Delta-like Protein 3 Prevalence in Small Cell Lung Cancer and DLL3 (SP347) Assay Characteristics

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**Context.**—Delta-like protein 3 (DLL3) is a protein that is implicated in the Notch pathway.

**Objective.**—To present data on DLL3 prevalence in small cell lung cancer and staining characteristics of the VENTANA DLL3 (SP347) Assay. In addition, the assay’s immunoreactivity with other neoplastic and nonneoplastic tissues is outlined.

**Design.**—Individual formalin-fixed, paraffin-embedded specimens of small cell lung cancer and tissue microarrays comprising neoplastic and nonneoplastic tissues were procured. Sections were cut and stained with DLL3 (SP347) assay. The slides were examined to determine prevalence, staining characteristics, and immunoreactivity.

In the United States, lung cancer is diagnosed in more than 222,500 patients and accounts for 155,870 deaths annually.1 Small cell lung cancer (SCLC) is a distinct type of lung cancer that represents approximately 15% of all primary lung cancers.2 It is a neuroendocrine tumor that is histologically defined by distinctive features including finely granular chromatin, nuclear molding, crush artifact, and Azzopardi phenomenon. Although treatment exists for primary SCLC, relapses are common; and despite the aggressive nature of SCLC, limited options are available as second-line treatment for this aggressive disease, with no third-line treatment available.3–6 Because of the lack of treatment options for SCLC, it is imperative to further examine the biology of SCLC cancers and investigate new therapeutic targets for SCLC.

Delta-like protein 3 (DLL3) is a transmembrane protein that has been implicated in SCLC tumorigenesis through its interactions with the Notch pathway. Specifically, DLL3 inhibits Notch activation, thereby promoting neuroendocrine tumorigenesis.7 This is in contrast to the other Notch family ligands (DLL1, DLL4, JAG1, and JAG2), which activate the Notch pathway in neuroendocrine tumors, leading to the suppression of tumor growth in these tumors.8 DLL3’s contribution to neuroendocrine tumorigenesis is further supported by studies demonstrating that it is a downstream target for achaete-scute homolog 1 (ASCL1), which has been linked with neuroendocrine cell fate decisions and tumorigenesis in SCLC.9

Studies have suggested that DLL3 is elevated in neuroendocrine tumors, including SCLC, whereas it is low in the majority of normal tissue in humans.8 This presents an opportunity to target and selectively deliver drugs to tumor cells highly expressing the DLL3 antigen by using antibody-drug conjugates specific to the DLL3 antigen. Recently, we used a rabbit monoclonal antibody in the development of the VENTANA DLL3 (SP347) Assay, which may be used to identify patients with high levels of DLL3 expression in SCLC. Here, we present data on the prevalence of DLL3 expression in SCLC, as well as the staining characteristics and immunoreactivity of the DLL3 (SP347) assay.

**MATERIALS AND METHODS**

**Specimen Cohort**

**Validation Cohort.**—A validation cohort of 1503 cases of formalin-fixed (10% neutral buffered formalin), paraffin-embedded SCLC whole-section samples was procured from an internal tissue bank at Ventana Medical Systems, Inc, and the following commercial tissue banks: Asterand Bioscience; Avadent BioSciences; Boca Biolistics; Conversant Bio; GLAS; ILSbio; Impath; PrecisionMed, Inc; TriMetis Life Sciences; and US Biomax, Inc. The cohort included primary and metastatic SCLC samples and consisted of resections, core needle biopsies, and fine-needle aspirates. Review of hematoxylin-eosin-stained slides from cases in the validation cohort confirmed morphologic features consistent with small cell

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carcinoma. These include small to medium-sized cells with minimal cytoplasm, indistinct nucleoli, and nuclear molding.

**Discovery Cohort.**—The discovery cohort used for immunoreactivity assessments included the following: (1) Cut slides (4 µm) from 2 tissue microarrays (TMAs) of normal tissue: one array (FD808i-1) containing 24 types of normal human tissue representing 72 individual cases, and another array (999l, Food and Drug Administration) containing 32 types of normal human tissue representing 78 individual cases procured from US Biomax; (2) Cut slides (4 µm) also procured from a tumor tissue array (808i-2, Food and Drug Administration) containing 54 cases of carcinoma from multiple locations plus 18 cases with 6 types of normal tissue adjacent to tumor (single cores per case) representing 72 individual cases, also from US Biomax; (3) Cut slides (4 µm) from an array (LUC1504) of SCLC tissues containing 150 cores including 5 normal/benign lung tissue and 70 cases of SCLC tissue procured from Pantomics.

