



A genomic analysis of large cell neuroendocrine carcinoma versus small cell lung cancer: which is which?

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Comment on: Miyoshi T, Umemura S, Matsumura Y, *et al.* Genomic profiling of large-cell neuroendocrine carcinoma of the lung. Clin Cancer Res 2016. [Epub ahead of print].

Submitted Oct 15, 2016. Accepted for publication Oct 26, 2016.

doi: 10.21037/tcr.2016.11.19

View this article at: <http://dx.doi.org/10.21037/tcr.2016.11.19>

Neuroendocrine tumors are a vastly diverse group of tumors developed by neuroendocrine cells. Neuroendocrine cells are found throughout the whole body, and function in hormone regulation and epithelium repair. In the lung, there are various types of neuroendocrine tumors, one being large cell neuroendocrine carcinoma (LCNEC). LCNEC is a rare and aggressive subtype of non-small cell lung cancer (NSCLC) and is usually treated as a type of NSCLC (1-4). However, in recent years, it has been shown that LCNEC shares similar histological, immunohistochemical (IHC), and molecular characteristics with small cell lung cancer (SCLC), despite the different characterization of the size of the cells (5). Consequently, there have been recent studies testing various combination therapies on both LCNEC and SCLC, but have resulted in somewhat poorer outcome amongst LCNEC patients than patients with SCLC (6). This highlights the ongoing ambiguity and lack of optimal clinical treatment of LCNEC versus SCLC, and motivates further genomic investigation of these two types of neoplasms. There are currently no approved targeted therapies specifically for LCNEC or SCLC; chemotherapy is presently the only therapeutic option (2).

Miyoshi and colleagues examined a Japanese patient cohort consisting of a total of 78 formalin-fixed paraffin-embedded (FFPE) LCNEC samples by using a 244 cancer-related gene targeted exon next-generation sequencing approach to discover genomic alterations. This cohort comprised of 55 surgically resected LCNEC, 13 advanced-stage LCNEC biopsies, and 10 combined LCNEC with NSCLC components. The vast majority of the patients

were ever smokers (97% and 98% in LCNEC and SCLC, respectively). They then compared the LCNEC genomic profile to 141 SCLC patients' genomic alterations: 90 biopsy samples; 50 surgically resected samples and one advanced SCLC sample from Miyoshi *et al.* previous data (7). Of these SCLC samples, 12 were of a combined type; however, it was not specified which SCLC was of a combined form. Comparison of the LCNEC and SCLC genetic alteration data was used to produce a molecular profile of LCNEC to predict molecular targeted therapies and cancer progression (8).

Miyoshi and colleagues identified a high prevalence of mutations in *TP53* and *RB1* genes in LCNEC; however, SCLC had a much higher frequency of *RB1* mutations when compared to LCNEC (Table 1). In addition, they identified targetable activating mutations in *KIT*, *EGFR*, *ERBB2*, and *FGFR1* genes, and found a higher copy number gain in *ERBB2* and *SETBP1* genes in LCNEC when compared to SCLC. Other mutated genes which were significantly more frequent in LCNEC included *LAMA1*, *PCLO*, *MEGF8*, and *RICTOR*. Yet, overall, Miyoshi and colleagues concluded that LCNEC and SCLC have similar genomic profiles. The majority of the genetic alterations were related to the PI3K/AKT/mTOR pathway, which could be a potential target pathway in LCNEC tumor formation and progression. In patients with LCNEC combined with adenocarcinoma, Miyoshi and colleagues found known oncogenic mutations within both cell components in the genes *EGFR* (E746_A750 del, exon 19) and *KRAS* (G12V), while a patient with LCNEC

Table 1 Significantly altered genes in LCNEC and SCLC patients (8-12)

