RESEARCH

DLL3 (delta-like protein 3) expression correlates with stromal desmoplasia and lymph node metastases in medullary thyroid carcinomas

M Ingenwerth[1], T Brandenburg[2], D Führer-Sakel[2], M Goetz[1], F Weber[3], H Dralle[3], H-U Schildhaus[1], K W Schmid[1] and S Theurer[1]

[1]Institute of Pathology, University Hospital of Essen, University of Duisburg–Essen, Essen, Germany
[2]Division of Laboratory Research, Department of Endocrinology, Diabetes and Metabolism and Clinical Chemistry, University Hospital Essen, University of Duisburg-Essen, Germany
[3]Department of General, Visceral and Transplantation Surgery, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

Correspondence should be addressed to S Theurer: sarah.theurer@uk-essen.de

Abstract

Medullary thyroid carcinomas (MTC) are rare and aggressive neuroendocrine tumors of the thyroid. About 70% of MTC are sporadic; approximately 50% of those harbor somatic *RET* mutation. DLL3 is widely expressed in many neuroendocrine tumors and has been evaluated as a potential therapeutic target. Since stromal desmoplasia in sporadic MTC has been identified as a reliable predictor of aggressive behavior and development of lymph node metastases, a possible correlation of DLL3 expression with the presence of stromal desmoplasia was of particular interest. 59 paraffin-embedded samples of sporadic MTC with (44 cases) and without (15 cases) stromal desmoplasia and known lymph node status were included. DLL3 expression was determined by immunohistochemistry; no expression (0%), low expression (1–49%) and high expression (≥50%) were correlated with clinicopathological data. The proportion of DLL3 positivity was significantly correlated with both stromal desmoplasia ($P < 0.0001$) and lymph node metastases ($P < 0.0001$). MTC without stromal desmoplasia consistently lack DLL3 expression. This is the first study to focus on MTC regarding DLL3 expression and the relationship to various factors. Our results demonstrate that expression of DLL3 in MTC represents a reliable surrogate marker for stromal desmoplasia and lymph node metastases and might be an indicator for aggressive clinical behavior. DLL3 expression in ≥50% of tumor cells virtually excludes MTC without stromal desmoplasia. DLL3 was discussed as a potential therapeutic target in malignant tumors of other locations with positive immunohistochemical reaction and might therefore be a new therapeutic option in MTC, as well.

Introduction

Medullary thyroid carcinomas (MTC) are rare and aggressive neuroendocrine thyroid tumors composed of cells with evidence of C-cell differentiation. They account for only 2–3% of thyroid malignancies, but are causing about 15% of thyroid cancer-associated deaths (1, 2). They can occur either as part of hereditary syndromes (<30% of all MTC) in multiple endocrine neoplasias (MEN) 2A or 2B with germline mutations in the RET proto-oncogene or as sporadic tumors with approximately 50% having a somatic RET mutation. Sporadic MTC occurs independently from the number of non-neoplastic C-cells within the thyroid although non-MEN2-associated C-cell hyperplasia (CCH) has been defined (3); in contrast, hereditary MTC in MEN-syndromes arise from preexisting

'neoplastic CCH'. Differentiating between neoplastic CCH and invasive hereditary micro-MTC (tumor size <1 cm) can be very challenging, since malignancy is defined by invasiveness of neoplastic C-cells. Demonstration of stromal desmoplasia is the most useful surrogate marker of invasive growth of neoplastic C-cells (4). Stromal desmoplasia, a newly tumor-built fibrous stroma, surrounding tumor cells, is the only known factor reliably predicting aggressive behavior and development of lymph node metastases in sporadic MTC. In a cohort of 41 sporadic MTC without stromal desmoplasia, investigated by Koperek and co-workers, none of the tumors had lymph node metastases (5). In contrast, serum calcitonin or CEA levels, or RET-mutational status are not statistically related to the biological behavior of the considered MTC there (6). Therapeutic options in advanced stage of disease are still limited. State of the art is initial surgical treatment with central and lateral cervical lymph node dissection (2, 7, 8, 9, 10). A systemic treatment with tyrosine kinase inhibitors may be considered in progressive disease with high tumor burden and no further surgical options available (8). Ten-year survival rate depends mainly on tumor stage with a very poor outcome in stage IV (advanced local disease with nodal spread or any tumor size with distant metastases) of only 21% (2).

