Diagnosis of small cell lung carcinoma metastases

Cancer is defined by unscheduled cellular growth and metastatic spread into other organs of the body. Tumor cells gain their capacities form genetic alterations, also known as mutations, leading to altered protein functions and signaling pathways. Tumors are highly dependent on these oncogenic driver alterations, called oncogene addiction. Some oncogenic signaling pathways can be blocked by specific therapies. These targeted personalized therapy approaches revolutionized cancer care.

The detection of targetable oncogenic signaling pathways was significantly improved using next generation sequencing (NGS) approaches detecting somatic mutations in tumor tissue. In the daily routine clinical practice of the pathology, tissue is formalin-fixed and paraffin-embedded (FFPE) which serves for immunohistochemistry (IHC) and molecular diagnostics. Since tumor material is often limited in the clinic, multiplexed amplicon-based NGS provides a sensitive, rapid and high throughput method that can deal with low input tumor DNA. Furthermore, this method enables rapid integration of new targets into molecular routine diagnostics. Thus, broad testing for clinical trials and implementing targeted therapies can be improved.

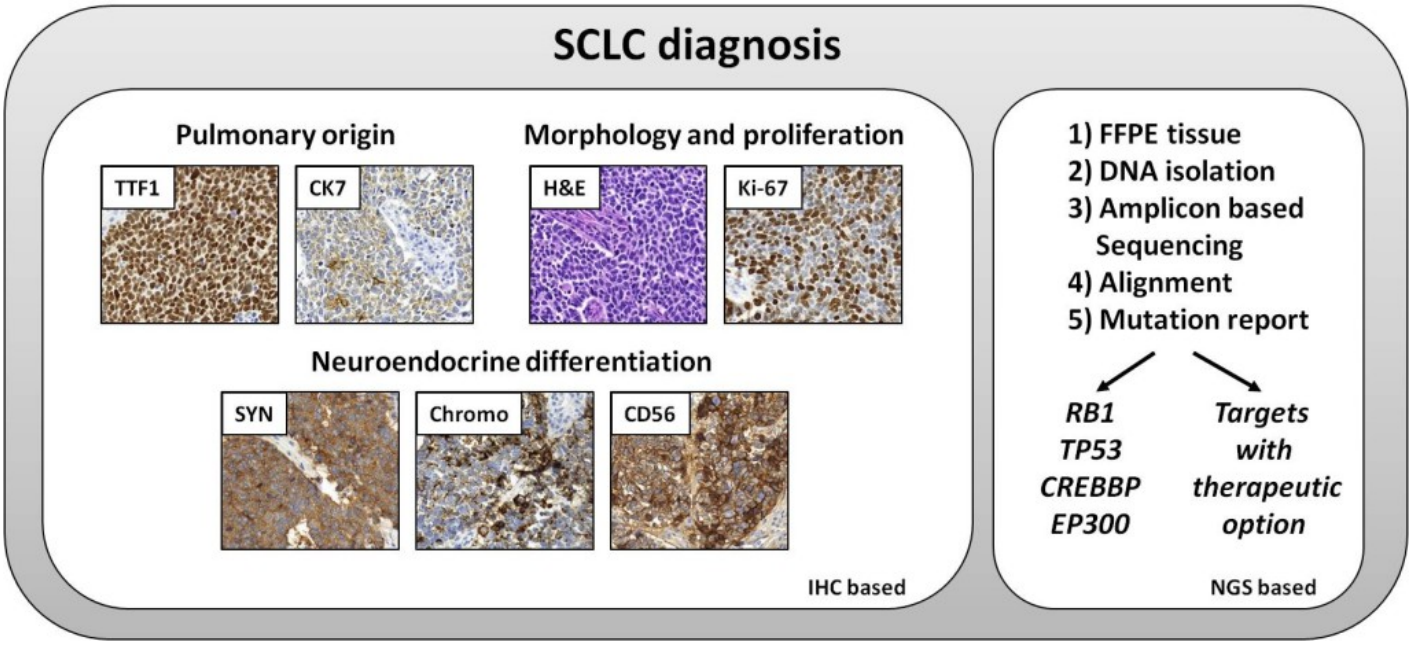

Fig. 1. Comprehensive SCLC diagnosis. Formalin fixed paraffin embedded (FFPE) tissue was used for immunohistochemistry (IHC) and DNA isolation for next generation sequencing (NGS). A Hematoxylin and Eosin (H&E) stain enables the detection of a characteristic small cell morphology. The Ki-67 stain is used to measure cell proliferation, significantly enhanced in SCLC. A pulmonary origin is determined by the expression of thyroid transcription factor 1 (TTF1) or cytokeratin 7 (CK7). Characteristic neuroendocrine differentiation is indicated by the expression of synaptophysin (SYN), chromogranin A (Chromo) or CD56. Mutations in the RB1, TP53, CREBBP

and EP300 genes were identified by amplicon-based NGS.

Lung cancer causes most cancer-related deaths worldwide, whereby metastases are the predominant reason for high mortality rates. Lung cancer comprises the entities of non-small cell lung carcinomas (NSCLCs) and small cell lung carcinomas (SCLCs). Thereby, SCLC is the most aggressive form of lung cancer and is built from highly invasive neuroendocrine differentiated rapidly proliferating tumors. Tumor cells detach early from their primary tumor site in the lung and frequently translocate to distant organs such as lymph nodes, bones, the liver and the brain to induce systemic metastases and leading to an enhanced deadly outcome. Thus, it is a compulsive issue to improve SCLC diagnostics, which is routinely performed by IHC.

The main oncogenic mutations identified in SCLCs are in the RB1 gene (encoding the retinoblastoma protein, RB) and in the TP53 gene (encoding for the tumor suppressor protein 53, p53). Other recurrent mutations were found in the histone modifier genes such as CREBBP and EP300.

We investigated mutations in these SCLC related genes by amplicon-based NGS in combination with comprehensive IHC in fifty randomly collected routine cases of SCLC metastases isolated from trachea and lymph nodes. In addition, 167 cases of routinely isolated primary SCLC that were previously classified by IHC, were analyzed by amplicon-based NGS, as well.

We identified non-synchronous mutations in CREBBP and EP300 in 100% of primary SCLC and SCLC metastases. Moreover, we found frequent synchronous mutations in RB1 and TP53. Interestingly, IHC analysis revealed enhanced proliferation indicated by Ki-67 stain and a collapsed keratin cytoskeleton in SCLC lymph node metastases compared to SCLC metastases isolated from trachea.

Both features were described as being part of epithelial to mesenchymal transition (EMT) which facilitates detachment from primary tumor site and increases motility and invasion capacities of tumor cells. EMT and a collapsed keratin cytoskeleton were frequently observed in SCLCs, but this phenomenon itself was not potent enough to trigger SCLC formation or EMT induction all alone. Most likely, signaling pathways involving NOTCH mediated signaling and a central RB-p53 signaling axis are the major course of SCLC pathology.

In the future, IHC may be routinely combined with multiplexed amplicon-based NGS in SCLC diagnostics. Thereby, NGS will extensively focus on targets with therapeutic options, which remains challenging for SCLC. Members of the NOTCH- or WNT- signaling pathway may serve as relevant targets to treat SCLC patients.

Publication

Implementing amplicon-based next generation sequencing in the diagnosis of small cell lung carcinoma metastases.
Meder L, König K, Fassunke J, Ozreti? L, Wolf J, Merkelbach-Bruse S, Heukamp LC, Buettner R.
Exp Mol Pathol. 2015 Dec