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Review

Insights into Novel Prognostic and Possible Predictive Biomarkers of Lung Neuroendocrine Tumors

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Abstract. *Primary lung neuroendocrine tumors (NETs) consist of typical and atypical carcinoids, large-cell neuroendocrine carcinomas and small-cell lung carcinomas. NETs are highly heterogeneous in histological characteristics, clinical presentation and natural history. While there are morphological and immunohistochemical criteria to establish diagnosis, there is a lack of universal consensus for prognostic factors or therapeutic targets for personalized treatment of the disease. Thus, identifying potential markers of neuroendocrine differentiation and prognostic factors remains of high importance. This review provides an insight into promising molecules and genes that are implicated in NET carcinogenesis, cell-cycle regulation, chromatin remodeling, apoptosis, intracellular cascades and cell–cell interactions. Additionally it supports a basis for classifying these tumors into categories that distinct molecular characteristics and disease natural history, which may have a direct impact on treatment options. In light of the recent approval of everolimus, mammalian target of rapamycin pathway inhibition and related biomarkers may play a central role in the treatment of pulmonary NETs. Future clinical trials that integrate molecular profiling are deemed necessary in order to treat patients with NET on a personalized basis.*

Lung cancer remains one of the most frequent and lethal types of cancer worldwide. The recent World Health Organization (WHO) classification distinguishes five major types of lung cancer: adenocarcinoma, squamous-cell carcinoma, small-cell lung carcinoma (SCLC), large-cell neuroendocrine carcinoma (LCNEC), and carcinoid tumors (1). Although the development of next-generation sequencing techniques has afforded comprehensive opportunities to study lung tumors and the emergence of novel therapeutics, very little has been translated into improving patient survival. Over the past 20 years, the average 5-year overall survival rate for lung cancer has remained stable at around 15% (2).

Primary lung neuroendocrine tumors (NETs) are highly heterogeneous in histological characteristics, clinical presentation and disease course. The histological variants of NETs, including typical carcinoid, atypical carcinoid, LCNEC and SCLC (3). Typical and atypical carcinoid are well-differentiated but differ in cellular atypia and mitotic yield; overall, these carcinoids have a more favorable prognosis compared to LCNEC and SCLC, which account for 15-20% of lung cancer-related deaths (2).

Genetic and molecular profiling of resected tumors have revealed potential targets for personalized anticancer therapeutics and pinpoint towards an emerging molecular taxonomy of cancer, in general (4, 5) and in lung neoplasia (6). Substantial research efforts are being made in order to determine molecular signatures that may have prognostic value and may constitute therapeutic targets (7). Thus, the aim of this review was to summarize and critically evaluate the emerging data on prognostic and possible predictive biomarkers in lung NETs.

Mammalian Target of Rapamycin Pathway

The mammalian target of rapamycin (mTOR) pathway plays a key role in cellular processes by regulating protein synthesis, cell growth and metabolic systems. Deregulation

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of the mTOR signaling pathway is implicated in several tumor types, including NETs, raising the potential for being an attractive target for therapy (8-10). High frequencies of gene copy-number alterations in genes encoding members of the PI3K–AKT–mTOR pathway were detected in SCLC (11). In an immunohistochemical study of AKT and mTOR expression in bronchopulmonary NETs, phosphorylated AKT and mTOR were found in the majority of cases, suggesting a central role for this pathway in NET carcinogenesis (12). It should be noted that higher expression of these molecules was shown in carcinoids compared to LCNECs and SCLCs. Interestingly, mTOR expression was inversely associated with greater tumor stage in LCNECs and SCLCs, with T1-T2 tumors having higher levels of phosphorylated mTOR than T3-T4 ones (12). The similarity in genomic profiling between LCNEC and SCLC was addressed in a recent study using capture sequencing of all the coding exons of 244 cancer-related genes (13). These results highlight that high-grade and more aggressive NETs may have molecular hallmarks distinct from those of low- and intermediate-grade tumors, which could have diagnostic and therapeutic clinical implications (12).