The development of MTC is not completely understood, but it seems to be proven, that the NOTCH pathway plays a pivotal role. In the thyroid the NOTCH pathway is usually active during the embryonal development and regulates proliferation and differentiation of C-cells (11). However, it is also well known that the NOTCH pathway is downregulated during tumor growth of neuroendocrine tumors. The NOTCH receptor, which is expressed on the cell membrane of neuroendocrine cells interacts with different ligands and acts variably as a tumor suppressor protein or oncogenic. One of these ligands, known as Delta-like ligand 3 (DLL3), is an inhibitory NOTCH ligand, which is overexpressed in high grade neuroendocrine tumors-like small cell lung cancer (SCLC) and other tumors of neuroendocrine origin-like small cell bladder cancer and metastatic neuroendocrine prostate carcinoma (11, 12, 13). Overexpression of DLL3 in neuroendocrine tissues can be immunohistochemically demonstrated as strong staining of the cell surface membrane, whereas non-neuroendocrine tissues usually do not express DLL3. In SCLC the percentage of cells expressing DLL3 (>/< 50% of tumor cells) correlates with time to tumor progression and is, therefore, a prognostic marker. Novel antibody-based therapies targeting DLL3 (Rovalpituzumab tesirine, Rova-T) were recently developed and are actually being

evaluated in several clinical studies in SCLC (12). Actually, little is known about DLL3 expression in MTC, however, in a phase I/II study, a small cohort of 13 MTC with >1% expression of DLL3 were included. Results of this study were promising for neuroendocrine carcinoma with DLL3 expression >50% (14). To our knowledge, no study focusing on MTC's DLL3 expression and their association to various factors exists. Thus, we investigated DLL3 expression in MTC additionally addressing clinicopathological parameters to determine potential therapeutic options with DLL3-targeting antibodies in different settings.

Material and methods

Clinical samples

59 paraffin-embedded samples of sporadic MTCs, seen between 2014 and 2019 at the Institute of Pathology, University Hospital of Essen, were retrieved from the files. Initial diagnosis was made according to the World Health Organisation (WHO) criteria outlined in 2017 (15) by KWS and ST, experts of thyroid pathology. To assure the diagnosis, every case was at least stained immunohistochemically for calcitonin and chromogranin a. All primary tumors and all lymph node metastases had a positive staining result. The presence of desmoplasia was confirmed in H&E-staining. 44/59 cases showed stromal desmoplasia; concomitant lymph node metastases were found in 39 of these 44 cases, 5 cases had tumor-free lymph nodes at initial presentation. Fifteen MTC completely lacked stromal desmoplasia – all lymph nodes investigated in these 15 cases were tumor-free. Available clinicopathological data are shown in Table 1. The study was approved by the ethics committee of the University Hospital of Essen (20-9139-BO).

Tissue microarray construction

For tissue microarray construction, the tumor areas were marked on slide and corresponding core biopsies of 2 mm diameter were taken in triplicates with a TMA Grand Master System (3DHistech, Hungary) according to the manufacturer's protocol. 10 cases were left out for whole slide stains to assess distribution of staining throughout the tumor.

Immunohistochemistry (IHC)

The TMAs and whole slides were cut into 4 µm thick slides and MAB against DLL3 (Ventana, clone SP347, ready to use)

Table 1 Clinicopathological data of MTC cohort.