### Immunohistochemistry Staining

Four-micrometer-thick tissue sections were cut from the cohort of cases and mounted on positively charged glass slides. The slides from both validation and discovery cohorts were stained with VENTANA DLL3 (SP347) Assay (P/N 790-7016, Ventana) along with OptiView DAB IHC Detection Kit (P/N 760-700, Ventana) on the BenchMark ULTRA automated staining platform using the recommended staining conditions of the DLL3 (SP347) assay package insert. Rabbit monoclonal negative control Ig (P/N 790-4795) was used as the negative reagent control. The samples were counterstained with hematoxylin II (P/N 790-2208, Ventana) and bluing reagent (P/N 760-2037, Ventana). Recommended tissue controls were used based on the DLL3 (SP347) assay package insert. For each case in the cohort and for the TMAs, one slide was stained with hematoxylin-eosin using either the VENTANA SYMPHONY system or Sakura Tissue-Tek Prisma & Film Automated Slide Stainer & Coverslipper according to the manufacturer’s instruction.

### Scoring

**Validation Cohort.**—Negative reagent control and DLL3 (SP347) assay-stained slides were evaluated for nonspecific background staining and cross-reactivity on a scale of 0 to 3 in...
Table 1. DLL3 (SP347) Reactivity in Small Cell Lung Cancer Samples of the Validation Cohort (N = 1362)

| Reactivity     | Cases No. | %    |
|----------------|-----------|------|
| Not reactive   | 322       | 23.6 |
| Reactive       | 1040      | 76.4 |

Abbreviation: DLL3, delta-like protein 3.

RESULTS

Validation Cohort

In the validation cohort of 1503 SCLC cases, 141 samples (9.4%) were deemed not evaluable or not acceptable (eg, no tissue/tumor present, crush, necrosis, or high background), and these samples were excluded from the analysis of DLL3 staining and reactivity. Of the 1362 evaluable samples, reactivity was observed in 1040 (76.4%) (Table 1). In addition, the samples exhibited a wide range of staining intensities (Figure 1) and percentage tumor cell staining (bimodal distribution of percentage staining, with 80% of the cases either falling below 25% or above 75% tumor cell staining) (Figure 2).

Staining Characteristics

Upon examination of the SCLC specimens, the following staining characteristics were identified: partial and complete membrane/cytoplasmic staining, granular staining pattern, and homogeneous or heterogeneous staining pattern (Figure 3, A through L).

Discovery Cohort

In the discovery TMAs, 29 samples were deemed not evaluable (eg, no tissue/tumor present, artifacts, or edge artifacts), and these cases were not used in the analysis of reactivity. Samples exhibited a range of staining intensity from weak or moderate to strong in both normal and neoplastic tissues and a range of percentage tumor cell staining in neoplastic tissues.

Reactivity was observed in 24 of 156 evaluable cores (15.8%) in the nonneoplastic TMAs (Table 2). The reactive nonneoplastic cores were composed primarily of normal tissues with a neural or neuroendocrine component. Reactivity was observed in 16 of 65 evaluable cores (24.6%) (1 core was normal tissue adjacent to tumor) in the carcinoma TMA (Table 3) and 80 of 143 evaluable cores (55.9%) in the SCLC TMA.

Table 2. Staining Intensity of Reactive Cores in the Nonneoplastic Tissue Microarrays

| Organ                | DLL3 Staining               | Intensity | Localization                          |
|----------------------|----------------------------|-----------|---------------------------------------|
| Cerebrum             | 2                          | Cytoplasm of neurons |                       |
| Adrenal gland        | 1                          | Cytoplasm of adrenal cortical zona reticularis cells |
| Adrenal gland        | 1                          | Cytoplasm of adrenal cortical zona reticularis cells |
| Pancreas             | 1                          | Cytoplasm of pancreatic acinar epithelium and islet cells |
| Pancreas             | 1                          | Cytoplasm of pancreatic acinar epithelium and islet cells |
| Pancreas             | 1                          | Cytoplasm of pancreatic acinar epithelium and islet cells |
| Hypophysis           | 2                          | Cytoplasm of neuroendocrine cells of anterior pituitary |
| Hypophysis           | 1                          | Cytoplasm of neuroendocrine cells of anterior pituitary |
| Hypophysis           | 1                          | Cytoplasm of neuroendocrine cells of anterior pituitary |
| Testis               | 2                          | Cytoplasm of immature seminiferous precursors and Leydig cells |
| Thyroid gland        | 1                          | Cytoplasm of thyroid follicular epithelium cells |
| Stomach              | 1                          | Cytoplasm of chief cells |
| Liver                | 1                          | Cytoplasm of hepatocytes |
| Liver                | 2                          | Cytoplasm of hepatocytes |
| Larynx               | 1                          | Nucleus of mucus glandular epithelium cells |
| Larynx               | 1                          | Nucleus of mucus glandular epithelium cells |
| Adrenal gland        | 2                          | Cytoplasm of adrenal cortical zona reticularis cells |
| Adrenal gland        | 2                          | Cytoplasm of adrenal cortical zona reticularis cells |
| Adrenal gland        | 1                          | Cytoplasm of adrenal cortical zona reticularis cells |
| Hypophysis           | 2                          | Cytoplasm of neuroendocrine cells of anterior pituitary |
| Hypophysis           | 2                          | Cytoplasm of neuroendocrine cells of anterior pituitary |
| Liver                | 2                          | Cytoplasm of hepatocytes |
| Liver                | 1                          | Cytoplasm of hepatocytes |