Preclinical data suggest that targeting mTOR pathway in pulmonary NETs is a feasible therapeutic strategy. Rapamycin has been shown to reduce carcinoid proliferation both *in vitro* (BON-1, H727 and MTC cell lines) and in BON-1 xenograft models (14, 15). Moreover, everolimus has been shown to reduce secretion of vascular endothelial growth factor and cell viability in human primary bronchial carcinoid cell cultures (16).

The RAD001 in Advanced Neuroendocrine Tumors, Fourth Trial (RADIANT-4) has increased the interest in the mTOR pathway in NETs (17, 18). This phase III, randomized clinical trial included 302 patients with advanced, progressive, non-functional, pulmonary or gastrointestinal NETs that received either the mTOR inhibitor everolimus (10 mg/day), or placebo, with supportive care optimized for the two groups. Patients treated with everolimus benefited with prolonged progression-free survival and had a 52% reduction in the estimated risk of progression or death (hazard ratio=0.48, 95% confidence interval=0.35-0.67, $p<0.00001$) (17). These results led to the expansion of Food and Drug Administration approval for everolimus to progressive, well-differentiated, nonfunctional NETs of lung or gastrointestinal origin that are unresectable, locally advanced, or metastatic (19). Currently, combinations of everolimus with other agents are being investigated in phase I and phase II clinical trials (18).

Potential markers predicting sensitivity to mTOR inhibitors have been also suggested, such as total mTOR level, AKT, p70S6K (RPS6KB2), and mitogen-activated protein/extracellular signal-regulated kinases (MAPK/ERK) levels in patients with pulmonary carcinoids (20). In SCLC cells, high expression of eukaryotic translation initiation factor 4E (eIF4E) was associated with increased resistance to everolimus treatment. The mTOR-independent activation of eIF4E pathway mediated by MYC has been suggested as a mechanism contributing to everolimus resistance and has provided the rationale for its inhibition in future studies (21).

Cell-cycle Regulatory Genes

The expression of tumor protein 53 (*TP53*) and retinoblastoma protein 1 (*RB1*) cell-cycle checkpoint genes in lung NETs has been assessed by whole-exome and targeted sequencing, with a significantly higher mutational load detected in carcinomas (70.0%) compared with carcinoids (10.2%) (22). Loss of RB1 was frequently encountered in all lung NET subtypes, mainly in SCLC (69.7%) and LCNEC (55.6%) compared to typical (24.5%) and atypical carcinoids (11.4%). Regarding SCLC in particular, homozygous deletions comprised 39.4% of the cases. Moreover, when loss of heterozygosity was also included, the *RB1* alterations prevailed among SCLC cases, at 91% (22). Loss of *TP53* was also frequent among high-grade NETs, as it was detected in 48.5% of SCLC and 40.7% of LCNEC cases, whereas fewer than one-third of carcinoids presented with *TP53* losses. Conversely, loss of heterozygosity of multiple endocrine neoplasia type 1 (*MEN1*) was more frequently detected in carcinoids (15.1% in typical and 22.9% in atypical) compared to LCNEC (3.7%) and SCLC (0%) (22). Thus, it has been suggested that *TP53* and *RB1* transcription patterns may help distinguish between SCLC- and NSCLC-like LCNECs (23).