	Total number of MTCs (n = 59)	Desmoplasia	No desmoplasia
Sex			
Male	27	23	4
Age: median (range)	53.4		
Female	32	21	11
Age: median (range)	56.2		
Age			
≤ 56 years	30	22	8
> 56 years	29	22	7
Lymph node status			
Metastases	39	39	0
No metastases	20	5	15
		44	15

was used, which was proven to be specific for DLL3-expression elsewhere (16, 17). IHC was performed with an OptiView Ventana System using the OptiView DAB IHC Detection Kit and the Cell Conditioning 1 pretreatment solution (CC1) according to the manufacturer's protocol (All Ventana). Pretreatment with the CC1 solution was for 80 min at 100°C, primary body incubation for 32 min at 36°C, linker component for 8 min at room temperature followed by HRP substrate for 8 min at room temperature. Slides were counterstained with hematoxylin. As specified by the manufacturer, staining reaction is specific when seen membranous or cytoplasmic. As negative control kidney and liver tissue were used. As positive control slide with non-small lung cancer was parallel stained showing a positive signal for DLL3.

Scoring IHC staining

IHC scoring was performed as described elsewhere (18, 19). In short, percentage of positive DLL3 tumor cells were determined by two pathologists (ST and MI) and the results were divided into three groups (no expression: 0%; low expression: 1–49%; high expression: ≥ 50%). Immunohistochemical reaction was scored positive with an expression membranous and/or cytoplasmic, at least detectable at 200-fold magnification. For calculating the odds ratio, percentage of positive DLL3 tumor cells were divided into two groups (no expression/low expression: < 49%; high expression ≥ 50%).

Statistics

Fisher's exact test was used to test independency of DLL3 positive tumor cell quantity and clinicopathological data followed by multivariate analyses. Differences between mean of DLL3 positive tumor cells were compared using

Man–Whitney U test. All analyses were performed using SPSS Version 22 (SPSS) or for Odds ratio https://www.medcalc.org/calc/odds_ratio.php. P values < 0.05 were considered significantly different.

Results

Expression analyses of DLL3 in MTCs

In MTC the proportion of DLL3 positive cells showed strong dependency to stromal desmoplasia and the positive lymph node status (Fig. 1A and Table 2) with a higher number of cases with DLL3 high expression in desmoplasia positive carcinomas ($P < 0.0001$), and cases with lymphonodal spread ($P < 0.0001$). Multivariate analyses showed significant results for desmoplasia ($P < 0.0001$) and lymph node metastasis status ($P < 0.0001$) as well. Odds ratio of DLL3 high expression is 42.7-fold higher in tumors with desmoplasia than in tumors without desmoplasia and additionally 53.5-fold higher for tumors with lymph node metastases. Primary tumors without desmoplasia ($n = 15$) never had lymph node metastases and never showed DLL3 high expression. Primary tumors with desmoplasia and concomitant lymph node metastases had a high association to DLL3 high expression ($P < 0.0001$). Total number of positive tumor cells in cases with lymph node metastases was significantly higher than in cases without lymph node metastases ($P < 0.0001$), similar to cases with and without desmoplasia ($P < 0.0001$) (Fig. 1C).

