Received: 16 March 2021 | Revised: 15 May 2021 | Accepted: 8 June 2021

DOI: 10.1111/cas.15017

REVIEW ARTICLE

Cancer Science WILEY

Delta-like canonical Notch ligand 3 as a potential therapeutic target in malignancies: A brief overview

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Funding information
JSPS KAKENHI, Grant/Award Number 18K16375; Osaka Medical College Internal Research Grant.

Abstract

Delta-like canonical Notch ligand 3 (DLL3) is a member of the Delta/Serrate/Lag2 (DSL) Notch receptor ligand family and plays a crucial role in Notch signaling, which influences various cellular processes including differentiation, proliferation, survival, and apoptosis. *DLL3* is expressed throughout the presomitic mesoderm and is localized to the rostral somatic compartments; mutations in *DLL3* induce skeletal abnormalities such as spondylocostal dysostosis. Recently, *DLL3* has attracted interest as a novel molecular target due to its high expression in neuroendocrine carcinoma of the lung. Moreover, a DLL3-targeting Ab-drug conjugate, rovalpituzumab tesirine (ROVA-T), has been developed as a new treatment with proven antitumor activity. However, the development of ROVA-T was suspended because of shorter overall survival compared to topotecan, the second-line standard treatment. Thus, several studies on the mechanism and function of *DLL3* in several malignancies are underway to find a new strategy for targeting *DLL3*. In this review, we discuss the roles of *DLL3* in various malignancies and the future perspectives of *DLL3*-related research, especially as a therapeutic target.

KEYWORDS

DLL3, malignancy, Notch signaling, ROVA-T, therapeutic target

1 | INTRODUCTION

Delta-like canonical Notch ligand 3 is a member of the DSL Notch receptor ligands, which include five ligands in mammals: DLL1, DLL3, DLL4, JAG1, and JAG2.[1] Delta-like canonical Notch ligand 3 plays a crucial role in Notch signaling, which influences various cellular processes, including differentiation, proliferation, survival, and apoptosis.[2] *DLL3* is expressed throughout the presomitic mesoderm and is localized to the rostral somatic compartments[3,4]; mutations in *DLL3* are known to induce skeletal abnormalities such as spondylocostal dysostosis.[3]

High DLL3 expression has been recently observed on the surface of SCLC and LCNEC cells. A DLL3-targeting Ab-drug conjugate,

Abbreviations: BiTE, bispecific T-cell engager; CAR, chimeric antigen receptor; CI, confidence interval; CRPC-NE, castration-resistant small-cell neuroendocrine prostate cancer; DLL, delta-like canonical Notch ligand; DOR, duration of response; DSL, Delta/Serrate/Lag2; EGFL7, epidermal growth factor-like domain multiple 7; GHPA, growth hormone-secreting pituitary adenoma; GI, gastrointestinal; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; JAG, jagged; LCNEC, large cell neuroendocrine carcinoma; MCC, Merkel cell carcinoma; miRNA, microRNA; NEC, neuroendocrine carcinoma; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; ROVA-T, rovalpituzumab tesirine; SCBC, small-cell bladder cancer; SCLC, small-cell lung cancer.

ROVA-T, a promising targeted therapy, showed efficient regression in SCLC and LCNEC.[4,5] Notably, our recent findings showed that GI neuroendocrine malignancies had high DLL3 expression, similar to neuroendocrine lung cancer, and that *DLL3* silencing inhibited their cell growth through apoptosis induction.[6] Thus, *DLL3* is a potential target for novel lung cancer treatments and has attracted attention as a therapeutic gene for several malignancies. However, in lung cancer, ROVA-T development was suspended because of shorter OS compared with the control, topotecan, which is the current standard care.

On the contrary, our previous findings indicate that DLL3 expression is frequently silenced by epigenetic modifications such as aberrant DNA methylation and histone acetylation in HCC cells and that DLL3 overexpression induces apoptosis in HCC cells.[7,8] Hepatitis B virus protein HBx also causes epigenetic modifications and suppresses DLL3 expression in HBV-associated HCC.[6,9] Thus, despite being a therapeutic target due to its high expression in some carcinomas, DLL3 expression could demonstrate different tendencies in each malignancy.

Based on this background, despite the potential of *DLL3* as a novel therapeutic target, and several ongoing studies on the mechanism and function of *DLL3* in several malignancies, it is necessary to determine the diseases in which *DLL3* can be targeted, and their characteristics. In this article, we summarize the characteristics of *DLL3*, discuss its roles in various malignancies, and elaborate on the future perspectives of *DLL3*-related research, especially as a therapeutic target.

2 | STRUCTURE AND FUNCTION OF DLL3

Delta-like canonical Notch ligand 3 is a structurally divergent DSL family member. Unlike other DSL ligands, DLL3 localizes in the Golgi apparatus and emerges on the cell surface when overexpressed.[10] Delta-like canonical Notch ligand 3 does not bind to Notch receptors, and inactivates Notch signaling in cis.[11] Delta-like canonical Notch ligand 3 also prevents the localization of Notch and/or DLL1 on the cell surface through intracellular retention.[12] Thus, DLL3 is regarded as a cell-autonomous inhibitor of Notch signaling.[13] It is also one of several notch ligands that is a direct downstream target of ASCL1, a transcription factor associated with pulmonary neuroendocrine cell development.[14-17] These findings suggest that DLL3 is related to neuroendocrine tumorigenesis, especially in lung cancer.