Walter *et al*. reported results of a comparative mRNA expression analysis among lung NETs subtypes (24). By investigating a panel of mRNAs implicated in cell-cycle regulation (such as cyclin-dependent kinases (CDKs)] and apoptosis, they found a lower expression of Achaete-scute homolog 1 (*ASCL1*), B-cell lymphoma 2 (*BCL2*), caspase 8 (*CASP8*), G_1/S-specific cyclin-E1 (*CCNE1*), *CDK1, CDK2, CDKN1A* and *CDKN2A* in carcinoids compared to carcinomas. An inverse association was found for *CCNE1* and CDK6 (24). These findings provide evidence that carcinoids and carcinomas control *RB1* expression in different ways, by modulating a different subset of cell-cycle regulators. In the analysis, SCLC had a profile discernible from that of LCNEC, by having a higher expression of *CDK2, CDKN1B, CDKN2A* and *PNN* genes compared to the latter (24). Interestingly, minimal *CCND1* and *CDKN2A* expression was correlated with N0 status, whereas low BCL2 expression was associated with absence of venous invasion. It should be noted that Walter *et al*. also identified β-actin (*ACTB*), *CDKN1B*, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), growth factor receptor-bound protein 2 (*GRB2*), Ras homolog gene family, member A

(*RHOA*) and syndecan-binding protein (*SDCBP*) as reference genes in NETs, due to their stable and reliable expression in mRNA expression analysis (25).

To date, various gene abnormalities have been reported to occur frequently in SCLC and LCNEC but are rarely observed in typical carcioids (26). All carcinoid tumors were characterized by diffuse RB1 expression, which was absent from SCLCs and LCNECs. On the contrary, all SCLCs and LCNECs demonstrated intense and diffuse P53 expression (27), in contrast to carcinoids (28). Endo *et al*. evaluated the expression patterns of developing neural cell-specific transcription factors (DNTFs) (including BRN2, thyroid transcription factor 1 (TTF1) and ASCL1)] in patients with resectable lung NETs (27). SCLCs and LCNECs unanimously expressed DNTFs, which were also expressed in a subset of carcinoids (27). DNTs have also been detected in the setting of diffuse idiopathic pulmonary neuroendocrine cell hyperplasia, which is a debatable entity regarding its premalignant potential (29). LCNECs and SCLCs have also demonstrated a higher expression of nuclear survivin, which is essential for the completion of mitosis, in contrast to lung adenocarcinoma (30). These findings may be suggestive of differences in the underlying molecular mechanisms of tumorigenesis between carcinoids and LCNEC/SCLC and may also have therapeutic implications (28).

Apoptosis

Evasion of apoptosis is a well-known hallmark of cancer, with members of the BCL2 family actively implicated in this process both by promoting or hindering apoptosis, such as BCL2-associated X (BAX) and BCL2 respectively (31, 32). The expression of BCL2 and its activated form, namely phosphorylated BCL2 (pBCL2), was recently evaluated in surgical specimens of resectable, early-stage lung NET (33). The highest BCL2 expression was detected in LCNECs, while SCLCs had the highest pBCL2 expression. Carcinoids were at the bottom of the list regarding both molecules under investigation. Interestingly, higher pBCL2 levels were inversely associated with progression-free and overall survival (33). In another study of mRNA expression analysis, the *BCL2*/*BAX* ratio increased throughout the spectrum of lung NETs, from typical and atypical carcinoids, LCNEC and SCLC (24). Furthermore, SCLC and lung cancer carcinomas with neuroendocrine differentiation did not express BCL2-associated athanogene 3 (BAG3), a BCL2-interacting protein that promotes anti-apoptotic activity; however, the number of examined samples was rather limited (34).

These findings were indirectly confirmed by a study on human lung cancer cells, where the up-regulation of *NOTCH1* was associated with decreased BCL2 expression which in turn, was presented as decreased apoptotic potential of cancer cells. This phenotype was significantly correlated with SCLC subtype (35).

CD44 is another molecule that promotes apoptosis and its role in lung NETs has been investigated. In fact, absence of CD44 expression has been correlated with significantly low 20-year survival in patients with pulmonary carcinoids (36). In parallel, Swarts *et al*. found that loss of expression of orthopedia homeobox (OTP) was an independent adverse prognostic factor of survival in patients with pulmonary carcinoids (36). Conversely, high CD44/OTP expression has been associated with improved recurrence-free and overall survival in patients with pulmonary carcinoids (37, 38). Thus, both CD44 and OTP have been suggested as novel candidates for the subclassification of pulmonary carcinoids in terms of prognosis (36, 38).