Distribution of DLL3 positive cells in MTC

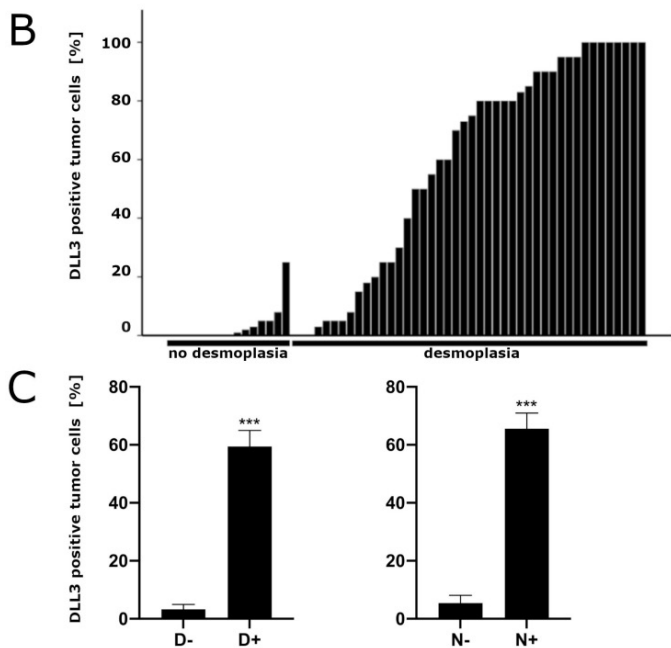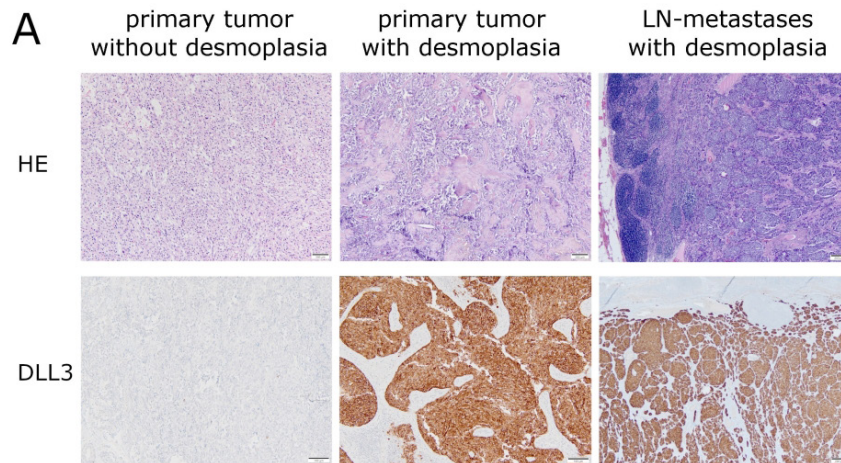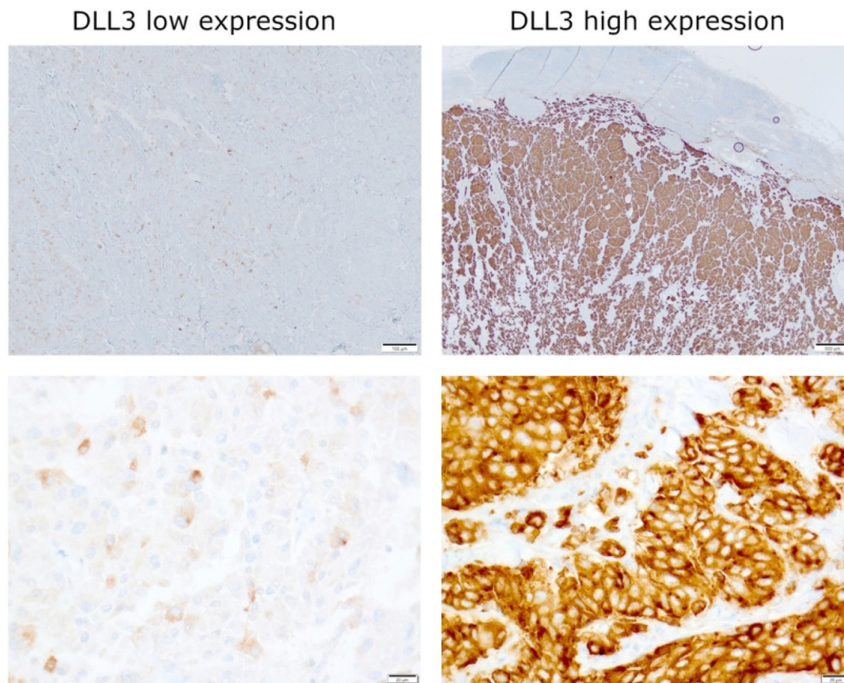Immunohistochemical staining of DLL3 shows a widely homogenous distribution over the entire tumor without

Figure 1

Expression of DLL3 in MTC with and without desmoplasia and its lymph node metastasis (A). Representative microphotographs of MTC with and without desmoplasia showing in H&E typical morphologic features. Immunohistochemistry for DLL3 shows a strong signal in tumors with desmoplasia as well in primary tumors as in lymphnode metastases. Tumors without desmoplasia are largely negative. (B) Bar chart divided into desmoplasia negative and positive tumors showing DLL3 expression of every single case. (C) Number of DLL3 positive tumor cells is significantly increased dependent on status of metastases and desmoplasia. ***$P < 0.0001$.

Table 2 Statistical analysis of DLL3 expression in relation to clinicopathological data and calculated odds ratio.

