Deleted in liver cancer protein family in human malignancies (Review)

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Abstract. The Deleted in Liver Cancer (DLC) protein family comprises proteins that exert their function mainly by the Rho GTPase-activating protein (GAP) domain and by regulation of the small GTPases. Since Rho GTPases are key factors in cell proliferation, polarity, cytoskeletal remodeling and migration, the aberrant function of their regulators may lead to cell transformation. One subgroup of these proteins is the DLC family. It was found that the first identified gene from this family, DLC1, is often lost in hepatocellular carcinoma and may be involved as a tumor suppressor in the liver. Subsequent studies evaluated the hypothesis that the DLC1 gene acts as a tumor suppressor, not only in liver cancer, but also in other types of cancer. Following DLC1, two other members of the DLC protein family, DLC2 and DLC3, were identified. However, limited published data are available concerning the role of these proteins in malignant transformation. This review focuses on the structure and the role of DLC1 and its relatives in physiological conditions and summarizes data published thus far regarding DLC function in the neoplastic process.

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1. Introduction

In recent years, significant progress has been made in understanding the biological functions mediated by Rho GTPases. As key regulators of diverse cellular pathways GTPases affect such crucial processes as transcriptional regulation, cell cycle progression, apoptosis, and membrane trafficking (1,2). This family of small (20-30 kDa) signaling G proteins (guanine nucleotide-binding proteins) constitutes a major branch of the Ras superfamily (3). Membership in the superfamily of Ras proteins is determined by the presence of the GTPase domain. A total of 23 Rho proteins have been identified, among which RhoA, Rac1 and Cdc42 are the best characterized (4). Rho GTPases are also involved in the cytoskeleton formation of the cell via the regulation of actin dynamics (5). RhoA induces stress fiber formation and focal adhesion assembly, thereby regulating cell shape, attachment and motility. On the other hand, Rac1 promotes lamellipodium formation and membrane ruffling. Cdc42 has been shown to act in the formation of filopodia, finger-like actin-rich protrusions, thought to be involved in the sensing of the extracellular environment (2,6).

As with other small GTP-binding proteins of the Ras superfamily, the Rho GTPases act by switching between an inactive GDP-bound and an active GTP-bound conformation, with the latter form capable of interacting with a wide range of downstream effectors, thereby activating them. The cycling of Rho GTPases between these GTP- and GDP-bound states is modulated by the three classes of regulatory proteins, the guanine nucleotide exchange factors (GEFs) which catalyze the exchange of GDP for GTP, the GTPase-activating proteins (GAPs) that promote hydrolysis of GTP to GDP, and the guanine nucleotide dissociation inhibitors (GDIs), which bind to the GDP-bound form and not only prevent nucleotide exchange, but also sequester Rho GTPases in the cytoplasm (4).

The effect on the wide spectrum of biological functions suggests the involvement of Rho GTPases and their regulators in cancer progression. Increasing evidence obtained from numerous *in vitro* and *in vivo* studies shows that deregulated signaling of Rho proteins may lead to tumorigenesis (7). The fact that no constitutively active Rho mutants have been reported in human tumors suggests that aberrant Rho GTPase signaling in cancer is caused by the alterations of their regulators (4,7). Findings of a large number of studies revealed that regulators of Rho proteins are over- or down-expressed in various types of human cancer (8-11). The most common

alteration reported for Rho regulators in cancer is inactivation of RhoGAPs. One branch of this protein family is a member of the deleted in liver cancer (DLC) family. In the late 80s, the identification of a gene found to be commonly deleted in liver tumors, the so-called DLC1, focused the attention on the role of this protein family in tumorigenesis. Subsequent studies revealed that this type of genetic loss is found in a number of other neoplasms (11-14).

To the best of our knowledge, no reports summarizing the nature of the DLC family proteins are currently available. This review focuses on the structure, function and expression of DLC1 and its two other homologs, DLC2 and DLC3, in physiological conditions and in human malignancies.

