<td></td>
</tr>
<tr>
<td>Pancreas cancer (20)</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Colon cancer (20)</td>
<td></td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Colorectal cancer (160)</td>
<td></td>
<td>65</td>
<td>(Park et al., 2007)</td>
</tr>
<tr>
<td>Endometrial cancers (20)</td>
<td></td>
<td>95</td>
<td>(Zukerberg et al., 2004)</td>
</tr>
<tr>
<td>Endometrial cancer (103)</td>
<td>Hyperplasia (22)</td>
<td>77.2</td>
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<td>Endometrioid (54)</td>
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<tr>
<td>Serous (14)</td>
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</tr>
<tr>
<td>Clear cell (13)</td>
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<td>84.6</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer (24)</td>
<td>Serous carcinomas (14)</td>
<td>79</td>
<td>(Dong et al., 2003)</td>
</tr>
<tr>
<td>Endometrioid carcinomas (10)</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer (70)</td>
<td>Serous (26)</td>
<td>96.5</td>
<td>(Sakamoto et al., 2008)</td>
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<tr>
<td>Endometrioid (17)</td>
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<td>94.2</td>
<td></td>
</tr>
<tr>
<td>Clear cell (17)</td>
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<td>Mucinous (6)</td>
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</tr>
<tr>
<td>Transitional (4)</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
A cyclin-like protein associated with Cdk3 through the yeast two-hybrid system was named as ik3-1 (Matsuoka et al., 2000).

The protein from neurons enhancing phosphorylation of Cdk5 by c-Abl was named as Cables1 (Zukerberg et al., 2000).

Cables1 affects the p53- and p73-induced cell death (Tsui et al., 2002).

Loss of Cables1 is associated with the development of endometrial hyperplasia and endometrial cancer (Zukerberg et al., 2004).

Loss of Cables1 can changes the cell characterize (increase growth rate, delay senescence and decrease serum dependence) (Kirley et al., 2005b).

Loss of Cables1 is phosphorylated by the Akt kinase and that serves as substrates for 14-3-3 binding (Shi et al., 2015b).

Loss of Cables1 makes germline expansion at the expense of oocyte quality in adult female mice (Lee et al., 2007).

Cables1 is required for embryonic neural development (Groeneweg et al., 2011).

Cables1 stabilizes p21 protein by antagonizing proteasome subunit alpha type 3 (Shi et al., 2015a).

Cables1 stabilizes p63 to ensure cell death after genotoxic stress (Wang et al., 2010).

Cables1 stabilizes p53 and p73-induced cell death in adult female mice (Lee et al., 2007).
Figure 2

A. 

Cyclin N domain (1-98)

PPXP rich domain

Thr309  Ser313  Thr415

Cyclin box fold region (478-626)

C-terminal

B. 

C. 

D. 

E. 

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N-cadherin
β-catenin
Rebo
Pim1/2
p21
14-3-3
PSMA3
c-Abl
p33
p3
p73
Cdk2
Akt
Cdk5
Wee1
Petaxa2
Trap
β-catenin
N-cadherin
Robo
Pim1/2
p21
14-3-3
PSMA3
Cdk5
Akt
p33
p3
p73
Cdk2
Wee1
Petaxa2
Trap
Figure 4