NOTCH Family

The NOTCH1 signaling pathway plays an important role in cell-cycle fate and is implicated in neuroendocrine differentiation of cells in the respiratory and gastrointestinal systems. NOTCH1 has a dual role in cancer, by acting either as a tumor-suppressor gene or as an oncogene, depending on tumor type (39, 40). It has been shown that NETs, including SCLC, are characterized by an increased expression of achaete-scute complex-like 1 (ASCL1) that promotes NET growth and is downregulated by NOTCH1 (39). Interestingly, a molecular pathway that promotes secondary SCLC has been identified and includes NOTCH inactivation with subsequent *ASCL1* up-regulation and activation of the Wnt cascade that is amplified by concurrent *RB1* and *TP53* mutations (41). Furthermore, the findings reported by Altree-Tacha *et al*. support the notion that ASCL1 has a high specificity for high-grade NETs compared to carcinoids (42). In SCLC, the up-regulation of the deactivated NOTCH1 pathway resulted in the suppression of cell growth and neuroendocirne differentiation, and induced an epithelial-like morphology of cell lines (35). However, NOTCH3, another member of the NOTCH pathway, did not have any effect on cell proliferation nor on neuroendocrine features of SCLC (43).

Epithelial–Mesenchymal Transition

Epithelial–mesenchymal transition (EMT) is considered to playa pivotal role in cancer propagation (44-48). This process, which normally occurs during embryonal development, consists of a cell shift from epithelial to mesenchymal phenotype (44, 45). *In vitro* induction of EMT in adult epithelial cells resulted in increased metastatic potential (49, 50). A cardinal step during EMT, the loss of cell-to-cell adherence, is mediated by the deregulation of cadherins (48, 51). In this context, two members of the

Table I. *Summary of biomarkers in lung neuroendocrine tumors*.