3 | ASSOCIATIONS AND ROLES OF *DLL3* IN LUNG CANCERS

The roles of *DLL3* are being mostly investigated in lung cancer. Increased *DLL3* expression was detected in SCLC by whole transcriptome RNA-sequencing.[5] Further investigation indicated that DLL3 expression is detectable on the membrane of SCLC and LCNEC tumor cells. Thus, ROVA-T was developed and was proved to demonstrate antitumor activity.[5] A phase I open-label study was undertaken in the USA, and the safety of ROVA-T, its tolerated dose, and dose-limiting toxic effects were determined.[4] Serious adverse events, grade 3 or worse, included thrombocytopenia, pleural effusion, and increased lipase levels. The maximum tolerated dose was 0.4 mg/kg every 3 weeks, whereas 0.3 mg/kg every 6 weeks was recommended as the appropriate dose and schedule in the phase II trial.[4]

Significant clinical findings were obtained from TRINITY, an open-label, single-arm, phase II study including patients with DLL3-expressing SCLC showing relapsed or refractory disease, previously treated with at least two chemotherapy lines.[18] The primary end-points in this trial were the ORR by central radiographic assessment according to RECIST version 1.1 and OS. The secondary end-points were DOR, disease control rate, and PFS. For all patients, the ORR was 12.4%, and the median OS was 5.6 months. The median DOR was 4.0 months, the median PFS was 3.5 months, and the disease control rate was 69.6%. In contrast, for patients with DLL3-high expression, the ORR was 14.3%, and the median OS was 5.7 months. The median DOR was 3.7 months, median PFS was 3.8 months, and disease control rate was 73.5%.[18] These results were comparable for the DLL3-high and DLL3-positive groups. Furthermore, the response of DLL3-high patients to ROVA-T was significantly higher than that of DLL3-nonhigh patients, showing some DLL3 expression.

Two randomized phase III studies, the TAHOE and MERU studies, were also carried out. The TAHOE study was an open-label, two-to-one randomized study comparing ROVA-T with topotecan, the second-line standard treatment for DLL3-high SCLC with first disease progression during or after first-line platinum-based chemotherapy.[19] The primary end-point was OS. The median OS of the ROVA-T group was 6.3 months (95% CI, 5.6-7.3), and that of the topotecan group was 8.6 months (95% CI, 7.7-10.1).[19] The median PFS of the ROVA-T group (3.0 months; 95% CI, 2.9-3.6) was also inferior to that of the topotecan group (4.3 months; 95% CI, 3.8-5.4), ORR was 15% in the ROVA-T group compared to 21% in the topotecan group, and the median DOR was 3.5 months (95% CI, 2.8-4.2) in the ROVA-T treatment group, compared to 4.9 months with topotecan (95% CI, 3.9-7.9). Based on these results, enrollment in the TAHOE study was discontinued.[19] The primary purpose of the MERU study was to evaluate the effect of ROVA-T, given as maintenance therapy following first-line chemotherapy compared to the placebo; the results could not meet their primary objective. Thus, the development of ROVA-T was suspended in August 2019.

Finally, in a phase I trial, the ORR was 38%, median PFS was 4.3 months, and DOR was 4.3 months in DLL3-high patients.[4] Although these results are superior to the data from TRINITY and TAHOE trials, the phase I study could contain an exploratory aspect, and the number of DLL3-high patients was also small (n = 26).[4] The recommended dose (0.3 mg/kg every 6 weeks) was applied in the TRINITY and TAHOE trials considering the results of the phase I study. These differences could be related to the unsuccessful results of the trials. The unique toxicity of ROVA-T due to pyrrolobenzodiazepine, which damages DNA, should also be considered. Indeed,

TABLE 1 Summary of Delta-like canonical Notch ligand 3 (DLL3) associations and functions in lung cancer

Types of lung cancer	Associations and functions	References
LCNEC	DLL3 is highly expressed on the cell surface in LCNEC and SCLC	4,5
SCLC	DLL3 overexpression promotes PI3K/Akt signaling through Notch signaling inhibition	13
	DLL3 promotes SCLC tumor growth, migration, and invasion by modulating Snail	14
	In the phase I study, a DLL3-targeting Ab-drug conjugate (ROVA-T) showed effectiveness and safety in patients	4
	In the phase II study, TRINITY, ROVA-T was found to be an effective treatment for DLL3-high and DLL3-positive groups	18
	In phase III studies, TAHOE and MERU, the development of ROVA-T was suspended in August 2019	19

Abbreviations: LCNEC, large cell neuroendocrine carcinoma; ROVA-T, rovalpituzumab tesirine; SCLC, small cell lung cancer.

pleural effusion, pericardial effusion, edema, cutaneous reactions, and thrombocytopenia were observed in the TAHOE trial, and these side-effects interfered with the good clinical course in patients with SCLC.[19] In particular, pleural effusion and pericardial effusion were not reported in preclinical experiments. These adverse events might have resulted in inadequate treatment with ROVA-T.

Regardless of these findings, DLL3 remains a pivotal target for SCLC and further investigations are required to gain a breakthrough in developing therapeutic strategies and mechanisms targeting DLL3 in other malignancies. A summary of DLL3 in lung cancer is shown in Table 1.

