Clinical relevance of circulating microRNAs as lung cancer biomarkers

Mattia Boeri, Gabriella Sozzi, Ugo Pastorino
Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy

Improvements in clinical management of lung cancer have been modest over the last 20 years and with almost 1.6 million deaths worldwide (19% of the total) it still has the highest mortality rate among cancers. So far no valid biomarker has proven to be useful in lung cancer clinical practice and in reducing mortality. Technical limitations, as well as genetic and biological tumor heterogeneity have likely limited the successful identification of tumor-specific markers. An interesting landmark to identify novel and more reliable biomarkers is searching for candidates by looking not only at the tumor itself but also at the interplay between the tumor and the host with the aim to identify changes related to the biological reactivity of the host to a developing cancer.

MicroRNAs (miRNAs) are 19 to 25 nucleotide-long non-coding RNAs regulating gene expression by binding complementary sequences of target mRNAs. MicroRNAs have a role in cell-to-cell communication and are actively released in the extracellular space included in vesicles or bounded to proteins protecting them from RNase activity. In this respect, circulating microRNAs represent ideal candidates since biomarkers could reflect both the early changes in the pulmonary environment or the cross-talk between the tumor and its surrounding microenvironment.

MicroRNAs-based liquid biopsies are emerging as promising tools for individual risk stratification, for the diagnosis and prognosis of lung cancer and more recently as predictive biomarkers of response to therapy. In our Institution, a specific circulating microRNA signatures classifier (MSC) was generated and validated in plasma samples collected from volunteers enrolled in Low Dose Computed Tomography (LDCT) lung cancer screening trials, in order to set-up a minimally invasive test able to detect lung cancer in its early stages. LDCT is considered the gold standard for the early detection of lung cancer. Results of a USA randomized screening trial reported a significant increase in the number of lung cancer diagnoses, especially at lower stages, and a reduction of mortality up to 20%. On the other hand, two major issues emerged from screening studies: the high number of false positive cases identified by LDCT (over 95%) and the overdiagnosis issue (estimated over 18% in the NLST trial), leading to further examinations or unnecessary surgical procedures with remarkable increasing of costs and morbidities.

The MSC stratifies patients in 3 levels of risk: high, intermediate and low. In large retrospective studies using plasma samples collected from more than 1000 volunteers enrolled in the INT/IEO and in the MILD screening trials, the MSC resulted in 87% sensitivity, 81% specificity and 99% negative predictive value. Moreover, the combined use of LDCT and the MSC test showed a 5-fold reduction in the number of false positive cases obtained with LDCT alone. Considering the 111 (3.3%) lung cancer cases detected during the first 5 years of these two screening trials, 84 had a plasma sample available to perform the MSC test. In this cohort the MSC was able to predict the disease occurrence two years before LDCT detection and the 5-year survival was respectively 88.9% for low risk MSC, 79.5% for intermediate risk MSC and 40.1% for high risk MSC (p=0.001).

Another possible use of liquid biopsy could be the monitoring of the disease status after treatment or surgical resection. The MSC test was thus employed to analyze 100 longitudinally-collected plasma samples obtained from 31 alive patients (28 disease-free and 3 relapsing) after surgical resection of primary lung tumors. Considering the 25 disease-free patients positive to the MSC test at the time of diagnosis, a reduction of MSC risk profile was observed in 19 (76%) post-surgery plasma samples. Whereas in the 3 relapsing patients an increase of the MSC risk level was observed at the time of detection of second primary tumor or metastatic progression.

So far the MSC test showed very promising results in screening settings, being able to improve individual risk assessment and the performance of LDCT. Further analysis will provide information about the sensitivity of the MSC in clinically detected lung cancer. The MSC risk levels are also being evaluated according to other risk factors such as smoking habits (measured as Pack Year), the inflammation levels (measured as C-reactive protein) or the forced expiratory volume (FEV-1). Finally, by targeted NGS we are profiling the mutational load of screening detected tumors in order to analyze association with the MSC risk profile.