	DLL3 expression (division for Fisher's exact test)			*p* value	DLL3 expression (division for odds ratio)		Odds ratio
	0%	1–49%	≥50%		<49%	≥50%	
Lymphnode status							
No metastases	10	9	1		19	1	
Metastases	2	10	27	<0.0001	12	27	42.7
Desmoplasia							
No desmoplasia	8	7	0		15	0	
Desmoplasia	4	12	28	<0.0001	16	28	53.5
Desmoplasia/LN combined							
No desmoplasia /LN−	8	7	0		15	0	
No desmoplasia/LN+	0	0	0		0	0	
Desmoplasia/LN−	2	2	1		4	1	
Desmoplasia/LN+	2	10	27	<0.0001	12	27	*

*Odds ratio cannot be calculated.

DLL3 low expression   DLL3 high expression

Figure 2
Immunohistochemical staining of DLL3 shows a widely homogenous distribution of the entire tumor with low or high expression in lower magnification in top row (200×). Bottom row shows the cytoplasmatic and membranous expression of DLL3 positive tumor cells in higher magnification (400×).

sparing tumor sections as shown by whole slide staining (Fig. 2). Cases with high expression (defined as ≥ 50% of tumor cells with a positive staining reaction) only show a low number of negative tumor cells. Cases with no/low expression (0 or < 50% positive tumor cells) only have few positive tumor cells with mostly weak expression.

Discussion

Medullary thyroid carcinoma is an aggressive disease, especially in advanced, (lymphonodal) metastasized situation, with limited therapeutic options. Recently, NOTCH-Inhibitor DLL3 was identified as a novel therapeutic target in SCLC and large cell neuroendocrine carcinoma of the lung (LSCLC). First results in anti-tumor effectiveness of the DLL3-targeting antibody Rovalpituzumab tesirine (Rova-T) for these tumors are promising (19, 20). DLL3 seems to be overexpressed in various aggressive carcinomas of neuroendocrine origin, including small cell lung cancer (SCLC) (12), neuroendocrine carcinoma of gastrointestinal tract (21) and the prostate (22). Virtually nothing is known about DLL3 expression in medullary thyroid carcinoma. In a phase I/II study 13 MTCs were included with DLL3 expression >1%. In this study efficacy of Rova-T therapy was tested in different neuroendocrine tumors, mainly addressing SCLC. Best clinical response to Rova-T was achieved in neuroendocrine carcinomas with DLL3 expression >50%. The objective response rate to Rova-T in MTC was given as 2 of 13. Unfortunately, no further information was given about the MTCs included concerning stromal desmoplasia, lymph node status, RET-mutational status or exact percentage of DLL3 expression. Essential knowledge about MTC in this study is mainly the fact that MTC can express DLL3 and some respond to DLL3-targeting antibody therapy (14). Aim of our present study was therefore to basically investigate DLL3 expression in MTC and its correlation to stromal desmoplasia as an indicator of aggressive biological behavior. This could lead to more information about which MTC particularly could be a potential candidate for DLL3-targeting antibody therapy. To our knowledge, our study is the first to focus on MTC regarding their DLL3 expression. Meanwhile a phase III trial investigating Rova-T as a therapeutic option in SCLC was put on hold due to a shorter overall survival in patients receiving Rova-T as a monotherapy in the second line. Further studies with Rova-T in combination with different chemotherapeutic agents focusing SCLC are still ongoing (23). Furthermore, some promising new agents targeting DLL3 were introduced and actually tested for efficacy at SCLC (12). Even if actual results are disappointing for SCLC, efficacy of Rova-T on neuroendocrine tumors of other origin, for example MTCs, remains still unclear, but possible.