4 | NOVEL DLL3-TARGETING TREATMENTS EXPECTED FOR LUNG CANCER

4.1 | Near-infrared photoimmunotherapy

A new cancer treatment technology, near-infrared photoimmunotherapy, which uses an Ab-photosensitizer conjugate followed by near-infrared light exposure and specifically damages cancer cells, was developed.[20] Cells incubated with ROVA-IR700, wherein ROVA-T was conjugated with an IR700 photosensitizer, were remarkably lysed upon near-infrared light exposure. Furthermore, xenografts in mice treated with ROVA-IR700 were observed to shrink.[20]

4.2 | AMG 757: a half-life extended, DLL3-targeted BiTE

Bispecific T-cell engager is a novel immune treatment that redirects a patient's T cells to kill tumor cells.[21] AMG 757 is a first-in-class, half-life-extended, BiTE molecule that activates T cells to reduce DLL3-expressing tumor cells.[22] AMG 757 was effective against SCLC cell lines in vitro and resulted in significant tumor regression through T cell activation in both patient-derived xenografts and orthotopic SCLC tumors in mouse models.[22] These findings suggest that AMG 757 can be used as a DLL3-targeting immune therapeutic for SCLC. A phase I study on AMG 757 in patients with SCLC is ongoing (NCT03319940).[23]

4.3 | AMG 119: a CAR T cell therapy targeting DLL3

Chimeric antigen receptor T cell therapy involves genetic modification of a patient's autologous T cells. It was developed to direct a patient's T cells to express the chimeric receptor for a tumor antigen and reinfuse these cells into the patient to attack and kill the target cells.[24,25]

AMG 119 is an adoptive cellular therapy comprising a patient's autologous T cells modified to express a transmembrane CAR that targets DLL3 and attacks DLL3-positive cells. Treatment with AMG 119 CAR T cells results in decreased SCLC cells expressing DLL3 in vitro and inhibition of tumor growth in an SCLC xenograft model in vivo.[26,27] A phase I study evaluating the safety, tolerability, and efficacy of AMG 119 for SCLC (NCT03392064) is also ongoing.

As these findings indicate, DLL3-targeting therapies still have great potential with the most advanced application in lung cancer (Table 2).

5 | ASSOCIATION AND ROLE OF *DLL3* IN VARIOUS MALIGNANCIES

5.1 | Liver cancer

In HCC, *DLL3* was found to be an aberrantly methylated gene.[28] *DLL3* expression was not observed in HCC cell lines (HuH1, HuH2, ALEX, and Kim1) by real-time PCR, and methylation of *DLL3* was detected in HuH2 and Kim1 cells, but not in ALEX analyzed by methylation-specific PCR. Expression of DLL3 in HuH2 and Kim1 cells was recovered after treatment with 5-aza-2'-deoxycytidine and trichostatin, which act as a demethylating agent and histone deacetylase inhibitor, respectively. Thus, *DLL3* was downregulated in HCC cells through aberrant promoter methylation.[7] Moreover,

TABLE 2 Summary of novel expected Delta-like canonical Notch ligand 3 (DLL3)-targeting treatments and new clinical trials for lung cancer

DLL3-targeting treatments	Functions and associations	Clinical trial number	References
NIR-PIT	A new cancer treatment technology: an Ab-photosensitizer conjugate followed by near-infrared light exposure that specifically damages cancer cells	–	20
AMG 757	A half-life extended, DLL3-targeted BiTE: AMG 757 was effective against SCLC cell lines in vitro and led to significant tumor regression through T cell activation	NCT03319940	22,23
AMG 119	A CAR T cell therapy targeting DLL3: AMG 119 shows potent killing of SCLC cells expressing DLL3 in vitro and inhibits tumor growth in an SCLC xenograft model in vivo	NCT03392064	26,27

Abbreviations: BiTE, bispecific T cell engager; CAR, chimeric antigen receptor; NIR-PIT, near-infrared photoimmunotherapy SCLC, small cell lung cancer.

DLL3 expression was found to be silenced in clinical specimens of HBV-associated HCC; HepG2.2.15 cells, which are transformed with the HBV gene, also showed low DLL3 expression by western blotting and real-time PCR, compared to the parental HepG2 cells. We then observed increased *DLL3* expression in HepG2.2.15 cells after trichostatin treatment, but not with 5-aza-2′-deoxycytidine. Therefore, the HBV protein HBx could cause epigenetic modifications such as histone acetylation, and suppress *DLL3* expression in HBV-associated HCC.[9] Overall, the expression and function of *DLL3* differed in HCC compared to other malignancies.

5.2 | Pancreatic cancer

Quantitative PCR for *DLL3* gene expression in 22 pancreatic cancer cell lines showed that PANC-1 and SU86.86 cells showed a 3-fold or higher copy number than that in human pancreatic epithelial cells.[29] Moreover, *DLL3* knockdown in SU86.86 cells caused considerable growth inhibition.[29] Similarly, high DLL3 expression was detected in HPAC and PANC-1 cells by western blot analysis.[30]

These findings suggest that targeting *DLL3* might be useful in pancreatic cancer; however, the location of DLL3 expression in pancreatic cancer is unclear. Thus, further experiments are needed to determine the possibility of widening the applications of targeting *DLL3*.