2. The DLC family consists of three structurally related proteins

The human genome encodes approximately 70 RhoGAPs that share a conserved GAP domain, whose functional role is to turn off Rho-mediated signaling (15). One subgroup of the human RhoGAPs contains DLC1 (also termed as STARD12 or ARHGAP7), a human homolog of the rat p122RhoGAP (14,16). The ArhGAP7/DLC1 gene is localized on chromosome 8p21-22 and encodes a 1091-amino acid protein with a predicted molecular mass of 122 kDa. By means of quantitative RT-PCR assay, it was determined that DLC1 is widely expressed in normal tissues, with high abundance in the lung and ovary, and moderately in the thyroid, spleen, intestine and kidney. The adrenal gland, liver and pancreas exhibit the lowest expression (17).

There are two additional members of the DLC family; DLC2 (or STARD13), located on chromosome 13q12 (18), and DLC3 (also known as KIAA0189 or STARD8), located on the X chromosome at q13 brand (19). The DLC2 encodes a 1113-amino acid protein with a molecular weight of 125 kDa, whereas the protein product of the DLC3 transcript has 1103 aa with a calculated molecular mass of 121 kDa. DLC2 has a broad tissue distribution, with the highest levels in the brain, heart and liver (9,19). The third member of the deleted in liver cancer protein family is also detected in a broad spectrum of human tissues, with high abundance in the lung, kidney and placenta (19).

3. Typical DLC protein possesses three domains: SAM, RhoGAP and START

DLC proteins have three distinct domains: the sterile α motif (SAM) localized at its N-terminus, a conserved RhoGAP (GAP) domain found close to the middle of the protein and the steroidogenic acute regulatory-related lipid transfer (START) domain at the C-terminus (Fig. 1) (18-20).

The biological activity of DLC1 is mainly executed by the RhoGAP domain (~150-200 aa), which promotes the hydrolysis of GTP bound to the Rho GTPases. This catalytic activity is mediated by an 'arginine finger' donated by GAP. It was reported that DLC1 and DLC3 contain a conserved 'arginine finger' at position 677 and 688, respectively (19,20). In *in vitro* assays both the full-length DLC1 and the isolated RhoGAP domain reveal activity on RhoA, RhoB and RhoC, to a lesser extent on Cdc42, and no activity on Rac1 (11,21). By inacti-

vating these small GTPases, DLC1 affects cell morphology and control actin cytoskeletal remodeling (16,22). Similar to DLC1, both DLC2 and DLC3 contain a RhoGAP domain and exhibit GAP activity for RhoA and Cdc42 *in vitro* (18,23).

SAM domains (~70 aa) were found in nuclear and signaling proteins (e.g., p73 and p63 proteins, Eph-related tyrosine kinases and Ets transcription factors) (24,25). The SAM domain was thought to act as a protein interaction module through the formation of homo- and hetero-oligomers with other SAM domains, or even RNA and DNA (26). Results of structural studies of the DLC2 SAM domain have shown that it may also bind lipids (27).

However, much less is known regarding the exact role of the SAM domain in DLC function. One structure-function study has shown that the SAM domain may serve as an autoinhibitory domain for RhoGAP catalytic activity (28). Zhong *et al* have identified the eukaryotic elongation factor-1A1 (EF1A1) as a novel target of the SAM domain of DLC1 (29). EF1A1 is involved in protein synthesis (30) and transporting β-actin mRNA (31). It regulates the actin network through its G-actin-binding and F-actin-bundling activity, and also by stabilizing microtubules (32-34). The expression of EF1A1 is increased in a wide variety of human cancers, including pancreas, lung, prostate, breast, colon and melanomas (30). All of these activities support the hypothesis that EF1A1 has a significant regulatory role in cell growth and the cytoskeletal network. Zhong *et al* have demonstrated that the DLC1 SAM domain is capable of adopting a four-helix fold that creates a unique hydrophobic motif, allowing it to bind directly to EF1A1. Findings of these authors show that this interaction facilitates EF1A1 distribution to the membrane periphery and membrane ruffles and suppresses cell migration in a GAP-independent manner (29).

