Editorial

Orthopedia homeobox protein (OTP) in the diagnostic and prognostic workup of pulmonary carcinoid tumors

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Pulmonary carcinoid (PC) tumors

Lung neuroendocrine neoplasms (NENs) account for approximately one fifth of all lung malignancies. They are a heterogenous group of neoplasms that can be classified as low- and intermediate-grade PCs and high-grade large-cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC). PCs are further divided into typical carcinoid (TC) and atypical carcinoid (AC) tumors based on the number of mitoses and presence of necrosis (1).

PCs are rare, well-differentiated tumors that account for <2% of all lung cancers. The only curative treatment for PC tumors is a complete surgical resection. Resected PC tumor patients generally have a good prognosis with a 5-year overall survival 87–100% for TC patients and 60–70% for AC patients (2,3). However, PCs show a risk for late recurrence and distant metastasis. The risk is higher for ACs (20–35% metastasize) and lower for TCs (5–10% metastasize). Clinical management of metastatic disease remains challenging and a curative treatment strategy is not available (1,4). Thus, the aim of treatment is to control tumor growth and possible functioning syndromes to improve both quality of life and survival.

Challenges in the histopathological evaluation of pulmonary carcinoid tumors

Diagnosis of a PC requires a careful histological evaluation. Here, distinguishing between PCs and high-grade LCNEC and SCLC is crucial as it impacts subsequent surgical management and additional therapeutic management and has a significant impact on prognosis. Typically, a biopsy or fine-needle aspiration is the first sample the pathologist receives. Evaluating these small specimens can be challenging, which created a need for specific immunohistochemical markers that can assist in differential diagnostics (5).

On the other hand, occurrence of PC tumor recurrence or metastasis cannot be reliably predicted with the current WHO classification. This leads to clinical and radiological surveillance, which causes unnecessary radiation exposure, is a constant reminder of the disease for the patients, and results in additional health care costs. Previous studies have reported several clinical and molecular markers associated with the risk of developing metastases and poor outcome (3). These include e.g., age, gender, performance status, TNM staging, mitotic index, and Ki-67 proliferation index at the

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time of diagnosis. However, more molecular parameters that distinguish PCs with a favorable outcome from those with a metastatic potential are still needed.

As PCs and other neuroendocrine tumors can metastasize, finding the primary tumor site is not always straightforward. Some patients present with symptoms related to the overproduction of biologically active hormones and peptides, which can guide identification of the primary tumor. In many cases, only fine needle aspiration or biopsy is available for assessing organ-specific immunohistochemical markers. Typically, a panel of cytoplasmic (chromogranin and synaptophysin), membranous (CD56), and nuclear (insulinoma-associated protein 1, INSM1) markers are used to confirm neuroendocrine differentiation (4). In addition, pan-cytokeratin can be used to exclude paraganglioma and pheochromocytoma. However, immunohistochemical markers that could point to the primary tumor are limited and include e.g., thyroid transcription factor 1 (TTF-1), glucagon, gastrin, serotonin, caudal type homeobox 2 (CDX2), PAX-family transcription factors, special AT-rich sequence-binding protein 2 (SATB2), and islet-1 (ISL1). Of these, only TTF-1 is useful for determining lung origin but has limited sensitivity (6).

Value of orthopedia homeobox protein (OTP) immunohistochemistry in PC diagnostics and prognostication

One potential candidate for differential diagnostics and a novel prognostic marker for PC tumors is OTP. OTP is a member of the homeobox protein family and has a well-defined role in embryonic neurodevelopment (7). The initial gene-expression studies by Swarts *et al.* a decade ago showed that OTP is downregulated in PC tumors from patients with poor disease outcome (8). Swarts *et al.* also used immunohistochemistry (*Table 1*) to study protein level expression and confirmed that the absence of nuclear OTP is associated with the occurrence of distant metastasis and poor disease outcome. OTP was found to be an independent prognostic marker but better predicted patient outcome when combined with CD44, a stem cell marker and cell-surface glycoprotein. These findings were later validated by several other researchers resulting in the addition of coexpression of nuclear OTP and CD44 as a prognostic factor in the current WHO classification (1,10,15-17).

