Editor's note:
In the era of personalized medicine, a critical appraisal new developments and controversies are essential in order to derived tailored approaches. In addition to its educative aspect, we expect these discussions to help younger researchers to refine their own research strategies.

Controversies on Lung Cancer: Pros and Cons

Commentary on tumor heterogeneity

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I would like to thank all three authors Drs. Ilie and Paul Hofman, and Mari Mino-Kennudson for their state of art overview on the role of liquid biopsy present and in future and the correlation to tissue biopsy (1-4). On this topic it could not be a classical pro&con discussion, as nobody in the pathologic community would neglect liquid biopsy. However, the strategy to what extend liquid biopsy can be integrated into the daily practice in pathology remains to be defined. In that regard we can see slight differences how liquid biopsy will be implemented in the US and in Europe.

As for the costs raised in Dr. Hofman's article it can be stated, that any kind of pathologic analysis is by far the cheapest diagnostic test among most clinical and radiological tests. And when correlated to the costs of targeted therapy, these costs can only be called "peanuts". In their analysis tumor heterogeneity either from the genetic point but also from morphology have been stressed as an argument for liquid biopsy. This aspect needs a commentary.

Morphologic heterogeneity of NSCLC is well known, and has been introduced into the classification of lung cancer. Adenocarcinomas are classified according to their predominant pattern, squamous cell carcinomas may be composed of well-differentiated and areas of undifferentiated carcinoma portions. Sarcomatoid carcinomas are characterized by a combination of well-differentiated carcinoma with sarcomatoid looking carcinomas. Adenosquamous carcinoma is composed of two different types of carcinoma. But does that mean these different components are genetically heterogeneous? The answer is yes and no. To bring light into this controversial discussed topic, we will discuss heterogeneity in primary tumors as well as metastasis, before asking, what liquid biopsy can add.

In some studies *EGFR*, *KRAS*, and *PI3K* mutations have been identified in both components of adenosquamous carcinomas (5), *EGFR* mutations were homogenously distributed in primary lung carcinomas. In contrast *ALK* rearrangements in the same series showed intra-tumor heterogeneity of rearrangements. In another study analyzing 629 patient tumor samples 9 out of 30 carcinomas with *ALK* rearrangements showed heterogeneity in the fusion transcript, whereas no heterogeneity was seen in 364 samples with *EGFR* mutations. Interestingly *ALK* fusions were positively associated with a micropapillary pattern and negatively associated with a lepidic pattern pointing to some morphologic and genetic associations (6). Even in sarcomatoid carcinomas, which present with heterogeneity of morphological patterns the concordance rate for mutations was relatively high with 61% (7). Similarly samples taken from different areas of surgically removed carcinomas showed a high degree of homogeneous mutational profiles for *EGFR* and *KRAS* (18 of 19 cases) (8). There was only one study showing a significant intra-tumoral heterogeneity. However, the authors did not differentiate driver mutations and other mutations. So they concluded heterogeneity on the basis of multiple mutations in subclones within the primary tumor (9). Additional mutations might reflect adaptations of subclones to hypoxia, changes in metabolism. This aspect

is elucidated in the study by de Bruin, which sequenced 25 spatially distinct regions from seven operable NSCLCs and found evidence of branched evolution, with driver mutations arising before and after subclonal diversification. There was pronounced intra-tumor heterogeneity in copy number alterations, translocations, and mutations associated with metabolic enzyme activity (10). This point to individual pathways of carcinogenesis, with either homogeneous or heterogeneous types of driver mutations.

What about heterogeneity in metastasis: When looking up primary tumor and metastasis, some studies showed the same type of mutation in both, whereas in other studies subclones do exist in the primary as well as in the metastatic site, which are genetically different.

In a review summarizing 26 different published studies a substantial concordance was observed between primary and metastatic tumors in terms of *EGFR*, *KRAS*, *BRAF*, *p16* and *p53* mutations. However, some level of discordance was seen in most studies; testing methodologies appeared to play a key role in this, along with underlying tumor heterogeneity (11). In the study by Chang *et al. p53* and *EGFR* mutation/overexpression status was different between primary tumors and lymph node metastases in only 5.4/7.2% and 28.6/33.9%, respectively. In most cases, the *p53* and *EGFR* mutations usually preceded lymph node metastasis, and these gene statuses in the primary cancer and their lymph node metastasis were concordant (92.9% and 69.6%). Therefore when *p53* mutations occur before the establishment of lymph node metastasis, they subsequently persist in the metastatic nodes (12).

In a series of 30 *EGFR* mutated adenocarcinomas the mutation was detected in 28 lymph node metastases. In 12 cases there were discordant *EGFR* mutations between primary tumors and metastasis. In 11 cases *EGFR* mutations were detected only in the primary tumor, whereas in 1 case only in lymph node metastases (13). In the study by Kim *et al.* 41 primary tumor and matched metastatic lymph nodes were analyzed by next generation sequencing. Two hundred and thirteen non-synonymous mutations, 32 deletions, and four insertions were discovered. Non-synonymous mutations were seen more often in the primary tumors when the mutation profiles between primary tumor and metastatic L/N were compared, 13 (31.7%) of 41 cases showed discrepant mutation profiles (14).