Biomarker category	Comments
mTOR pathway	• Higher expression in carcinoids (10) • mTOR expression inversely associated with greater tumor stage in LCNEC and SCLC (10) • Preclinical data: mTOR inhibitors reduce carcinoid proliferation, cell viability and VEGF secretion (12-14) • RADIANT-4 phase III clinical trial: everolimus was associated with 52% reduction in the estimated risk of progression or death ($p<0.00001$) >> FDA approval (15-17) • Everolimus in combination with other agents in ongoing phase I and II clinical trials (16) • Total mTOR levels, AKT, p70S6K (RPS6KB2), and ERK1/2 (MAPK3/1) protein levels predict sensitivity to mTOR inhibitors in pulmonary carcinoids (18) • Increased elF4E levels are associated with increased resistance to everolimus in SCLC (19)
Cell cycle regulation genes	• *RB1* and *TP53*: a significantly higher rate of mutations in carcinomas than in carcinoids ($p<0.0001$) (20) • *RB1* and *TP53* transcription patterns may help distinguish between SCLC- and NSCLC-like LCNEC tumors (20, 21) • Lower expression of *ASCL1, BCL2, CASP8, CCNE1, CDK1, CDK2, CDKN1A* and *CDKN2A* in carcinoids compared to carcinomas (22) • Higher expression of *CCNE1* and *CDK6* in carcinoids compared to carcinomas (22) • Higher expression of *CDK2, CDKN1B, CDKN2A* and *PNN* in SCLC compared to LCNEC (22) • Minimal *CCND1* and *CDKN2A* expression correlated with N0 status (22) • *ACTB, CDKN1B, GAPDH, GRB2, RHOA* and *SDCBP*: reference genes in NETs (23) • SCLC and LCNEC have similar DNTF expression associated with malignant neuroendocrine phenotype (25) • LCNEC and SCLC: higher expression of nuclear surviving (28)
Apoptosis	• High pBCL2 expression: increased risk of disease progression and death (31) • pBCL2: highest expression in SCLC, followed by LCNEC and lowest expression in carcinoid (31) • BCL2/BAX ratio: increasing values from TC to AC, LCNEC and SCLC (22) • Absence of CD44 expression: significantly low 20-year survival in patients with pulmonary carcinoids adenocarcinomas (34) • Loss of expression of OTP: independent adverse prognostic factor of survival in pulmonary carcinoids (34) • High CD44/OTP expression: improved RFS and OS in pulmonary carcinoids (35, 36)
Notch family	• Increased expression of ASCL1 promotes NET growth and is downregulated by NOTCH1 (37, 39) • ASCL1: high specificity for high-grade NETs compared to carcinoids (40) • Up-regulation of the deactivated NOTCH1 pathway resulted in suppression of cell growth and neuroendocirne differentiation (33)
Epithelial-mesenchymal transition	• E-Cadherin/β-catenin complex loss of integrity: independently associated with LCNEC and SCLC subtypes, lymph node infiltration and decreased RFS and OS (52) • SNAI1 was the predictive marker with the highest significance to disease-related mortality (52)
Ki-67 labeling index	• Ki-67 labeling index is highly expressed in LCNEC and SCC compared to lung adenocarcinoma (28) • Cutoff value for Ki-67 labeling index of 20%, optimally discriminating between TC/AC and LCNEC/SCLC with 100% sensitivity and specificity (56)
Chromatin-remodelling genes	• KMT2 family of covalent histone modifiers was mutated in 13.6% carcinoids *versus* 26.7% carcinomas ($p=0.11$) and the ARID family, involved in the SWI–SNF complex had mutations in 9.0% carcinoids *versus* 10.0% carcinomas ($p<0.99$) (20) • Most frequently encountered mutated genes were *MEN1, PSIP1* and *ARID1A* (26)
MicroRNAs	• *miR-22, miR-29a, miR-29b, miR-29c, miR-367*; miR-504, miR-513C, miR-1200*: adverse association with tumor grade (59) • *miR-18a, miR-15b*, miR-335*, miR-1201*: positive association with the tumor grade (59) • let-7d, miR-19, miR-576-5p, miR-340*, and miR-1286: significantly associated with OS (59)
Receptor Tyrosine Kinases	• LCNEC and the SCLC tumors had significantly higher scores for c-KIT, IGF1R, and KDR and lower scores for ERBB2, FGFR1, c-MET and ROS1, compared with adenocarcinomas (61) • LCNEC and the SCLC tumors had significantly higher scores for c-KIT, KDR and RET and lower scores for EGFR and IGF1R when compared with squamous cell carcinoma (61) • *RET* mutations in SCLC are rare; M918T *RET* somatic mutation in metastatic SCLC tumor was related with activation of ERK signaling, *MYC* expression and increased cell proliferation (63) • Clinical data regarding patients with NET harbouring *ALK* rearrangements are derived from case reports (69)
Heat shock protein	• High expression of HSP90 in NETs (73) • Small molecule HSP90 inhibitors reduced the viability of NCI-H727 human bronchopulmonary NET cell line (73)
Neuroendocrine (NE) tissue markers	• NE-negative patients: a significantly better prognosis than their NE-positive counterparts (74) • Inverse association between CgA levels above 100 pmol/l and OS (75)
4F2hc (CD98)	• CD98 expression increased from low to high grade tumors and high 4F2hc levels were associated with poor OS (77,78) • CD98 expression was associated with high GLUT1, HIF-1α, p-AKT, p-mTOR and p-S6K levels (77, 78)

Table I. *Continued*

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Biomarker category	Comments
sREST	• High levels of sREST in SCLC cells (79) • Preclinical studies with small-interfering RNAs (si-RNAs): encouraging results in suppressing sREST and restoring REST level (79)
YAP1	• High-grade NETs are specifically characterized by loss of YAP1 (80) • YAP1-negative cases: more chemosensitive compared to YAP1-positive (80)