A few years later, Nonaka *et al.* reported OTP to be a useful diagnostic marker for PCs. In their series of altogether more than 1,000 samples from pulmonary neuroendocrine hyperplasias, PCs, LCNECs, SCLCs, non-pulmonary NENs, endocrine tumors, adenocarcinomas, and squamous-cell carcinomas, OTP expression was mainly observed in pulmonary neuroendocrine hyperplasias and PCs (*Table 1*) (9). In addition, normal tissues from various organs were studied and no OTP expression was observed. Similarly, Viswanathan *et al.* reported that they did not observe OTP expression in a small series of lung adenocarcinomas (n=8) and squamous cell carcinomas (n=10) (14).

OTP expression has also been studied on cell blocks produced from fine-needle aspirations with or without corresponding surgical resection samples (*Table 1*) (12-14). These studies strengthened the interpretation that nuclear OTP immunohistochemistry is highly sensitive and specific in distinguishing PCs from other pulmonary neuroendocrine and non-neuroendocrine malignancies both in cytologic and surgical specimens.

Interestingly, OTP expression is not observed in synaptophysin-positive neuroendocrine cells (so-called Kulchitsky cells) of the normal bronchus, but OTP is diffusely and strongly expressed in neuroendocrine hyperplasia (9). This suggests that OTP activation may be an important step for carcinoid tumorigenesis. However, downregulation seems to be related to tumor progression since loss of OTP protein expression correlates with prognosis (8,10,15,16). Recently, the authors of the paper commented on, examined DNA methylation data and found that that changes in DNA methylation patterns were associated with the difference in OTP expression levels (18). They suggested that OTP expression is a unique feature of PCs with a favorable prognosis; in patients with a poor prognosis, OTP expression is lost most likely due to changes in DNA methylation levels. This finding may lead to future therapeutics for PC patients that target histone modifiers or chromatin remodeling complexes.

Technical aspects of evaluating immunohistochemical OTP expression

Most of the previous studies on immunohistochemical OTP expression in PCs and other tumors were conducted with either rabbit anti-OTP polyclonal antibody (pAb) (HPA039365; Atlas Antibodies; later Sigma Aldrich) or with rabbit anti-OTP pAb (HPA059342; Sigma Aldrich) (*Table 1*). Only one study that focused on tumors with neuroendocrine differentiation at relatively uncommon sites (e.g., prostate, breast, and gynecological tumors) used another primary

Table 1 Previous studies that analyzed OTP expression in lung neuroendocrine neoplasias using immunohistochemistry

Authors, year (ref)	Number of lung NENs	Number of other tumors	DIPNECH [nuclear pos (%)/all]	TC [nuclear pos (%)/all]	AC [nuclear pos (%)/all]	LCNEC [nuclear pos (%)/all]	SCLC [nuclear pos (%)/all]	Primary antibody
Swarts *et al.* 2013 (8)	352	Not included	Not included	175 (78%)/225	31 (49%)/63	1 (4%)/24	4 (12%)/34	pAb, HPA039365, Atlas Antibodies
Nonaka *et al.* 2016 (9)	266	753	7 (100%)/7	105 (85%)/123	10 (48%)/21	0 (0%)/11	2 (2%)/93	pAb, HPA039365, Atlas Antibodies
Papaxoinis *et al.* 2017 (10)	86	Not included	7 (88%)/8	55 (80%)/69	9 (53%)/17	Not included	Not included	pAb, HPA039365, Atlas Antibodies
Papaxoinis *et al.* 2018 (11)	166	Not included	16 (100%)/16	117 (89%)/132	21 (62%)/34	Not included	Not included	pAb, HPA039365, Atlas Antibodies
Yoxtheimer *et al.* 2018 (12)	30	20	Not included	4 (50%)/8	1 (17%)/6	1 (20%)/5	0 (0%)/11	pAb, HPA039365, Sigma Aldrich
Hanley *et al.* 2018 (13)	15	44	Not included	9 (100%)/9	1 (17%)/6	Not included	Not included	pAb, HPA039365, Sigma Aldrich
Viswanathan *et al.* 2019 (14)	42	18	Not included	9 (82%)/11	10 (83%)/12	0 (0%)/9	0 (0%)/10	pAb, HPA039365, Sigma Aldrich
Centonze *et al.* 2023 (15)	317	Not included	Not included	196 (77%)/253	25 (43%)/58	Not included	Not included	pAb, HPA059342, Sigma Aldrich
Reuling *et al.* 2022 (16)	171	Not included	Not included	96 (86%)/112	45 (76%)/59	Not included	Not included	pAb, HPA059342, Sigma Aldrich

