Letter to the Editor

Epithelial-to-mesenchymal transition and EGFR status in NSCLC: the role of vimentin expression

Running title: Vimentin in NSCLC

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In a recent article by Lindsay et al. published in *Annals of Oncology*, vimentin expression was detected in circulating tumor cells (CTCs) from patients with non-small cell lung cancer (NSCLC) [1]. The authors reported a significant difference in the percentage of patients with vimentin-positive CTCs between EGFR-mutant and EGFR-wild-type disease. However, such a substantial difference was not observed for other oncogenic drivers, *e.g.* ALK-rearrangement and KRAS mutations.

Although vimentin has long been known to play a role in carcinogenesis, its potential involvement in resistance to cancer therapies has also been described, especially EGFR-directed tyrosine kinase inhibitors (TKIs) [2]. Vimentin is a type III intermediate filament protein constitutively expressed in mesenchymal cells (*e.g.* fibroblasts, macrophages, endothelial cells). Its expression may be higher in epithelial-derived tumor cells, and metastatic cancer cells show higher levels than primary tumors [3]. Vimentin expression may change substantially through transcriptional regulation during tumor progression. Epithelial-to-mesenchymal transition (EMT) is a process of phenotypic changes that occur during carcinogenesis but can also influence sensitivity to EGFR-TKIs.

This understanding of vimentin-related biological functions led researchers to hypothesize a prognostic role for vimentin expression, and findings from numerous studies on this topic were summarized in an important meta-analysis including 32 studies with 4118 patients [4]. The pooled hazard ratio (HR) for overall survival (OS) showed a significant association between vimentin overexpression and poorer prognosis in univariate analysis but not in multivariate analysis. In a study by Ren et al. on around 200 patients, mesenchymal phenotype (*i.e.* high H-score of vimentin, fibronectin or N-cadherin) was significantly more frequent in patients with wild-type EGFR than in those with mutated EGFR [5]. This is in contrast to Lindsay et al.'s study which reported a higher percentage of vimentin-positive CTCs in EGFR-mutated patients. Although the results from these 2 studies are not comparable, their radically different significance suggests that the analysis of vimentin alone may not be sufficient to evaluate the correlation between EMT and EGFR status.

The above findings and the conclusions drawn thereon induced us to explore the role of vimentin expression by immunohistochemistry (Benchmark^{XT} Ultra, Ventana Medical Systems, Tucson, AZ) in patients evaluated for EGFR mutational status. We selected 77 patients with non-squamous NSCLC whose tumor tissue had been assessed for both EGFR activating mutations and vimentin expression. Eleven patients were EGFR-mutant. We evaluated the association between EGFR status and the percentage of vimentin-positive tumor cells in terms of mean values and using 3 different cut-offs to define vimentin positivity (*i.e.* ≥1%, ≥10% and ≥50%). The mean value of vimentin was 39.0%, standard deviation 68.69% (45.36% for EGFR mutant, 37.94% for EGFR wild-type). The comparison of means between the 2 groups using the Mann-Whitney test was not significant ($P = 0.31$). Similarly, the chi-squared test did not reveal a significant association between EGFR status and the percentage of vimentin positivity at any of the cut-off values (Table 1). These findings suggest that the evaluation of vimentin expression alone is not sufficient to study the role of EMT in patients with EGFR-activating mutations.

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Disclosure

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References

1. Lindsay CR, Faugeroux V, Michiels S et al. A prospective examination of circulating tumor cell profiles in non-small-cell lung cancer molecular subgroups. Ann Oncol 2017; 28: 1523-1531.
2. Bronte G, Bravaccini S, Bronte et al. Epithelial-to-mesenchymal transition in the context of epidermal growth factor receptor inhibition in non-small-cell lung cancer. Biol Rev Camb Philos Soc 2018; 93: 1735-1746.
3. Kidd ME, Shumaker DK, Ridge KM. The role of vimentin intermediate filaments in the progression of lung cancer. Am J Respir Cell Mol Biol 2014; 50: 1-6.
4. Ye Z, Zhang X, Luo Y et al. Prognostic values of vimentin expression and its clinicopathological significance in non-small cell lung cancer: a meta-analysis of observational studies with 4118 cases. PLoS One 2016; 11: e0163162.
5. Ren S, Su C, Wang Z et al. Epithelial phenotype as a predictive marker for response to EGFR-TKIs in non-small cell lung cancer patients with wild-type EGFR. Int J Cancer 2014; 135: 2962-2971.

Table 1. Mean of vimentin-positive tumor cells and number of patients with vimentin positivity at different cut-off values in EGFR-mutant *vs.* EGFR-wild-type NSCLC

	EGFR-mut (*n* =11)	EGFR-wt (*n* = 66)	*P*
Mean Vim+ tumor cells	45.36%	37.94%	0.313
Vim+ (1-100%)	7	35	0.513
Vim– (0%)	4	31	
Vim+ (10-100%)	6	30	0.576
Vim– (0-9%)	5	36	
Vim+ (50-100%)	6	17	0.053
Vim– (0-49%)	5	49	

EGFR, epidermal growth factor receptor; mut, mutant; wt, wild-type; Vim+, vimentin-positive; Vim–, vimentin-negative; *n,* number of patients; *P* value <0.05, statistically significant