RFS: Recurrence-free survival; OS: overall survival; SCLC: small-cell lung carcinoma; LCNEC: large-cell neuroendocrine carcinoma; mTOR: mammalian target of rapamycin; IGF1R: insulin growth factor 1 receptor; KDR: kinase insert domain receptor; EGFR: epidermal growth factor receptor; ERBB2: erb-b2 receptor tyrosine kinase 2; FGFR1: fibroblast growth factor receptor 1; c-MET: MET proto-oncogene; ROS1: ROS proto-oncogene 1; RET: ret proto-oncogene; YAP1: Yes-associated protein 1; ASCL1: Achaete-scute homolog 1; BCL2: B-cell lymphoma 2; CASP8: caspase 8; CCNE1: G_1/S-specific cyclin-E1.

SNAIL protein family (namely SNAI1 and SNAI2), which suppress E-cadherin transcription, have been implicated in lung NETs (52, 53).

A recent study on surgically resected human lung tissue with NET demonstrated a low expression of E-cadherin and β-catenin in highly malignant tumors (54). Disintegration of E-cadherin/β-catenin complex was independently associated with LCNEC and SCLC subtypes, lymph-node infiltration and decreased progression-free and overall survival (54). Furthermore, SNAI2 expression independently predicted lymph node involvement and reliably differentiated SCLC from LCNEC (54). SNAI1 was the predictive marker with the highest significance of association with disease-related mortality (7-fold risk for death due to the disease). Additionally, increased expression of E-cadherin and β-catenin was correlated with the histological subtype of typical carcinoid (54).

Ki-67 Labeling Index

Ki-67 is a generally accepted proliferation marker (55) and the Ki-67 antigen-labeling index (LI), *i.e.* the percentage of labeled nuclei after immunohistochemical staining, is an emerging biomarker in NETs (56). The role of Ki-67 LI in lung NET has been the subject of several independent investigations, with potential diagnostic, prognostic, and grading implications (57). However, differences in Ki-67 LI are reported between the four histological variants (57). There is overlap between the histological categories, and Ki-67 LI is not perfectly congruent with other defining criteria (57). Ki-67 is highly expressed in LCNEC and SCC compared to lung adenocarcinoma (30). Interestingly, a recent study on biopsy specimens suggested a cut-off value for Ki-67 LI of 20% that optimally discriminates between typical/atypical carcinoid and LCNEC/SCLC with 100% sensitivity and specificity (58).

Chromatin-remodelling Genes

Chromatin-remodeling genes have been suggested to play a key role in tumorigenesis; in fact, deregulation of these genes may be considered as a driver event of carcinogenesis in pulmonary carcinoids (28). Simbolo *et al*. showed that chromatin-remodeling genes were mutated in carcinoids (45.5%) and carcinomas (55.0%) at similar rates (22). Specifically, the histone-lysine *N*-methyltransferase 2 (KMT2) family of covalent histone modifiers (*KMT2A, KMT2C* and *KMT2D*) was mutated in 13.6% carcinoids versus 26.7% carcinomas and the AT-rich interaction domain (ARID) family, involved in the switch/sucrose non-fermentable (SWI–SNF) complex (*ARID1A, ARID1B*, and *ARID2*), had mutations in 9.0% carcinoids versus 10.0% carcinomas (22). In another study, Fernandez-Cuesta *et al*. performed gene copy-number analysis, and genome/exome and transcriptome sequencing of pulmonary carcinoids, and covalent histone modifiers were found to be mutated in 40% of the cases, whereas mutated members of the SWI–SNF complex were detected in 22.2% of them. The most frequently encountered altered genes were *MEN1*, PSIP1 and SFRS1 interacting protein 1 (*PSIP1*), and *ARID1A* (28).