Abbreviation: DLL3, delta-like protein 3.
DISCUSSION

This is the first study in the literature, to our knowledge, to use the VENTANA DLL3 (SP347) Assay to examine the prevalence of DLL3 protein expression within a cohort of SCLC. In the validation cohort of cases, a high percentage (76.4%) had reactivity to our antibody, exemplified by observable staining with the OptiView detection kit. In addition, the validation cohort of cases showed a bimodal prevalence of DLL3 protein expression.
neuroendocrine carcinoma. 42 (88%) had positive staining. Lastly, in a Japanese cohort of 63 patients with SCLC, 52 (83%) were deemed positive for DLL3 expression. In summary, DLL3 (SP384) prevalence data are consistent with the data in the literature using other antibodies, showing that there are high rates of DLL3 protein expression in SCLC.

Additionally, reactivity was observed in a small percentage of nonneoplastic and neoplastic tissues, and was significantly higher within discovery SCLC TMAs in our study. This is consistent with a study by Saunders et al.8 That showed low prevalence of DLL3 in normal, nonneoplastic tissues. Also, this study demonstrated that the reactive tissues were primarily of neural or neuroendocrine origin, which corresponds with the known characteristics of DLL3 in mRNA studies.8,12

Herein, we present data that show the prevalence of DLL3 expression in a cohort of 1503 cases and also show that the VENTANA DLL3 (SP347) Assay is reactive with recognizable staining patterns that can be used for determining the level of DLL3 expression in SCLC.

### Table 3. VENTANA DLL3 (SP347) Assay Staining Intensity and Percentage Tumor Cells Staining of Cores in Neoplastic Tissue Microarrays

| Organ | Pathology | DLL3 Staining Intensity | % DLL3-Positive Tumor Cells |
|-------|-----------|-------------------------|-----------------------------|
| Cerebrum | Glioblastoma | 1 | 5 |
| Cerebrum | Malignant ependymoma | 1 | 40 |
| Cerebrum | Malignant oligodendroglioma | 2 | 70 |
| Pancreas | Islet cell tumor | 1 | 100 |
| Testis | Seminoma | 2 | 20 |
| Testis | Embryonal carcinoma | 1 | 50 |
| Thyroid | Medullary carcinoma | 1 | 50 |
| Lung | Small cell undifferentiated carcinoma | 2 | 100 |
| Small intestine | Malignant interstitialoma | 1 | 100 |
| Rectum | Moderate malignant interstitialoma | 1 | 100 |
| Liver | Hepatocellular carcinoma | 1 | 20 |
| Cervix | Squamous cell carcinoma | 1 | 2 (staining in immune cells) |
| Rectum | Malignant melanoma | 2 | 100 |
| Lymph node | Diffuse B-cell lymphoma of right thigh | 1 | 3 |
| Cardiac pericardium | Normal tissue adjacent to tumor | 1 | NA (cytoplasmic staining on lining cells) |

Abbreviations: DLL3, delta-like protein 3; NA, not applicable.

distribution of percentage of cells staining and a wide range of staining intensities.

Three studies of DLL3 expression-level data8,10,11 showed similar prevalence results. However, all 3 of these studies used a mouse monoclonal antibody, in contrast to the SP347 rabbit monoclonal antibody, and their analysis of positivity had different definitions. In the first study describing DLL3 prevalence in SCLC, the authors used an H-score of 100 or higher as a positive cutoff and found that 120 (72%) of 167 treatment-naïve and 17 (85%) of 20 recurrent and treatment-refractory SCLC cases were positive for DLL3 expression.8 In the second and third studies, positivity was defined as 1% or more of tumor cells staining. The second study showed that of 48 patients with SCLC or large cell

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