The START domain (~210 aa) is a well-conserved lipid-binding domain, which is primarily found in proteins that transfer lipids between organelles and are involved in lipid metabolism as well as in modulation of signaling events involved in lipid processing. In mammals, 15 START domain-containing proteins were identified (35). Among these proteins, steroidogenic acute regulatory protein (StAR) and metastatic lymph node 64 (MLN64) appear to be the most well-known representatives (36). Both StAR and MLN64 bind cholesterol (37), and are also known to play a role in lipid transport into mitochondria (38,39). In particular, StAR localizes to the mitochondria and stimulates the translocation of cholesterol from the outer to the inner mitochondrial membranes (40). DLC2 has been found to localize in mitochondria and was found in proximity to the lipid droplets through the START domain (41). Future research is required in order to establish the lipid ligand of DLC2-START and clarify whether the START domain of DLC2 and its homologs, DLC1 and DLC3, play a role in mitochondrial lipid transport.

Between the SAM and RhoGAP domains there is a long unstructured region that contains a phosphorylation-independent binding site, which Kawai *et al* termed as the FAT (focal adhesion targeting) domain, due to the fact that its presence determines of DLC focal adhesion localization (42). Independent studies have reported that DLC1, DLC2 and DLC3 are recruited to focal adhesion sites via their FAT sequence, which binds to the Src homology 2 (SH2) domains of focal
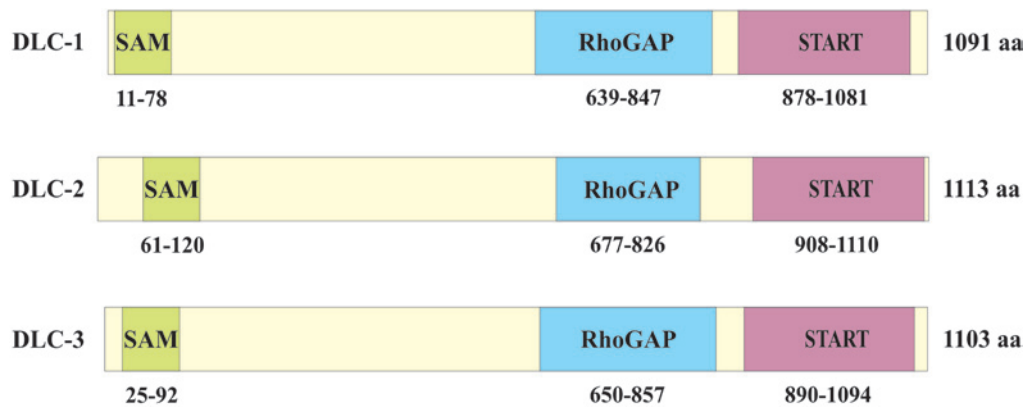
Figure 1. Schematic representation of DLC family proteins showing three major functional domains (SAM, RhoGAP and START domain).

adhesion proteins and interacts with tensins (43-45). However, the same region of DLC1 has been found to possess the ability to interact with the PTB domain of tensin2 (46-48) and tensin1 (44). The role of the interaction with the PTB domain in the localization of DLC1 to focal adhesions remains controversial and requires further investigation. Using pull-down assay, the interaction between the other members of the DLC family, DLC2 and DLC3, and tensin2 PTB was documented (46).

The central region of DLC1 was reported to target caveolae by interacting with caveolin-1 (48). Although the physiological function and significance of DLC1 in caveolar localization remain unclear, it appears that caveolae are one of the compartments whereby DLC1 may exhibit its tumor-suppressive role and affect the cytoskeletal reorganization by its RhoGAP activity.

The C-terminal region of DLC1 is known to interact with the phospholipase C-δ1 (PLCδ1), as well as enhance its activity (16). DLC3 has also been shown to activate PLCδ1 in an *in vitro* assay (23). It is suggested that this interaction supports involvement of DLC proteins in modulating actin cytoskeleton.

4. Expression analysis of DLCs reveals their down-regulation in various types of human cancer

DLC1 was first identified as a tumor suppressor gene in hepatocellular carcinoma (14). Nevertheless, the accumulating data have shown its involvement in the pathogenesis of various types of human cancer. In accordance with this theory, down-regulation of DLC1 was observed in a wide range of tumor tissues, including liver, breast, lung, ovarian, kidney, colon, stomach and prostate (Table I) (9-11,17,49-55). Underexpression of DLC1 was associated with either heterozygous deletions of the DLC1 gene or hypermethylation of the gene promoter region (11,50,51,53,56). The downregulation of DLC1 was also documented in breast cancer cell lines derived from aggressive metastatic tumors (57).