OTP, orthopedia homeobox protein; NEN, neuroendocrine neoplasia; DIPNECH, diffuse idiopathic neuroendocrine cell hyperplasia; TC, typical carcinoid; AC, atypical carcinoid; LCNEC, large-cell neuroendocrine carcinoma; SCLC, small cell lung carcinoma; pAb, polyclonal antibody.

antibody (rabbit anti-OTP pAb, NBP1–85861, Novus Biological) (19). However, the widely used rabbit anti-OTP pAbs HPA039365 and NBP1–85861 have been discontinued, and a lack of reliable monoclonal antibodies (mAbs) against OTP hinders the clinical implementation of OTP immunohistochemistry in routine diagnostics. Moreover, immunohistochemistry is highly automatized in most clinical pathology laboratories, which creates a need for OTP staining protocols with automated platforms.

As a solution, the group who originally found OTP to be prognostic in PC patients, reported in this journal how they developed and verified two new mAbs (clone CL11222 and CL11225) against OTP (20). Additionally, they optimized the staining protocols for two widely used automated immunohistochemical staining instruments, namely Dako Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, USA) and Autostainer 480S (ThermoFisher Scientific, Waltham, MA, USA).

Moonen *et al.* employed the widely used pAb HPA039365 as a reference when developing, verifying, and optimizing the new mAbs (20). They evaluated the specificity of OTP with tissue material consisting of TMA slides with more than 25 normal tissue types and whole sections from hypothalamus. Patient material consisted of 86 pulmonary NENs in TMA format and 40 other NENs of non-pulmonary origin.

Staining results showed that moderate nuclear OTP expression was observed in a subset of neurons in the hypothalamus, but other normal tissues were negative for OTP, as expected. All non-pulmonary NENs were negative for mAbs. Of pulmonary NENs, 74% of TCs and 64% of ACs showed nuclear positivity while almost all high-grade NENs were negative.

Moonen *et al.* also evaluated interobserver variability with κ scores and intraclass correlation coefficient (20). The results revealed substantial agreement on the Dako platform between the two mAb clones and almost perfect agreement on the ThermoFisher platform. In fact, interobserver variation is considerable in the histopathologic classification of PCs, especially in ACs (21). Still, correct classification is crucial, since the subtype has a practical impact on patient management; after resection, AC patients require more frequent follow-up and possible additional treatments while TC patients can be followed with a longer interval. As

evaluation of OTP immunohistochemistry shows almost perfect agreement, this marker could be used to improve prognosis prediction of PC tumors that are difficult to classify.

Conclusions

OTP is a highly sensitive and specific marker of PCs and is preferentially expressed in TCs versus ACs. As OTP seems to be homogeneously expressed through the tumor, evaluating OPT on biopsy specimens is appropriate. In addition, OTP immunohistochemistry can assist in confirming lung origin in metastatic well-differentiated NENs. With the new mAbs, OTP immunohistochemistry can be implemented in both pre-operative and post-operative histological analyses to improve PC differential diagnostics and prognostication. In future clinical practice, OTP immunohistochemistry may enable clinicians to reduce radiological follow-up and associated distress in PC tumor patients.

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