KMT2D exhibited truncating nonsense/frameshift/splice site mutations in 8% of SCLC tumors and 17% of SCLC cell lines in the study by Augert *et al*. These mutations in human SCLC cell lines were associated with reduced lysine methyltransferase 2D protein levels and reduced monomethylation of histone H3 lysine 4 that are associated with transcriptional enhancers (59).

MicroRNAs

MicroRNAs (miRNAs) are small, non-coding, single-stranded RNAs that regulate the cell cycle through epigenetic modifications, which are deregulated in lung cancer (60).

Mairinger *et al*. conducted a miRNA profiling study in 12 pulmonary NETs and found 12 miRNAs with highly significantly different expression (61). Among them, eight (*miR-22, miR-29a, miR-29b, miR-29c, miR-367*; miR-504, miR-513C* and *miR-1200*) had an adverse association and four (*miR-18a, miR-15b*, miR-335** and *miR-1201*) had a positive association with tumor grade. This finding signifies a differential role for each miRNA in lung NET. Interestingly, five miRNAs (*let-7d, miR-19, miR-576-5p, miR-340**, and *miR-1286*) were significantly associated with increased overall survival, suggesting that they may have an important role in disease progression (61). Larger studies might be able to pinpoint differences in miRNA expression among subtypes of lung NETs.

Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) have a cardinal role in cancer-associated molecular signaling cascades, and they may be promising targets for drug development (62). Expression of RTKs [c-KIT, insulin growth factor 1 receptor (IGF1R), kinase insert domain receptor (KDR), epidermal growth factor receptor (EGFR), erb-b2 receptor tyrosine kinase 2 (ERBB2), fibroblast growth factor receptor 1 (FGFR1), MET proto-oncogene; c-MET, ROS proto-oncogene 1 (ROS1) and ret proto-oncogene (RET)] was evaluated by immunohistochemical staining in a comparative study that included lung NETs, adenocarcinomas and squamous cell carcinomas (63). Of interest, no difference was noted among lung NET subtypes (LCNEC and SCLC) but significant differences in immunohistochemical score were noted between NETs and the other lung carcinomas under investigation (63). Both LCNEC and SCLC had significantly higher immunohistochemical scores for c-KIT, IGF1R and KDR, and lower scores for ERBB2, FGFR1, c-MET and ROS1 compared with lung adenocarcinomas. In addition, LCNECs and SCLCs had significantly higher scores for c-KIT, KDR and RET and lower scores for EGFR and IGF1R when compared with squamous cell carcinoma (63).

The RET tyrosine kinase receptor plays a key role in neuroendocrine development and *RET* mutations have been long known in patients with multiple endocrine neoplasia type 2 (MEN2) syndromes (64). RET mutations have also been described in SCLC; in the setting of metastatic SCLC, Dabir *et al*. identified the M918T *RET* mutation, which was associated with activation of ERK signaling, MYC expression and increased cell proliferation (65). Furthermore, non-germline RET mutations in exon 11 were identified in two patients with SCLC (66). However, *RET* mutations in SCLC are considered relatively rare, taking into consideration the results of previous studies, which performed genomic analyses of SCLC cell lines and human tissue, and failed to provide sufficient evidence for the presence of *RET* mutations (67-69).

Regarding anaplastic lymphoma kinase (*ALK*) gene rearrangement, Nakamura *et al*. did not find any *ALK* rearrangement in 227 pulmonary NETs (70). In addition, clinical data regarding patients with NET harboring *ALK* rearrangements were derived from case reports. The efficacy of ALK inhibitors, such as crizotinib, is rather questionable in these cases (71).

Heat-shock Protein 90

Heat-shock protein 90 (HSP90) constitutes a molecular chaperone regulating key oncoproteins that induce carcinogenesis (72). High expression of HSP90 has been found in NETs, whereas HSP90 inhibitors have shown efficacy in gastrointestinal and pancreatic NETs in the preclinical setting (73-75). AUY922 and HSP990 are novel small molecule HSP90 inhibitors that reduce the viability of NCI-H727 human bronchopulmonary NET cell line in a dose-dependent manner (75).