Study	Next-generation sequencing methodology	Altered genes in SCLC patients	Altered genes in LCNEC patients
Miyoshi <i>et al.</i> 2016 (78 LCNEC patients; 90 SCLC patients)	Targeted exon sequencing of 244 genes	<i>TP53</i> (81%)*, <i>RB1</i> (41%)*, <i>MLL2</i> (12%), <i>NOTCH</i> family (1/2/3) (11%)*	<i>TP53</i> (71%)*, <i>RB1</i> (26%)*, <i>MLL3</i> (11%), <i>LAMA1</i> (10%), <i>NOTCH1</i> (10%)*, <i>MLL2</i> (9%)
Rekhtman <i>et al.</i> 2016 (45 LCNEC patients)	Targeted exon sequencing of 241 genes; tumor/normal blood sequencing	NA	<i>TP53</i> (78%)*, <i>RB1</i> (38%)*, <i>STK11</i> (33%)*, <i>KEAP1</i> (31%), <i>KRAS</i> (22%)*, <i>PTPR</i> (22%)
George <i>et al.</i> 2015 (110 SCLC patients)	Whole genome sequencing	<i>TP53</i> (98%)*, <i>RB1</i> (98%)*, <i>KIAA1211</i> (18%), <i>COL22A1</i> (18%), <i>FMN2</i> (18%), <i>CREBBP</i> (15%), <i>NOTCH1</i> (15%)*	NA
Vollbrecht <i>et al.</i> 2015 (19 LCNEC patients; 17 SCLC patients)	Targeted sequencing of 48 gene hotspot panel	<i>TP53</i> (65%)*, <i>ERBB2</i> (24%)*, <i>PIK3CA</i> (24%), <i>ATM</i> (24%)	<i>TP53</i> (63%)*, <i>ERBB2</i> (16%)*, <i>KRAS</i> (11%)*
Karlsson <i>et al.</i> 2015 (32 LCNEC patients)	Targeted sequencing of 26 gene hotspot panel	NA	<i>TP53</i> (88%)*, <i>STK11</i> (16%)*, <i>PTEN</i> (13%)

*, indicates genes common amongst LCNEC and SCLC; LCNEC, large cell neuroendocrine carcinoma; SCLC, small cell lung cancer; NA, not applicable.

combined with squamous cell carcinoma had a *PIK3CA* gene activating mutation in both cell components (8).

In a recent report, Rekhtman *et al.* compared the genomic profiles of LCNEC and NSCLC. In agreement with Miyoshi and colleagues' data, Rekhtman *et al.* also found *TP53* and *RB1* genes to be the two most commonly mutated genes in LCNEC. In the Rekhtman *et al.* whole LCNEC cohort, they included additional genes *STK11*, *KEAP1*, and *KRAS* to be among the next most commonly mutated genes in LCNEC; however, in the SCLC-like LCNEC subset, (defined as LCNEC with co-altered inactivation of *RB1* and *TP53*), there was a complete absence of *STK11* and *KRAS* mutations, and an enriched existence of *MYCL*, *SOX2*, and *FGFR1* amplifications. They also identified the PI3K/AKT/mTOR pathway to be among the most frequently altered gene family/functional group. Furthermore, Rekhtman *et al.* discovered about 30% of the NSCLC-like LCNEC to have a distinct mutation profile, mostly consisting of loss-of-function mutations in the NOTCH family genes, which are key regulators of neuroendocrine differentiation. The most prominent alteration in SCLC-like LCNEC versus SCLC was an increased frequency of *KEAP1-NFE2L2* aberrations, which rarely occur in traditional SCLC patients, but are known to be prevalent in *STK11/KRAS* wildtype squamous cell carcinomas (9).

A small percentage of neuroendocrine tumors

may display combined histologies (i.e., SCLC with NSCLC components, and LCNEC with other NSCLC components). In their study, Miyoshi and colleagues included 10 combined LCNEC and 12 combined SCLC. The combined forms clearly indicate the heterogeneity of these tumors and investigating their genetics may offer clues to their potential different cells of origin. However, often the different components of combined forms are tightly intermixed and it may be challenging separating them; in this case sophisticated technology such as laser capture microdissection may be necessary. Unfortunately Miyoshi and colleagues apparently only used coring of tissue blocks and light microscopy, which may not have adequately separated the two components and therefore yielded inconclusive results of this sub-analysis.

The best recognized combined forms of neuroendocrine tumors with non-neuroendocrine tumors in the mixed SCLC-NSCLC histologies, which have been found in a variable number of cases, range from less than 1% to up to 28%. This variability may depend of the availability of tumor tissue and small biopsies do not allow the opportunity to study the tumor in their entirety (13). According to different reports from George *et al.* and Swanton *et al.*, loss of the tumor suppressor genes, *TP53* and *RB1*, is obligatory in SCLC, and inactivating mutations in the NOTCH family genes occur in 25% of human SCLC (10,14).

There has been more comprehensive reports examining LCNEC combined NSCLC or SCLC components. In an older report from Wagner *et al.* in 2009, loss of heterozygosity (LOH) analysis was used, which showed similar genetic abnormalities in the individual components of the combined SCLC cases (15). In addition, Buys *et al.* investigated a patient with combined small cell carcinoma with two different NSCLC components. They used a whole genome analysis by tiling-path array comparative genomic hybridization to evaluate the clonal relationship, which resulted in divergent clonal evolution (16). The analysis for combined LCNEC genomic alteration in this article indicated, five in ten LCNECs with other NSCLC components harbored the same key driver mutations in both components. For combined LCNEC, the median number of genetic mutations was 3.5 and 4 in LCNEC and NSCLC component respectively. The median concordance rate between LCNEC and associated NSCLC components was 71% (range, 60% to 100%).

