

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

Ki-67 expression in pulmonary tumors

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Submitted Sep 13, 2016. Accepted for publication Sep 28, 2016.

doi: 10.21037/tlcr.2016.10.13

View this article at: <http://dx.doi.org/10.21037/tlcr.2016.10.13>

Ki-67 as a proliferative marker

Ki-67 was introduced more than two decades ago as a measure of the *tumor proliferative fraction*, in the need for a useful marker that might help clinicians to guide therapy and predict the prognosis of patients with cancer (1). Although several methods have been used to determine the *tumor proliferative fraction* in the research setting, the only practical methods used in the pathology laboratory were Ki-67 and mitotic count (2). Mitotic count was long used by surgical pathologists as diagnostic and prognostic criteria in malignant tumors, but is subjective, has poor reproducibility and is dependent on laboratory techniques (3-5).

Alternatively, the *Ki-67 proliferative index (PI)*, determined as the percentage of Ki-67 positive cells by immunohistochemistry (IHC) could be assessed using a commercially available Ki-67 antibody to estimate the growth fraction of a tumor cell population. Ki-67 is a non-histone DNA-binding nuclear protein that is expressed in all phases of the cell cycle in proliferating cells, but not in quiescent (G0) cells (6). In contrast, counting mitotic figures measures only a portion of those cells in the G2 phase and in all M-phase cells. Ki-67 was first developed by Gerdes and colleagues in 1983 and has been used to distinguish growing from non-growing cells (7). In many malignancies the percentage of Ki-67 positive cells is correlated with parameters reflecting tumor aggressiveness or progression (8). These findings demonstrate the potential value of the Ki-67 antigen for the cytopathologic

or histopathologic study of tumors, although neither its biochemical structure nor its function have been fully elucidated to date.

Ki-67 PI correlates with other markers of cell proliferation (9,10), but overestimates the proliferative activity of tumors and is therefore not an exact reflection of tumor growth (11). Sarbia *et al.* (12) found a significant association between Ki-67 PI and the mitotic activity in tumor tissue.

Currently, immunohistochemical staining for Ki-67 is a widely accepted method for evaluating proliferative activity in various tumor types (13-19), but in clinical practice is only incorporated in the diagnostic algorithms of neuroendocrine tumors of gastrointestinal tract.

In lung, reports suggest a key role of Ki-67 in (I) the prognosis of non-small cell lung carcinoma; (II) prediction of brain metastases in patients with lung adenocarcinoma and (III) in the diagnosis, classification and prognosis of pulmonary neuroendocrine tumors.

Ki-67 as a prognostic marker in non-small cell lung cancer (NSCLC)

Metaanalyses of numerous studies performed on early-stage resected NSCLC suggested that high Ki-67 values are correlated with poor prognosis (20), a shorter disease-free survival (DFS) (21,22) and a shorter recurrence-free survival (RFS) after lung tumor resection (23). Similarly,

significant correlations between high Ki-67 expression and clinicopathologic characteristics (males, higher tumor stage, and poor differentiation) were found in Asian patients with NSCLC (24). However, the informative value of the vast majority of studies on this issue is limited by the small sample numbers investigated, different Ki-67 clones used in different studies, and the use of various Ki-67 cutoff points for defining a tumor positive or negative (25). Moreover, subgroup analyses of different NSCLC histologies demonstrate that the prognostic impact of Ki-67 PI depends on the NSCLC type (26) and studies of NSCLC cohorts with mixed histologies will not lead to meaningful results. Despite the large number of published analyses exploring the prognostic role in NSCLC, Ki-67 is still not considered an established factor for routine use in clinical practice. A recent study investigated the Ki-67 PI in three large, independent NSCLC cohorts and found the need for use of different cutoff values for each histologic NSCLC type (26). Ki-67 PI was a highly significant and independent predictor for DFS for lung adenocarcinoma and adenosquamous carcinoma, in both the test and validation cohorts, but not for OS and disease-specific survival (DSS) (26). Interestingly, the authors found that in squamous cell carcinoma a high PI was reversely correlated with a better OS (cut-off value of 50%; HR =0.65; P=0.007). A standardized assessment of proliferation by Ki-67 IHC might become a useful biomarker in the daily routine diagnostic setting of NSCLC.