Following the findings of dysregulation of the DLC1 gene function in a variety of solid tumors, downregulation of DLC2 and DLC3 genes was also shown to be involved in human neoplasm development. DLC2 was found to be downregulated in breast, lung, ovarian, renal, uterine, gastric, colon, rectal and liver tumors (Table II) (9,19,58). Notably, the comparison of DLC1 and DLC2 gene expression in the same cell lines revealed that DLC2 is more frequently downregulated than DLC1 in HCC cell lines (9). The expression of the third member of the deleted in liver cancer protein family was shown to be significantly low or undetectable in the majority of the human breast, prostate and ovarian cancer cell lines. A cancer profiling array has revealed a decreased level of DLC3 in primary tumors originating from kidney, lung, uterine, ovarian and breast tissues (Table II) (19).

5. DLCs act as tumor suppressor genes

In recent years extensive studies have revealed DLC1 to be a bona fide tumor suppressor gene not only in HCC, but also in other types of cancer. Accumulating evidence has attributed the suppressive function of DLC1 to RhoGAP activity. As mentioned above, DLC1 was shown to exhibit *in vitro* GAP activity for RhoA and Cdc42 (11). Negative regulation of the Rho/ROCK/MLC pathway in HCC cell lines by DLC1 was shown to be RhoGAP-dependent (59). DLC1 RhoGAP defective mutant failed to inhibit stress fiber formation in HCC lines (60), whereas the overexpression of DLC1 resulted in morphological change with disruption of actin stress fibers (22,59). Using various cancer cell models, DLC1 was shown to inhibit cell proliferation, suppress cell migration and invasion, and induce apoptosis (44,52,54,60,61). Additionally, DLC1 was reported to act as a suppressor of metastatic foci formation in breast cancer cells. Restoration of DLC1 expression in metastatic cell lines has been shown to result in the inhibition of cell migration and invasion as well as a significant reduction in metastases in athymic mice (57).

DLC2 has been identified as the second tumor suppressor protein of the DLC family and is considered to be equally important. The specific GAP activity on RhoA and Cdc42 suggested that DLC2 inhibits Rho-mediated cytoskeletal reorganization and counteracts Ras-mediated cell transformation in mouse fibroblasts (18). Further research has shown that DLC2 inactivates RhoA, leading to the inhibition of the chemically-induced stress fiber formation. This inactivation may be followed subsequently by a change of the cell morphology, from angular and spindle-shaped to round-shaped with dendritic protrusion formation, as was shown in mouse fibroblasts and hepatoma cells. Moreover, the overexpression of DLC2 suppresses cell proliferation, motility and

Table I. DLC-1 expression in various types of solid tumors.

Tissue	Tumor tissues		Carcinoma cell lines		References
Liver	20%	(6 of 30)	40%	(2 of 5)	(52)
	68%	(27 of 40)	43%	(3 of 7)	(11)
	52%	(Total: 99)		(17)	
Breast			70%	(12 of 17)	(50,54)
	57%	(8 of 14)	62.5%	(5 of 8)	(10)
	76%	(Total: 50)		(9)	
			33%	(3 of 9)	(52)
Lung	95%	(20 of 21)	58%	(11 of 19)	(55)
	90%	(Total: 21)		(9)	
Ovary	79%	(Total: 14)		(9)	
Kidney	75%	(Total: 20)		(9)	
Colon			70%	(12 of 17)	(50,54)
	43%	(Total: 35)		(9)	
Stomach			78%	(7 of 9)	(49)
	41%	(Total: 27)		(9)	
Prostate			50%	(2 of 4)	(54)
	37%	(10 of 27)	60%	(3 of 5)	(53)
Nasopharynx			91%	(11 of 12)	(51)
Esophagus			40%	(6 of 15)	(51)
Cervix			63%	(5 of 8)	(51)

The percentages indicate the frequency of tumor samples in which DLC-1 expression was decreased when compared to the adjacent normal tissue.

anchorage-independent growth in the human hepatoma cell line, HepG2 (62). DLC2 was also reported to have an inhibitory effect on the growth of breast cancer cells *in vitro* (63).