Considering these data, LCNEC combined with NSCLC components may behave and respond more like their NSCLC component, suggesting tumor heterogeneity and early evolution of the combined LCNEC. Because it is suggested that they perform like their NSCLC counterpart, examination of the two separate types of LCNEC combined NSCLC components could provide more insight. Further investigation of the *KEAP1/NFE2L2*, *PIK3CA* genes in LCNEC combined squamous cell carcinoma, and the *EGFR*, *KRAS*, and *NOTCH* family genes in LCNEC combined adenocarcinoma may offer a more targeted approach to the treatment of LCNEC. Together with Rekhtman *et al.* data, the combined mutation profiles could be used as markers to differentiate the different subtypes within LCNECs and advance targeted treatment for patients.

A case study by De Pas *et al.* in 2011 reported a never smoker patient with LCNEC harboring an activating mutation on the *EGFR* gene (L747_A755>AT, exon 19). This patient was treated with gefitinib, an approved drug for specific *EGFR* mutated NSCLC. After 2 months of therapy, the patient showed dramatic response to the treatment, and after 5 months, the patient showed complete response of the lung primary lesions (17). This suggests LCNEC carrying activating *EGFR* gene mutations could be treated with gefitinib with positive effects.

Because the PI3K/AKT/mTOR pathway was emphasized in Miyoshi and colleagues' discussion, perhaps the dual PIK3CA/mTOR inhibitor, PI-103, could be a potential targeted therapy for LCNEC carrying a *PIK3CA* mutation.

This drug has been tested and was shown to be active in NSCLC cell lines with activating *PIK3CA* mutations (18). Patients with *PIK3CA* and *EGFR*-mutated lung cancers may not respond to *EGFR* targeted therapies like gefitinib because of the double mutation. All these factors should be taken into consideration when evaluating the complex molecular profile of LCNEC for targeted therapy selection.

Miyoshi and colleagues have demonstrated the power of next-generation sequencing, and this proposal supports the ongoing movement of genomics delivering a "personalized" treatment approach by assessing the patient's tumor mutations and selecting the appropriate therapy for an improved response, subsequently increasing overall survival (19-21). Although the study was not a complete, comprehensive investigation of LCNEC, Miyoshi and colleagues provided valuable data in the advancement of LCNEC analysis, diagnosis, and treatment. Because of the distinct molecular characteristics compared to other lung cancer types, LCNEC should be treated as a separate group and not lumped into traditional NSCLC or SCLC treatments. Moreover, smoking patients need to be taken into consideration; it is known that NSCLC patients who have smoked have a 10-fold higher mutational load than never-smokers, and C>A/G>T transversions predominantly occur in NSCLC tobacco users, whereas C>T/G>A transitions most frequently occur in NSCLC never-smokers (22). It seems that LCNEC do not have distinct, targetable genes or mutations (*Table 1*), similar to other cancers such as pancreatic (23). Nonetheless, possible future studies with more refined methodologies or technologies, such as laser capture microdissection or tissue macrodissection instead of tissue block coring, tumor/normal (blood) pair sequencing, or using single cell or droplet digital PCR, could minimize tumor component/normal cell contamination and dissect tumor heterogeneity. In addition, the authors did not specify the sequencing mean read depth of coverage; a higher read depth of coverage will provide higher accuracy and detection of true somatic mutations. We believe further investigation within a larger and more diverse cohort comparing LCNEC to SCLC and NSCLC would be beneficial. The Cancer Genome Atlas (TCGA) project has only sequenced lung adenocarcinoma and squamous cell carcinoma cases (585 and 504, respectively), but no SCLC cases, mainly because of difficult collection of large, resected material (24). Since LCNEC are treated more like NSCLC based on morphology, perhaps there is potential to obtain more LCNEC surgical samples more easily for

genomic analysis, based on this presumption. Furthermore, using RNA sequencing, whole exome or whole genome sequencing would provide a stronger interpretation of the LCNEC genomic landscape.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Shaohua Cui (Department of Pulmonary Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.11.19>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: McCutcheon JN, Zhao X, Giaccone G. A genomic analysis of large cell neuroendocrine carcinoma versus small cell lung cancer: which is which? *Transl Cancer Res* 2016;5(Suppl 6):S1088-S1092. doi: 10.21037/tcr.2016.11.19