In our study 59 MTCs were investigated. 44 of these tumors had a striking stromal desmoplasia, 15 lacked stromal desmoplasia completely. The ratio of MTC with

and without stromal desmoplasia correlates with findings of published studies on stromal desmoplasia (5, 7, 24). 28 out of our 59 MTCs stained with DLL3, showed a high expression (defined as ≥ 50% of tumor cells with a positive staining reaction). All of these 28 cases had a striking stromal desmoplasia and no case without stromal desmoplasia had high expression of DLL3. Stromal desmoplasia in medullary thyroid carcinoma is reported to be a reliable predictor of aggressive behavior with development of lymph node metastases. According to larger studies of Koperek *et al.* and Scheuba *et al.*, the demonstration of stromal desmoplasia is highly associated with lymphonodal spread and was thus recognized as an independent risk factor for a negative outcome. They also describe that cases lacking stromal desmoplasia consistently lack develop lymph node metastases (5, 7). Therefore they, and some other groups, suggest to make the decision about extend of surgical intervention regarding neck dissection of lymph nodes dependent on the presence of stromal desmoplasia. Some even suggest to totally avoid lymphadenectomy in patients without stromal desmoplasia for sporadic MTC (7, 8, 25, 26). Stroma desmoplasia is defined as a newly built fibrous stroma, surrounding tumor cells and is detected morphologically by using routine hematoxylin and eosin staining. So far, no reliable marker staining desmoplasia or predicting aggressiveness of MTCs is known. We could show a significant correlation between desmoplasia, as well as lymph node metastases status, and DLL3 high expression, so DLL3 is the first known immunohistochemical indicator for aggressive behavior in MTC and staining can support diagnosis of desmoplasia. It also could be a supportive tool in the decision about the extent of surgical lymph node dissection, as DLL3 high expression seems to exclude low aggressive behavior of MTC. Cases without DLL3 expression but with desmoplasia exist (4 out of 44 cases with stromal desmoplasia had no expression of DLL3). These cases do not differ morphologically from other MTCs with low/high expression. None out of 15 cases without stromal desmoplasia had a high expression of DLL3. In vitro studies show that high expression of DLL3 leads to more aggressive behavior by promoting tumor growth, migration and invasion in small lung cancer cells via SNAIL overexpression (25, 26). Furthermore, SNAIL overexpression is known to induce fibrosis and in some cancer types epithelial-mesenchymal transition (27), features which maybe explain stromal desmoplasia in MTC.

In our study we examined cases with initial diagnosis from 2014 to 2019. As MTC is a slowly progressive tumor with possibly long disease courses, despite existing metastasis, tumor-associated deaths are not necessarily to be expected after short observation periods (28). Thus, death as endpoint of observation is not suitable in this case. Presence of metastases alone is a reliable marker for unfavorable course with tumor-associated deaths after years and therefor overall survival (29). As mentioned above, we assume to be proven, that stromal desmoplasia is a reliable surrogate marker for development of lymph node metastases and therefore aggressive disease course. Hence, we postulate that the correlation of DLL3 expression with stromal desmoplasia is sufficient to suggest that DLL3 expression predicts a bad outcome.

In Summary, we can report, that DLL3 is expressed in medullary thyroid carcinoma and shows a high association to stromal desmoplasia and lymph node metastases, whereas DLL3 is usually not expressed by MTCs without stromal desmoplasia. DLL3 is therefore a reliable surrogate marker for aggressive behavior of medullary thyroid carcinoma. Further studies are necessary to determine potential therapeutic options with DLL3-targeting antibodies.

References

1 Randle RW, Balentine CJ, Leverson GE, Havlena JA, Sippel RS, Schneider DF & Pitt SC. Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the past 30 years. *Surgery* 2017 **161** 137–146. (https://doi.org/10.1016/j.surg.2016.04.053)

2 Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F, *et al.* Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015 **25** 567–610. (https://doi.org/10.1089/thy.2014.0335)

3 Schmid KW. Histopathology of C cells and medullary thyroid carcinoma. *Recent Results in Cancer Research* 2015 **204** 41–60. (https://doi.org/10.1007/978-3-319-22542-5_2)

4 LiVolsi VDR, Komminoth P, Mete O, Mulligan L, Schmid KW, Waguespack SG, Elisei R & Eng C. Multiple endocrine neoplasia type

2. In *WHO Classification of Tumors of Endocrine Organs*, pp. 248–252. Eds RO RLloyd, G Klöppel & J Rosai. Lyon: International Agency for Research on Cancer (International Arctic Research Center), 2017.