5.3 | Breast cancer

Wnt signaling in human mammary epithelial cells, as achieved by ectopic Wnt-1 expression, elicits a DNA damage response followed by Notch activation; furthermore, DLL3 expression is significantly increased in Wnt-1-expressing human mammary epithelial cells at both the protein and RNA levels.[31] However, none of these cells exhibited high DLL3 expression by IHC. In contrast, low DLL3 expression was observed in two of 19 breast NEC cases.[32] Considering these results, *DLL3* could be a challenging therapeutic target in breast cancer at present.

5.4 | Gastrointestinal neuroendocrine carcinoma

Expression of DLL3 in GINEC has both similar and different characteristics, which are essential when considering DLL3 as a therapeutic target. Localization and expression levels of DLL3 were thus examined in GINEC cells. Expression of DLL3 in clinical specimens of the GI tract including the stomach, duodenum, jejunum, ileum, and rectum was examined by IHC. Delta-like canonical Notch ligand 3 was found to be expressed in the cytoplasm of cells in the deep layer of the GI tract mucosa. These tendencies were similar to chromogranin A, a representative neuroendocrine cell marker.[33] Double-fluorescence IHC showed that the expression of DLL3 and chromogranin A was synchronized. Thus, DLL3 was expressed mainly in the neuroendocrine cells of the GI tract. For experiments on GINEC cell lines, only ECC4 (small cell carcinoma of the rectum), ECC10, and ECC12 (small cell carcinoma of the stomach) were available.[34] Notably, DLL3 mRNA and protein expression was significantly (several thousand-fold compared to other cancer cell lines such as colon cancer cells) upregulated in these cell lines, similar to SCLC cells.[6] These features suggest the usefulness of DLL3 as a novel therapeutic target. Moreover, silencing of *DLL3* in these cells induced cell growth inhibition through internal apoptosis.[6]

However, unlike SCLC, DLL3 is expressed in the cytoplasm, and not on the cell surface. Interestingly, electron microscopy analysis showed that DLL3 was expressed in neurosecretory granules,

specific to GINEC cell lines. Although further investigation is required to determine whether *DLL3*-targeting agents are useful for GINEC, *DLL3* does show potential as a novel therapeutic target.

5.5 | Small-cell bladder cancer

Small-cell bladder cancer, which accounts for 2%-5% of all bladder tumors, has a poor prognosis.[35,36] There is no standard therapy for advanced SCBC, and early relapses result in poor overall outcomes. Given this situation, more effective treatments and biomarkers are required for SCBC. In a study including 46 samples, unsupervised hierarchical clustering of gene expression patterns and gene expression correlated with clinical phenotypes indicated that the "normal-like" type had a superior OS than the "metastasis-like" type.[37] Notably, more than 10% of DLL3 expression was associated with shorter OS and PFS, and more than 30% of CD56 (neuroendocrine marker) expression was also associated with shorter PFS and OS in SCBC.[37] High expression of DLL3 protein was associated with shorter OS and PFS. The efficacy of a DLL3-targeting agent has also been observed in a patient-derived xenograft model of SCBC.[37] This was the first study to report increased DLL3 expression as a poor prognostic biomarker for SCBC, and that *DLL3* has potential as a new therapeutic target in SCBC.

5.6 | Neuroendocrine prostate cancer

A subset of patients with advanced prostate cancer show histologic transformation to small-cell neuroendocrine prostate cancer. Castration-resistant small cell neuroendocrine prostate cancer is typically associated with poor outcomes, and patients are treated with platinum-based chemotherapy regimens.[38] Because the clinical behavior of CRPC-NE shares similarities with SCLC, the association of DLL3 expression with the CRPC-NE phenotype in prostate cancer was investigated and the antitumor activity of SC16LD6.5 (humanized Ab against DLL3) was evaluated in DLL3-expressing prostate cancer models.[38] Delta-like canonical Notch ligand 3 was found to be expressed in most CRPC-NE and some castration-resistant prostate adenocarcinoma cases, but not in the localized benign prostate cancer. Moreover, a single dose of SC16LD6.5 induced a complete and durable response in DLL3-expressing prostate cancer xenografts.[38] Overall, these findings indicate that *DLL3* is a potential therapeutic target in neuroendocrine prostate cancer.

5.7 | Gynecological cancer

Endometrial cancer is a common female neoplasm, and its incidence and/or mortality have been increasing recently.[39] Despite its detection at an early stage and a 5-year survival rate of more than 90%, advanced stages of the disease or high-risk histopathology lead to worse survival rates in endometrial cancer.[5,40] Expression of DLL3 was found to be significantly upregulated in endometrial cancer; DLL3 overexpression, advanced tumor stage grades, and lymph node metastasis were all found to be independent prognostic predictors for endometrial cancer.[41] In ovarian cancers, the expression of Notch components (NOTCH2, NOTCH3, DLL3, MAML1, and ADAM17), which are the top five most relevant genes, is associated with poor OS; furthermore, the expression of these genes is increased with progressing cancer stages.[42] Thus, *DLL3* plays a pivotal role in promoting gynecological cancer cell growth and causes poor outcomes in these cancer types. However, further investigation is needed to determine whether *DLL3* can be considered a therapeutic target in gynecological cancer.