Finally the genetic makeup of carcinomas can change under the pressure of tyrosine kinase inhibitor (TKI) therapy. In some cases a minor subclone does exist, which contain a resistance mutation in addition to the driver gene mutation (Del exon 19 + T790M mutation) (15), but there are also secondary somatic mutations arising under the pressure of targeted therapy (16,17). This happens in the *EGFR* gene as well as in the *ALK* and *ROS1* genes. And the trans-differentiation of an adenocarcinoma into a small cell carcinoma under targeted therapy seems to be preceded by a loss of RB and mutations in TP53, somatic mutations, which are not present in the original tumor.

When dissecting the process of invasion and metastasis additional modifications of the genetic machinery is required to communicate with the environment, avoid attack by the immune system, and many more aspects (18). This gives rise to genetic and epigenetic modifications, which I avoid to call heterogeneity. Even within the primary tumor different metabolic requirements might result in genetic/epigenetic modifications: Tumor cells in the center are exposed to hypoxia and acidic pH, whereas tumor cells in the periphery are better supplied by oxygen and nutrients. Tumor cells at the periphery are more exposed to stroma cells, and have to communicate with these cells, a task, which tumor cells in the center portion might not need. These are all adaptations in addition to the primary genetic changes driving carcinogenesis.

When coming to circulating tumor DNA (cfDNA) the question of tumor heterogeneity has not been extensively addressed. In comparing tissue biopsies and cfDNA in most cases there is concordant finding of the same mutations in both. However, there are samples with discordant findings: mutated cfDNA and wild type tissue DNA and the reverse. In the case of biopsies the explanation is usually the small size of the tissue, explaining a negative finding. But analysis of cfDNA so far is limited as in most studies single genes are analyzed, whereas the discussion on heterogeneity in tumors is based on the analysis of multiple genes. Therefore let us await more data on next generation sequencing using plasma samples, before we can consider that cfDNA analysis might be useful to pick up heterogeneous tumor cells clones.

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Footnote

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Ilié M, Hofman P. Rebuttal from Dr. Hofman and Dr. Ilié. Transl Lung Cancer Res 2016;5:428-9.
Mino-Kenudson M. Rebuttal from Dr. Mino-Kenudson. Transl Lung Cancer Res 2016;5:430-2.

References

1. Ilié M, Hofman P. Pros: Can tissue biopsy be replaced by liquid biopsy? Transl Lung Cancer Res 2016;5:420-3.
2. Mino-Kenudson M. Cons: Can liquid biopsy replace tissue biopsy?—the US experience. Transl Lung Cancer Res 2016;5:424-7.
3. Ilié M, Hofman P. Rebuttal from Dr. Hofman and Dr. Ilié. Transl Lung Cancer Res 2016;5:428-9.
4. Mino-Kenudson M. Rebuttal from Dr. Mino-Kenudson. Transl Lung Cancer Res 2016;5:430-2.
5. Vassella E, Langsch S, Dettmer MS, et al. Molecular profiling of lung adenosquamous carcinoma: hybrid or genuine type? Oncotarget 2015;6:23905-16.
6. Cai W, Lin D, Wu C, et al. Intratumoral Heterogeneity of ALK-Rearranged and ALK/EGFR Coaltered Lung Adenocarcinoma. J Clin Oncol 2015;33:3701-9.
7. Fallet V, Saffroy R, Girard N, et al. High-throughput somatic mutation profiling in pulmonary sarcomatoid carcinomas using the LungCarta™ Panel: exploring therapeutic targets. Ann Oncol 2015;26:1748-53.
8. Kim HK, Lee HY, Choi YL, et al. Assessment of intratumoral heterogeneity of oncogenic driver mutations in surgically-resected lung adenocarcinoma: implications of percutaneous biopsy-based molecular assay for target-directed therapy. Anticancer Res 2014;34:707-14.
9. Zhang J, Fujimoto J, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science 2014;346:256-9.
10. de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science 2014;346:251-6.
11. Sherwood J, Dearden S, Ratcliffe M, et al. Mutation status concordance between primary lesions and metastatic sites of advanced non-small-cell lung cancer and the impact of mutation testing methodologies: a literature review. J Exp Clin Cancer Res 2015;34:92.
12. Chang YL, Wu CT, Shih JY, et al. Comparison of p53 and epidermal growth factor receptor gene status between primary tumors and lymph node metastases in non-small cell lung cancers. Ann Surg Oncol 2011;18:543-50.
13. Park S, Holmes-Tisch AJ, Cho EY, et al. Discordance of molecular biomarkers associated with epidermal growth factor receptor pathway between primary tumors and lymph node metastasis in non-small cell lung cancer. J Thorac Oncol 2009;4:809-15.
14. Kim EY, Cho EN, Park HS, et al. Genetic heterogeneity of actionable genes between primary and metastatic tumor in lung adenocarcinoma. BMC Cancer 2016;16:27.
15. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res 2006;66:7854-8.
16. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med 2011;3:75ra26.
17. Politi K, Fan PD, Shen R, et al. Erlotinib resistance in mouse models of epidermal growth factor receptor-induced lung adenocarcinoma. Dis Model Mech 2010;3:111-9.
18. Popper HH. Progression and metastasis of lung cancer. Cancer Metastasis Rev 2016;35:75-91.

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