Ki-67 as a predictive marker of brain metastases in NSCLC

It is important to identify patients with NSCLC who are at greater risk of developing brain metastases since they may exist in the absence of neurological symptoms (27). Furthermore, reports show that prophylactic cranial irradiation may be an effective modality for preventing brain metastases in patients with NSCLC treated with adjuvant chemoradiation (28). Despite advances in diagnostic and therapeutic modalities, and sophisticated clinical practice guidelines, it remains unclear whether patients with early stage NSCLC should be screened for brain metastases or not (29-31). The metastatic cascade is rather complex and involves reciprocal interactions between tumor cells and host tissues, including alterations in tumor cell proliferation, adhesion, proteolysis, invasion, and angiogenesis (32).

In a recent study we evaluated patients with NSCLC

with and without brain metastasis in a unique series that had tumor material from both the primary lung tumor and matched metachronous brain metastasis (33). We have found that patients with high Ki-67, low caspase-3, high VEGF-C, and low E-cadherin in their primary NSCLC tumors have a higher risk of developing brain metastases when compared with a control group of NSCLC diagnosed during the same period of time. Patients with Ki-67 PI of $\geq 30\%$ had a 12-times increased risk of developing brain metastases ($P < 0.001$) and a worse prognosis compared to those with lower proliferative activity.

Our study suggests that patients with NSCLC and a specific biomarker expression may benefit from an individualized surveillance regimen and preventive therapeutic intervention designed to prevent or delay the development of brain metastases. These biomarkers are promising for predicting the development of brain metastases, and additional studies on larger series of patients are needed to validate the findings from our study.

Ki-67 in pulmonary neuroendocrine tumors

Pulmonary neuroendocrine tumors include typical carcinoid (TC) tumor, atypical carcinoid (AC) tumor, large-cell neuroendocrine carcinoma (LCNEC) and small cell lung carcinoma (SCLC) (34-36) and represent 20% of lung tumors. An initial accurate diagnosis is essential for patients with pulmonary neuroendocrine tumors because there are dramatic differences in outcome and therapeutic approach (37-42). The distinction between TC, AC, and LCNEC is based on the mitotic count and the presence of necrosis (43). While the mitotic count is an important component of the classification of pulmonary neuroendocrine tumors, it can be difficult to assess in limited biopsies. Mitoses can be difficult to distinguish from apoptotic cells and may be obscured by crush artefact (4,44-46). Also, evaluating mitotic count is time consuming and is subject to interobserver variability (37).

Studies performed using the current classification of pulmonary neuroendocrine tumors have shown an association between the Ki-67 PI and the grade of the neuroendocrine carcinoma (41,45,47-56). Although recent studies have attempted to identify the cutoff points for the Ki-67 proliferative indices for each diagnostic category of pulmonary neuroendocrine tumor (41,48,56), a consensus on how the Ki-67 proliferative index should be integrated into the diagnostic algorithm has not been established in detail (34,57).

While the presence of necrosis had been in Arrighoni's original definition of AC, the mitotic count he had proposed to define AC was between 5–10 mitoses per high power fields (HPFs) (58). Subsequently it was determined that the optimal cut-off values for mitotic counts are 0–1 mitoses per 10 HPFs for TC, 2–10 mitoses per 10 HPFs for AC, and greater than 10 mitoses per 10 HPFs for LCNEC (38). Based on these criteria the 10-year OS is 87%, 35%, and 9% for patients with TC, AC and LCNEC, respectively. A statistically significant difference in survival was also confirmed in more recent studies (46,59,60). We would suggest performing a Ki-67 IHC on all cases of pulmonary neuroendocrine tumors. For cases that have a Ki-67 proliferative index in the expected range for that diagnostic entity, the Ki-67 stain would support the H&E diagnosis. Ultimately, the final diagnosis, must be based on the H&E findings, and therefore, we recommend that after re-review, if there is no change in interpretation that the histologic evaluation stands regardless of the mitotic count, though a higher than expected Ki-67 proliferative activity could be mentioned in a note with the statement that the prognostic/biologic significance of the increased proliferative index is uncertain. We believe that immunohistochemical staining for Ki-67 is extremely helpful in the diagnosis of pulmonary neuroendocrine tumors and should be integrated as a valuable component in their diagnostic algorithm.

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Related to: Kriegsmann M, Warth A. What is better/reliable, mitosis counting or Ki67/MIB1 staining? *Transl Lung Cancer Res* 2016;5:543-6.

Kriegsmann M, Warth A. Ki-67 expression in pulmonary tumors—reply. *Transl Lung Cancer Res* 2016;5:552-3.

Chirieac LR. Tumor cell proliferation, proliferative index and mitotic count in lung cancer. *Transl Lung Cancer Res* 2016;5:554-6.

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Cite this article as: Chirieac LR. Ki-67 expression in pulmonary tumors. *Transl Lung Cancer Res* 2016;5(5):547-551. doi: 10.21037/tlcr.2016.10.13