5.8 | Isocitrate dehydrogenase-mutant glioma

Isocitrate dehydrogenase-mutant glioma is a distinct molecular subtype of glioma with poor prognosis, and 80%-90% of low-grade gliomas are IDH-mutant.[43,44] RNA sequencing analysis of more than 20 cancer types in The Cancer Genome Atlas dataset revealed that IDH-mutant glioma showed the highest *DLL3* expression.[5] Furthermore, DLL3 expression was extraordinarily high and homogeneous in IDH-mutant glioma by IHC, and an anti-DLL3 Ab-drug conjugate was effective for patient-derived IDH-mutant glioma tumorsphere cultures.[45] MicroRNAs are key to glioma development and progression.[46,47] Expression of DLL3 as a proneural marker was upregulated in secondary glioblastoma, compared with the primary tumor, and was downregulated by inhibiting miRNA-128a.[48] These findings indicate a relationship between DLL3 expression and miRNA in glioma, and could contribute to a breakthrough in additional treatment for DLL3-expressing malignancies.

5.9 | Growth hormone-secreting pituitary adenoma

Growth hormone-secreting pituitary adenomas constitute approximately 20% of all pituitary adenomas, which are the third most common intracranial neoplasm.[49] Approximately one-third of GHPAs show invasive and aggressive clinical courses, and invasion is a crucial factor in their treatment and clinical progress.[50] Epidermal growth factor-like domain multiple 7 plays a pivotal role in physiologic and pathological angiogenesis. In fact, high EGFL7 expression is associated with a poor clinical course in several malignancies, resulting in enhanced tumor cell migration and invasion by promoting cell motility through the Notch signaling pathway.[51,52] Moreover, EGFL7 interacts with all four Notch receptors, including DLL3, and modulates its signaling. The expression levels of EGFL7 and Notch2 are markedly higher in invasive GHPA and *EGFL7* knockdown was found to downregulate Notch2 and DLL3.[53] These findings suggest that EGFL7 could be a novel therapeutic target through the inactivation of DLL3 expression in malignancies.

TABLE 3 Summary of Delta-like canonical Notch ligand 3 (DLL3) associations and functions in various neuroendocrine malignancies

Types of cancer	Associations and functions	References
Breast neuroendocrine carcinoma	Low or no expression of DLL3 was found by IHC	31,32
	DLL3 expression was significantly increased in Wnt-1-expressing human mammary epithelial cells	
Gastrointestinal neuroendocrine carcinoma	DLL3 expression was upregulated in ECC4, ECC10, and ECC12 cell lines	6,34
	DLL3 knockdown caused inhibition of cell growth by internal apoptosis	
Small-cell-bladder cancer	High DLL3 protein expression was associated with a shorter OS and PFS	35-37
Neuroendocrine prostate cancer	DLL3 expression was found in most CRPC-NE and some CRPC-Adeno	38
	A single dose of SC16LD6.5 (humanized Ab against DLL3) induced a complete and durable response in DLL3-expressing prostate cancer xenografts	

Abbreviations: CRPC-Adeno, castration-resistant prostate adenocarcinoma; CRPC-NE, castration-resistant small cell neuroendocrine prostate cancer; IHC, immunohistochemistry; OS, overall survival; PFS, progression-free survival.

TABLE 4 Summary of Delta-like canonical Notch ligand 3 (DLL3) associations and functions in other malignancies

Types of cancer	Associations and functions	References
Liver cancer	DLL3 expression in HCC cells was downregulated by aberrant methylation	7-9,28
	DLL3 expression in HBV-associated HCC was inhibited by histone acetylation	
Pancreatic cancer	In pancreatic cancer cell lines, DLL3 expression was 3-fold higher than in human pancreatic epithelial cells	29,30
	DLL3 knockdown caused significant growth inhibition	
Gynecological cancer	DLL3 expression was significantly upregulated in endometrial cancer	39-42
	DLL3 overexpression, advanced tumor stage grades, and lymph node metastasis were all independent prognostic predictors for endometrial cancer	
	DLL3 expression was associated with poor overall survival and increased with progressing cancer stages in ovarian cancers	
Isocitrate dehydrogenase-mutant glioma	DLL3 expression was extraordinarily high and homogeneous	45,48
	microRNA-128a was related to DLL3 expression	
Growth hormone-secreting pituitary adenoma	EGFL7 knockdown resulted in DLL3 downregulation	51-53
Merkel cell carcinoma	High DLL3 expression was observed	54,55
	DLL3 overexpression was significantly associated with Merkel cell polyomavirus expression	

Abbreviations: EGFL7, epidermal growth factor-like domain multiple 7; HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

5.10 | Merkel cell carcinoma

Merkel cell carcinoma is a rare malignancy and a subset of neuroendocrine carcinomas of the skin. Considering the neuroendocrine features of MCC, the expression of DLL3 and ROVA-T treatment response was evaluated in MCC.[54,55] High DLL3 expression was detected in MCC, and a partial response to ROVA-T therapy was observed in some patients enrolled in an open-label study (NCT02709889). However, DLL3 expression in patients with MCC was not associated with OS.[55] Thus, extensive studies are required to confirm whether DLL3 might be a prognostic biomarker in MCC and whether ROVA-T is useful in large studies.

A summary of DLL3 in various neuroendocrine malignant cell types is shown in Table 3 and other malignant types in Table 4. Notably, in malignancies tending to have high DLL3 expression, treatment methods are not well established due to their relative rarity (Figure 1).