5 Koperek O, Scheuba C, Cherenko M, Neuhold N, De Micco C, Schmid KW, Niederle B & Kaserer K. Desmoplasia in medullary thyroid carcinoma: a reliable indicator of metastatic potential. *Histopathology* 2008 **52** 623–630. (https://doi.org/10.1111/j.1365-2559.2008.03002.x)

6 Chuang LL, Hwang DY, Tsai KB, Chan HM, Chiang FY & Hsiao PJ. A cohort study on 10-year survival of sporadic medullary thyroid carcinoma with somatic RET mutation. *Kaohsiung Journal of Medical Sciences* 2016 **32** 545–551. (https://doi.org/10.1016/j.kjms.2016.08.012)

7 Scheuba C, Kaserer K, Kaczirek K, Asari R & Niederle B. Desmoplastic stromal reaction in medullary thyroid cancer-an intraoperative 'marker' for lymph node metastases. *World Journal of Surgery* 2006 **30** 853–859. (https://doi.org/10.1007/s00268-005-0391-4)

8 Hao Z & Wang P. Lenvatinib in management of solid tumors. *Oncologist* 2020 **25** e302–e310. (https://doi.org/10.1634/theoncologist.2019-0407)

9 Dralle H, Musholt TJ, Schabram J, Steinmuller T, Frilling A, Simon D, Goretzki PE, Niederle B, Scheuba C, Clerici T, *et al*. German Association of Endocrine Surgeons practice guideline for the surgical management of malignant thyroid tumors. *Langenbeck's Archives of Surgery* 2013 **398** 347–375. (https://doi.org/10.1007/s00423-013-1057-6)

10 Machens A & Dralle H. Biomarker-based risk stratification for previously untreated medullary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* 2010 **95** 2655–2663. (https://doi.org/10.1210/jc.2009-2368)

11 Cook M, Yu XM & Chen H. Notch in the development of thyroid C-cells and the treatment of medullary thyroid cancer. *American Journal of Translational Research* 2010 **2** 119–125.

12 Owen DH, Giffin MJ, Bailis JM, Smit MD, Carbone DP & He K. DLL3: an emerging target in small cell lung cancer. *Journal of Hematology and Oncology* 2019 **12** 61. (https://doi.org/10.1186/s13045-019-0745-2)

13 von Arx C, Capozzi M, Lopez-Jimenez E, Ottaiano A, Tatangelo F, Di Mauro A, Nasti G, Tornesello ML & Tafuto S. Updates on the role of molecular alterations and NOTCH signalling in the development of neuroendocrine neoplasms. *Journal of Clinical Medicine* 2019 **8** 1277. (https://doi.org/10.3390/jcm8091277)

14 Mansfield AS, Hong DS, Hann CL, Farago AF, Beltran H, Waqar SN, Hendifar AE, Anthony LB, Taylor MH, Bryce AH, *et al*. A phase I/II study of rovalpituzumab tesirine in delta-like 3-expressing, advanced solid tumors 2020 **38** (15_suppl) 3552–3552.

15 DeLellis RA, Al Ghuzlan A, Albores Saavedra J, Baloch ZW, Basolo F, Elisei R, Kaserer k, LiVolsi V, Matias-Guiu X, Mete O, *et al*. Medullary thyroid carcinoma. In *WHO Classification of Tumors of Endocrine Organs*, pp. 108–113. Eds RV lloyd, RY Osamura, G Klöppel & J Rosai. Lyon: IARC, 2017.