Neuroendocrine Tissue Markers

Synaptophysin , chromogranin A (CgA), neural cell adhesion molecule and CD56 are well-established neuroendocrine tissue markers (40). Hamanaka *et al*. evaluated tissue and serum neuroendocrine markers to differentiate SCLC from LCNEC in patients with both resectable and advanced disease (tissue retrieved by biopsy) (76). The striking finding of the study was that neuroendocrine-negative patients demonstrated a significantly better prognosis than their neuroendocrine-positive counterparts. This SCLC subset with good prognosis, identified by low neuroendocrine marker expression, was found only in resectable cases (76). Pericleous *et al*. confirmed these observations by demonstrating an inverse association between serum CgA levels above 100 pmol/l and overall survival (77). It should be noted that special attention is needed in the interpretation of the results, taking into consideration the fact that comorbidities of each patient may alter biomarker levels, such as in the case of CgA (78).

Other Markers

CD98 is a molecule involved in cell–cell signaling and its deregulation has been described in malignant tumors. In pulmonary NETs, expression of CD98 increased from low- to high-grade tumors and high levels were associated with poor overall survival. Furthermore, its expression was associated with high levels of glucose transporter 1 (GLUT1), hypoxia-inducible factor 1-alpha (HIF1α), p-AKT, p-mTOR and p-S6K protein that are known to be implicated in NET carcinogenesis and are highly expressed in NETs (79, 80).

SCLC cells have been found to have high levels of soluble RE1-silencing transcription factor (sREST), which is an SCLC-specific isoform of RE1-silencing transcription factor that regulates expression of neuronal markers. This finding combined with the low level of REST expression suggest a mechanism of alternative splicing of REST in SCLC that is regulated by neural-specific Ser/Arg repeat-related protein of 100 kDa (nSR100). This pathway is implicated in tumor–extracellular matrix interactions that promote tumor proliferation. Preclinical studies with small-interfering RNAs have demonstrated encouraging results in suppressing sREST and restoring the REST level (81).

Yes-associated protein 1 (YAP1), the main Hippo pathway effector, is an oncogene implicated in the pathogenesis of lung cancer. Ito *et al*. showed high-grade NETs to be specifically characterized by loss of YAP1. Additionally, YAP1-negative cases were more chemosensitive compared to YAP1-positive ones. Thus, YAP1 is an emerging marker of neuroendocrine nature and a potential predictive factor of response to treatment of pulmonary NETs (82). Table I summarizes the biomarkers included in the present study.

Conclusion

Histological classification of lung NET is controversial due to lack of absolute concordance among the current classifications (19, 83). Interobserver variability can be overridden both by consensus meetings and by integrating molecular prognostic markers such as Ki67 and OTP; thus, a more accurate characterization and a better prediction of survival can be provided (84). The majority of studies evaluating the genomics of lung NETs were performed on paraffin-embedded human tissues from patients with resectable early-stage disease. Thus, these results cannot be generalized to all patients with lung NET because the majority of them have unresectable disease at diagnosis. Further translational research is considered indispensable in order to determine easily druggable molecular targets, such as gain of function mutations. These findings do offer important insight into the molecular pathways involved in each NET subtype and might facilitate design of novel therapeutic protocols.

Currently, clinical oncology is more personalized than ever before and different problems are treated differently. The development of multivariable predictive models evaluating baseline patient characteristics and molecular fingerprints of tumor biology are enhancing patient-specific therapeutic strategies. However, therapeutic approaches in patients with primary lung NETs need more randomization and comprehensive evidence-based data regarding the efficacy and the long-term outcomes of implicated treatments. In this context, ongoing (83) and future clinical trials integrating molecular profiling are deemed necessary in order to treat patients with lung NETs on a personalized basis.

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