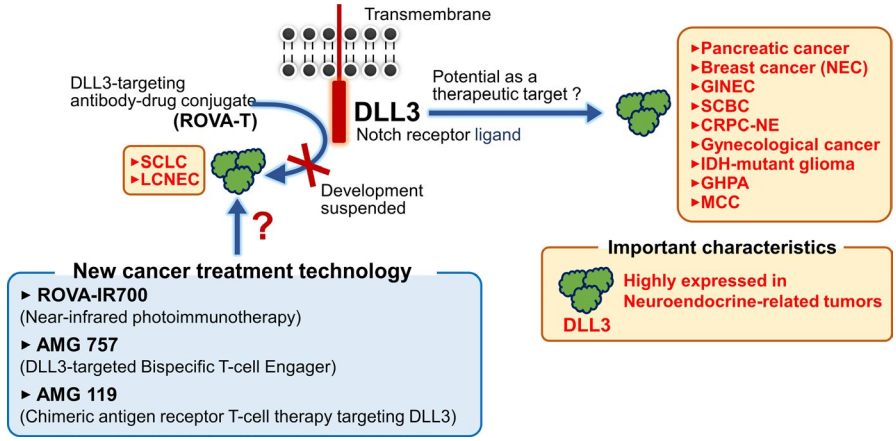

FIGURE 1 Expression and therapeutic potential of DLL3 in malignancies: a schematic representation DLL3 affects Notch signaling as a Notch receptor ligand. Recently, a DLL3-targeting antibody-drug conjugate (ROVA-T) was developed and examined in small cell lung cancer. However, the treatment effects did not overcome those of the current standard therapy. The impact of ROVA-IR700 application using near-infrared photoimmunotherapy has been investigated. Moreover, novel treatments such as AMG 757 and AMG 119 have been developed, and further clinical trials are ongoing. High expression of DLL3 was confirmed in other malignancies, especially in neuroendocrine-related tumors such as gastrointestinal neuroendocrine carcinoma. DLL3 may thus have potential for therapeutic breakthrough in rare tumors. CRPC-NE, castrationresistant small cell neuroendocrine prostate cancer; DLL3, Delta-like 3; GHPA, growth hormone-secreting pituitary adenoma; GINEC, gastrointestinal neuroendocrine carcinoma; IDH, isocitrate dehydrogenase; LCNEC, large cell neuroendocrine carcinoma; MCC, Merkel cell carcinoma; NEC, neuroendocrine carcinoma; SCBC, small-cell-bladder cancer; SCLC, small cell lung cancer

6 | FUTURE PERSPECTIVES

Given the findings of high DLL3 expression in SCLC and LCNEC, DLL3 is an attractive therapeutic target, and ROVA-T has been developed as a treatment. However, its phase III trial was canceled due to shorter OS compared to the second standard therapy. Despite these insufficient findings, DLL3 remains a novel target for SCLC. Although the development of ROVA-T was suspended, new technologies targeting DLL3, like near-infrared photoimmunotherapy, AMG 757, and AMG 119, have been developed.

In SCBC, neuroendocrine prostate cancer, IDH-mutant glioma, and MCC, experiments were carried out using ROVA-T, and its effectiveness was confirmed. Indeed, ROVA-T was found to be highly effective in SCLC, LCNEC, and SCBC. However, there are limitations in using ROVA-T as a therapeutic agent. One of these issues is the cell surface or cytoplasmic expression of DLL3.

In GINEC, DLL3 is expressed in the cytoplasm. Thus, delivery to the target organ needs to be considered, and further investigation regarding the effects of targeting *DLL3* in GINEC is necessary. The development of a novel inhibitor targeting DLL3 is also required. We have now started searching for compounds that inhibit DLL3 using an original screening assay. Once the appropriate compound is selected, it should be immediately tested for anticancer effects in DLL3-expressing malignancies.

7 | CONCLUSIONS

In this review, we discussed the functions of *DLL3* in various malignancies and the future perspectives of *DLL3*-related research, especially as a therapeutic target. *DLL3* plays a pivotal role in maintaining malignant growth and is related to poor prognosis, especially in relatively rare neuroendocrine subtypes. The findings of the reviewed reports could contribute to breakthroughs in treating these malignancies.

ACKNOWLEDGMENTS

This work was supported in part by JSPS KAKENHI Grant Number 18K16375 and by an Osaka Medical College (OMC) Internal Research Grant. We thank our collaborators, including our colleagues at Osaka Medical College, especially at the Department of Anatomy and Cell Biology. We would also like to thank Editage for English language editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. D'Souza B, Miyamoto A, Weinmaster G. The many facets of Notch ligands. *Oncogene*. 2008;27:5148-5167.
2. Radtke F, Raj K. The role of Notch in tumorigenesis: oncogene or tumour suppressor? *Nat Rev Cancer*. 2003;3:756-767.
3. Bulman MP, Kusumi K, Frayling TM, et al. Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. *Nat Genet*. 2000;24:438-441.
4. Rudin CM, Pietanza MC, Bauer TM, et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell

lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. *Lancet Oncol*. 2017;18:42-51.
5. Saunders LR, Bankovich AJ, Anderson WC, et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells *in vivo*. *Sci Transl Med*. 2015;7:302ra136.
6. Matsuo K, Taniguchi K, Hamamoto H, et al. Delta-like 3 localizes to neuroendocrine cells and plays a pivotal role in gastrointestinal neuroendocrine malignancy. *Cancer Sci*. 2019;110:3122-3131.
7. Maemura K, Yoshikawa H, Yokoyama K, et al. Delta-like 3 is silenced by methylation and induces apoptosis in human hepatocellular carcinoma. *Int J Oncol*. 2013;42:817-822.
8. Mizuno Y, Maemura K, Tanaka Y, et al. Expression of delta-like 3 is downregulated by aberrant DNA methylation and histone modification in hepatocellular carcinoma. *Oncol Rep*. 2018;39:2209-2216.
9. Hamamoto H, Maemura K, Matsuo K, et al. Delta-like 3 is silenced by HBx via histone acetylation in HBV-associated HCCs. *Sci Rep*. 2018;8:4842.
10. Geffers I, Serth K, Chapman G, et al. Divergent functions and distinct localization of the Notch ligands DLL1 and DLL3 *in vivo*. *J Cell Biol*. 2007;178:465-476.
11. Ladi E, Nichols JT, Ge W, et al. The divergent DSL ligand Dll3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *J Cell Biol*. 2005;170:983-992.
12. Chapman G, Sparrow DB, Kremmer E, Dunwoodie SL. Notch inhibition by the ligand DELTA-LIKE 3 defines the mechanism of abnormal vertebral segmentation in spondylocostal dysostosis. *Hum Mol Genet*. 2011;20:905-916.
13. Deng SM, Yan XC, Liang L, et al. The Notch ligand delta-like 3 promotes tumor growth and inhibits Notch signaling in lung cancer cells in mice. *Biochem Biophys Res Commun*. 2017;483:488-494.
14. Furuta M, Kikuchi H, Shoji T, et al. DLL3 regulates the migration and invasion of small cell lung cancer by modulating Snail. *Cancer Sci*. 2019;110:1599-1608.
15. Furuta M, Sakakibara-Konishi J, Kikuchi H, et al. Analysis of DLL3 and ASCL1 in surgically resected small cell lung cancer (HOT1702). *Oncologist*. 2019;24:e1172-e9.
16. Henke RM, Meredith DM, Borromeo MD, Savage TK, Johnson JE. Ascl1 and Neurog2 form novel complexes and regulate Delta-like3 (Dll3) expression in the neural tube. *Dev Biol*. 2009;328:529-540.
17. Augustyn A, Borromeo M, Wang T, et al. ASCL1 is a lineage oncogene providing therapeutic targets for high-grade neuroendocrine lung cancers. *Proc Natl Acad Sci USA*. 2014;111:14788-14793.
18. Morgensztern D, Besse B, Greillier L, et al. Efficacy and safety of rovalpituzumab tesirine in third-line and beyond patients with DLL3-expressing, relapsed/refractory small-cell lung cancer: results from the phase II TRINITY study. *Clin Cancer Res*. 2019;25:6958-6966.
19. Blackhall F, Jao K, Greillier L, et al. Efficacy and safety of rovalpituzumab tesirine compared with topotecan as second-line therapy in DLL3-high SCLC: results from the phase 3 TAHOE study. *J Thorac Oncol*. 2021. https://doi.org/10.1016/j.jtho.2021.02.009
20. Isobe Y, Sato K, Nishinaga Y, et al. Near infrared photoimmunotherapy targeting DLL3 for small cell lung cancer. *EBioMedicine*. 2020;52:102632.
21. Klinger M, Benjamin J, Kischel R, Stienen S, Zugmaier G. Harnessing T cells to fight cancer with BiTE® antibody constructs–past developments and future directions. *Immunol Rev*. 2016;270:193-208.
22. Giffin MJ, Cooke K, Lobenhofer EK, et al. AMG 757, a half-life extended, DLL3-targeted bispecific T-cell engager, shows high potency and sensitivity in preclinical models of small-cell lung cancer. *Clin Cancer Res*. 2021;27:1526-1537.
23. Smit MAD, Borghaei H, Owonikoko TK, et al. Phase 1 study of AMG 757, a half-life extended bispecific T cell engager (BiTE) antibody construct targeting DLL3, in patients with small cell lung cancer (SCLC). *J Clin Oncol*. 2019;37:TPS8577. https://doi.org/10.1200/JCO.2019.37.15_suppl.TPS8577
24. Miliotou AN, Papadopoulou LC. CAR T-cell therapy: a new era in cancer immunotherapy. *Curr Pharm Biotechnol*. 2018;19:5-18.
25. Frederickson RM. A new era of innovation for CAR T-cell therapy. *Mol Ther*. 2015;23:1795-1796.
26. Byers LA, Chiappori A, Smit MAD. Phase 1 study of AMG 119, a chimeric antigen receptor (CAR) T cell therapy targeting DLL3, in patients with relapsed/refractory small cell lung cancer (SCLC). *J Clin Oncol*. 2019;37:TPS8576. https://doi.org/10.1200/JCO.2019.37.15_suppl.TPS8576
27. Owen DH, Giffin MJ, Bailis JM, Smit MAD, Carbone DP, He K. DLL3: an emerging target in small cell lung cancer. *J Hematol Oncol*. 2019;12:61.
28. Yoshikawa H, de la Monte S, Nagai H, Wands JR, Matsubara K, Fujiyama A. Chromosomal assignment of human genomic NotI restriction fragments in a two-dimensional electrophoresis profile. *Genomics*. 1996;31:28-35.
29. Mullendore ME, Koorstra JB, Li YM, et al. Ligand-dependent Notch signaling is involved in tumor initiation and tumor maintenance in pancreatic cancer. *Clin Cancer Res*. 2009;15:2291-2301.
30. Song HY, Wang Y, Lan H, Zhang YX. Expression of Notch receptors and their ligands in pancreatic ductal adenocarcinoma. *Exp Ther Med*. 2018;16:53-60.
31. Ayyanan A, Civenni G, Ciarloni L, et al. Increased Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism. *Proc Natl Acad Sci USA*. 2006;103:3799-3804.
32. Vranic S, Palazzo J, Sanati S, et al. Potential novel therapy targets in neuroendocrine carcinomas of the breast. *Clin Breast Cancer*. 2019;19:131-136.
33. Gut P, Czarnywojtek A, Fischbach J, et al. Chromogranin A – unspecific neuroendocrine marker. Clinical utility and potential diagnostic pitfalls. *Arch Med Sci*. 2016;12:1-9.
34. Fujiwara T, Motoyama T, Ishihara N, et al. Characterization of four new cell lines derived from small-cell gastrointestinal carcinoma. *Int J Cancer*. 1993;54:965-971.
35. Fahed E, Hansel DE, Raghavan D, Quinn DI, Dorff TB. Small cell bladder cancer: biology and management. *Semin Oncol*. 2012;39:615-618.
36. Siefker-Radtke AO, Dinney CP, Abrahams NA, et al. Evidence supporting preoperative chemotherapy for small cell carcinoma of the bladder: a retrospective review of the M. D. Anderson cancer experience. *J Urol*. 2004;172:481-484.
37. Koshkin VS, Garcia JA, Reynolds J, et al. Transcriptomic and protein analysis of small-cell bladder cancer (SCBC) identifies prognostic biomarkers and DLL3 as a relevant therapeutic target. *Clin Cancer Res*. 2019;25:210-221.
38. Puca L, Gavyert K, Sailer V, et al. Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. *Sci Transl Med*. 2019;11:eaav0891.
39. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. *Lancet*. 2016;387:1094-1108.
40. Colombo N, Creutzberg C, Amant F, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. *Ann Oncol*. 2016;27:16-41.
41. Wang J, Zhang K, Liu Z, et al. Upregulated delta-like protein 3 expression is a diagnostic and prognostic marker in endometrial cancer: a retrospective study. *Medicine*. 2018;97:e13442.
42. Jia D, Underwood J, Xu Q, Xie Q. NOTCH2/NOTCH3/DLL3/MAML1/ADAM17 signaling network is associated with ovarian cancer. *Oncol Lett*. 2019;17:4914-4920.
43. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *New Engl J Med*. 2009;360:765-773.

44. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science*. 2008;321:1807-1812.
45. Spino M, Kurz SC, Chiriboga L, et al. Cell surface Notch ligand DLL3 is a therapeutic target in isocitrate sehydrogenase-mutant glioma. *Clin Cancer Res*. 2019;25:1261-1271.
46. Ventura A, Jacks T. MicroRNAs and cancer: short RNAs go a long way. *Cell*. 2009;136:586-591.
47. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer*. 2006;6:857-866.
48. Ma X, Yoshimoto K, Guan Y, et al. Associations between microRNA expression and mesenchymal marker gene expression in glioblastoma. *Neuro Oncol*. 2012;14:1153-1162.
49. Iuchi T, Saeki N, Tanaka M, Sunami K, Yamaura A. MRI prediction of fibrous pituitary adenomas. *Acta Neurochir*. 1998;140:779-786.
50. Dhandapani S, Singh H, Negm HM, Cohen S, Anand VK, Schwartz TH. Cavernous sinus invasion in pituitary adenomas: systematic review and pooled data meta-analysis of radiologic criteria and comparison of endoscopic and microscopic surgery. *World Neurosurg*. 2016;96:36-46.
51. Wu F, Yang LY, Li YF, Ou DP, Chen DP, Fan C. Novel role for epidermal growth factor-like domain 7 in metastasis of human hepatocellular carcinoma. *Hepatology*. 2009;50:1839-1850.
52. Massimiani M, Vecchione L, Piccirilli D, et al. Epidermal growth factor-like domain 7 promotes migration and invasion of human trophoblast cells through activation of MAPK, PI3K and NOTCH signaling pathways. *Mol Hum Reprod*. 2015;21:435-451.
53. Wang J, Liu Q, Gao H, et al. EGFL7 participates in regulating biological behavior of growth hormone-secreting pituitary adenomas via Notch2/DLL3 signaling pathway. *Tumour Biol*. 2017;39:1010428317706203.
54. Bhatia S, Afanasiev O, Nghiem P. Immunobiology of Merkel cell carcinoma: implications for immunotherapy of a polyomavirus-associated cancer. *Curr Oncol Rep*. 2011;13:488-497.
55. Xie H, Kaye FJ, Isse K, et al. Delta-like protein 3 expression and targeting in Merkel cell carcinoma. *Oncologist*. 2020;25:810-817.

How to cite this article: Matsuo K, Taniguchi K, Hamamoto H, et al. Delta-like canonical Notch ligand 3 as a potential therapeutic target in malignancies: A brief overview. *Cancer Sci*. 2021;112:2984–2992. https://doi.org/10.1111/cas.15017