Supporting the assertion that DLC family members play a significant role in human malignancies, the findings of three reports indicate that DLC3 acts as a potential tumor suppressor (19,23,44). Kawai *et al* have demonstrated that DLC3 serves as a GAP for both RhoA and Cdc42 in *in vitro* assays. Furthermore, the overexpression of DLC3 in HeLa cells disrupts actin stress fibers and changes cell morphology in a GAP-dependent manner (23). The introduction of DLC3 into human breast and prostate cancer cell lines has been shown to inhibit cell proliferation, colony formation and growth in soft agar (19).

With reference to the above-mentioned studies, the DLC family proteins may fulfill their suppressive function by decreasing the levels of active, GTP-bound Rho proteins or inhibiting the GDP-GTP cycling process, affecting cytoskeletal remodeling, cell shape, proliferation and migration, as well as induction of apoptosis. However, little is known about the molecular mechanisms through which DLC family proteins are capable of suppressing cell motility and cell growth. In their study, Holeiter *et al* have shown that enhanced migration of cells lacking DLC1 is dependent on the Rho effector protein Dia1 and does not require the activity of Rho kinase (ROCK) (64). Leung *et al* provide evidence for a key mechanism through which DLC proteins act as tumor suppressors (65). This study demonstrated that the expression

Table II. Expression of DLC2 and DLC3 in human tumors.

Tumors (total no. of samples)	DLC2 (%)	DLC3 (%)	References
Kidney (20)	65	95	(9,19)
Lung (21)	91	85	(9,19)
Uterus (42)	64	5	(9,19)
Ovary (14)	86	57	(9,19)
Breast (50)	72	52	(9,19)
Colon (35)	46	34	(9,19)
Stomach (27)	37	33	(9,19)
Rectum (18)	61	33	(9,19)

The percentages indicate the frequency of tumors exhibiting a decrease in DLC2 and DLC3 expression when compared to the adjacent normal tissue.

of DLC2 protein is involved in the inactivation of the Raf-1-ERK1/2-p70S6K signaling pathway, which is crucial for cell proliferation. This inhibitory activity of DLC2 is attributed to RhoGAP function (65).

By binding to phospholipase C-δ1, DLC1 enhances the hydrolysis of PIP2, which interacts with various actin-regulatory

proteins that affect the architecture of the actin cytoskeleton (16,22,66). In addition to the PLCδ1-enhancing and RhoGAP activities, proper focal adhesion localization and interaction with tensins have been demonstrated to be essential for the growth-suppression function of DLC1 (44,45,47,48). DLC1 mutants deficient in tensin binding disrupt the focal adhesion localization and lose their ability to suppress colony formation (44,45). Additional investigation is required to establish whether this localization is crucial for the tumor suppression activity of other members of the DLC family.

The DLC protein family contribution in normal tissue development was also documented (67). Although mice heterozygous for the DLC1 gene showed no physical abnormalities, mouse embryos, which were homozygous, died before day 10.5 postcoitus with severe defects in a number of organs, including the neural tube, brain, heart and placenta. In addition, mouse embryonic fibroblasts isolated from DLC1 mutant embryos revealed disruption of the actin filament and altered focal adhesions (67).

6. Conclusion

Deleted in liver cancer family proteins are a group of Rho GTPases whose aberrant function suggests dysregulation of numerous cell processes that can promote tumorigenesis. Accumulating evidence indicates that DLC proteins are regulated in various ways: at a genetic level by gene deletion, at an epigenetic level by the aberrant promoter methylation, or at a cellular level by the regulation of protein stability and localization. Future studies are required to establish which form of DLC regulation occurs most commonly in cancer and whether restoration of the proper function of the DLCs is capable of restraining cell transformation. Few studies regarding the function of the two family members, DLC2 and DLC3 are currently available. Additional studies may reveal which member of this family plays a key role in individual types of neoplasia, leading to identification of the protein that is due to become the subject of subsequent studies as a potential drug for targeted therapy.

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