16 Huang RSP, Holmes BF, Powell C, Marati RV, Tyree D, Admire B, Streator A, Newell AEH, Perez J, Dalvi D, *et al*. Delta-like protein 3 prevalence in small cell lung cancer and DLL3 (SP347) assay characteristics. *Archives of Pathology and Laboratory Medicine* 2019 **143** 1373–1377. (https://doi.org/10.5858/arpa.2018-0497-OA)

17 Brcic L, Kuchler C, Eidenhammer S, Pabst D, Quehenberger F, Gazdar AF & Popper H. Comparison of four DLL3 antibodies

performance in high grade neuroendocrine lung tumor samples and cell cultures. *Diagnostic Pathology* 2019 **14** 47. (https://doi.org/10.1186/s13000-019-0827-z)

18 Morgenszstern D, Besse B, Greillier L, Santana-Davila R, Ready N, Hann CL, Glisson BS, Farago AF, Dowlati A, Rudin CM, *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. *Clinical Cancer Research* 2019 **25** 6958–6966. (https://doi.org/10.1158/1078-0432.CCR-19-1133)

19 Rudin CM, Pietanza MC, Bauer TM, Ready N, Morgenszstern D, Glisson BS, Byers LA, Johnson ML, Burris 3rd HA, Robert F, *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: Oncology* 2017 **18** 42–51. (https://doi.org/10.1016/S1470-2045(16)30565-4)

20 Lashari BH, Vallatharasu Y, Kolandra L, Hamid M, Uprety D. Rovalpituzumab Tesirine: A novel DLL3-targeting antibody-drug conjugate. *Drugs in RandD* 2018 **18** 255–258. (https://doi.org/10.1007/s40268-018-0247-7)

21 Matsuo K, Taniguchi K, Hamamoto H, Ito Y, Futaki S, Inomata Y, Shima T, Asakuma M, Lee SW, Tanaka K, *et al*. Delta-like 3 localizes to neuroendocrine cells and plays a pivotal role in gastrointestinal neuroendocrine malignancy. *Cancer Science* 2019 **110** 3122–3131. (https://doi.org/10.1111/cas.14157)

22 Puca L, Gavyert K, Sailer V, Conteduca V, Dardenne E, Sigouros M, Isse K, Kearney M, Vosoughi A, Fernandez L, *et al*. Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. *Science Translational Medicine* 2019 **11** eaav0891. (https://doi.org/10.1126/scitranslmed.aav0891)

23 Severino M. Phase III trial evaluating rova-T in small cell lung cancer put on hold, 2018. (available at: https://www.targetedonc.com/view/phase-iii-trial-evaluating-rovat-in-small-cell-lung-cancer-put-on-hold)

24 Aubert S, Berdelou A, Gnemmi V, Behal H, Caiazzo R, D'Herbomez M, Pigny P, Wemeau JL, Carnaille B, Renaud F, *et al*. Large sporadic thyroid medullary carcinomas: predictive factors for lymph node involvement. *Virchows Archiv* 2018 **472** 461–468. (https://doi.org/10.1007/s00428-018-2303-7)

25 Furuta M, Kikuchi H, Shoji T, Takashima Y, Kikuchi E, Kikuchi J, Kinoshita I, Dosaka-Akita H & Sakakibara-Konishi J. DLL3 regulates the migration and invasion of small cell lung cancer by modulating Snail. *Cancer Science* 2019 **110** 1599–1608. (https://doi.org/10.1111/cas.13997)

26 Deng SM, Yan XC, Liang L, Wang L, Liu Y, Duan JL, Yang ZY, Chang TF, Ruan B, Zheng QJ, *et al*. The Notch ligand delta-like 3 promotes tumor growth and inhibits Notch signaling in lung cancer cells in mice. *Biochemical and Biophysical Research Communications* 2017 **483** 488–494. (https://doi.org/10.1016/j.bbrc.2016.12.117)

27 Wang Y, Shi J, Chai K, Ying X & Zhou BP. The role of snail in EMT and tumorigenesis. *Current Cancer Drug Targets* 2013 **13** 963–972. (https://doi.org/10.2174/15680096113136660102)

28 Schmid KW & Böcker W. Schilddrüse. In *Pathologie - 4 - Endokrine Organe*, Vol. 4. Ed W Remmele. Berlin, Heidelberg, Germany: Springer-Verlag, 1997.

29 Bergholm U, Bergstrom R & Ekbom A. Long-term follow-up of patients with medullary carcinoma of the thyroid. *Cancer* 1997 **79** 132–138. (https://doi.org/10.1002/(sici)1097-0142(19970101)79:1<132::aid-cncr19>3.0.co;2-5)