Validation of PEDF as a potential biomarker for NSCLC

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In the search for more efficient biomarkers for non-small cell lung cancer (NSCLC) we combined prefractionation techniques and protein separation by differential in-gel electrophoresis (2D-DIGE), to quantify protein changes between biological fluids (serum and pleural effusion) from NSCLC subjects and benign controls (pneumonia and tuberculosis individuals, respectively).

Pigment epithelium-derived factor (PEDF) was the sole protein identified in both fluids as differentially expressed in NSCLC patients. PEDF is a 50 kDa secreted glycoprotein that exhibits a duality with regards to apoptosis and survival [1]. PEDF has been reported to be present in serum at a concentration of 5 µg/mL [2], corroborating a relative success in mining down the proteome. It could be concluded that although immnunodepletion of high-abundance species improves protein profiling and several relevant proteins were revealed, most of the proteins identified still correspond to moderate and highly abundant ones. Two arguments may be exposed: first, the impossibility of covering the dynamic range of plasma and plasma-derived fluids, and, second, the gap between the broader linearity of DIGE in comparison with the reached in MS identification, as many of the unidentified spots corresponded to the less intense. Our study revealed one PEDF spot altered in serum with an average fold increase of 1.4 in NSCLC samples, while in the case of pleural effusion there were two altered forms showing changes of 1.5 and 2 fold increase in lung cancer patients.

The next step after discovering a potential biomarker is the confirmation of the variation observed on 2D gels; thus, immunodetection of PEDF was carried out in 1D-blots. When 10 µg of depleted protein were resolved results were contradictory: in the case of serum (Figure 1) the tendency was an elevated expression in NSCLC though levels were overlapped between cancer and benign control samples, whereas in pleural effusion (Figure 2) the pattern was opposed as the manifested by DIGE analysis. This discrepancy could be explained considering the superior sensitivity of antibody reactions and the great enrichment in PEDF levels achieved after prefractionation that would mask the significant differences found by DIGE methodology. Another reason might be the existence of more PEDF isoforms not distinguishable by 1D separation.

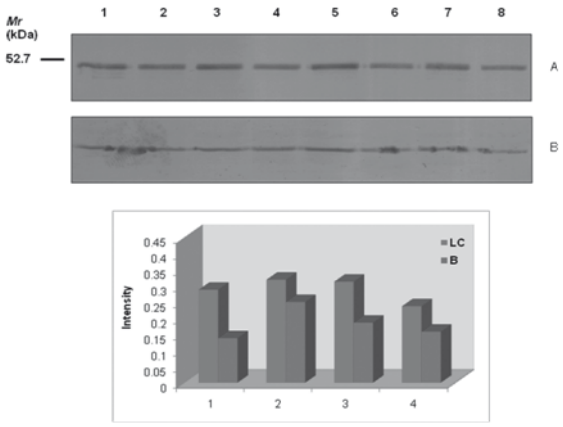

Figure 1. *1D immunodetection of PEDF on serum depleted samples. Immunoquantification of PEDF in gels resolving 10 µg of protein (B) was not consistent between cancer and control subjects, when dilution of the samples was performed (A) PEDF levels proved to be significantly increased in NSCLC. Lanes 1, 3, 5, 7: NSCLC samples, lanes 2, 4 ,6, 8: pneumonia samples. LC: lung cancer; B: benign.*

These two hypothesis were tested. First, 1D-blots were repeated diluting 1/10 the samples, finding in serum the same pattern initially obtained in 2D gels: a 1.5 fold increase in PEDF expression in cancer patients ($p = 0.043$) (Figure 1). For pleural effusion (Figure 2), although a slight overall increase was seen in NSCLC, the pattern was not repetitive.

To assess if there were variations in other PEDF isoforms not visualized on DIGE gels the molecule was specifically immunodetected in 2D-blots. For both fluids a wide plethora of isoforms was manifested with more than 12 spots detected (Figure 3),

which expanded about 1 pH unit. This variability agrees with the observed by [2] for purified human PEDF and is caused by posttranslational modifications. In serum all the isoforms observed displayed a higher intensity in NSCLC, while for pleural effusion a mixed pattern was generated with an average 1.3 fold increase in NSCLC.

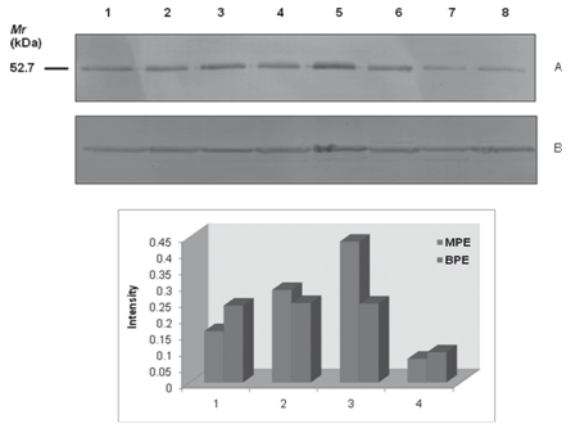

Figure 2. *1D immunodetection of PEDF on depleted pleural effusions. Blotting of 10 µg of protein (B) displayed a tendency to increased PEDF levels on benign samples, while repetition on diluted fractions partially reverse this pattern, although no statistically differences were achieved. Lanes 1, 3, 5, 7: NSCLC samples, lanes 2, 4 ,6, 8: tuberculosis samples. MPE: malignant pleural effusion; BPE: benign pleural effusion.*

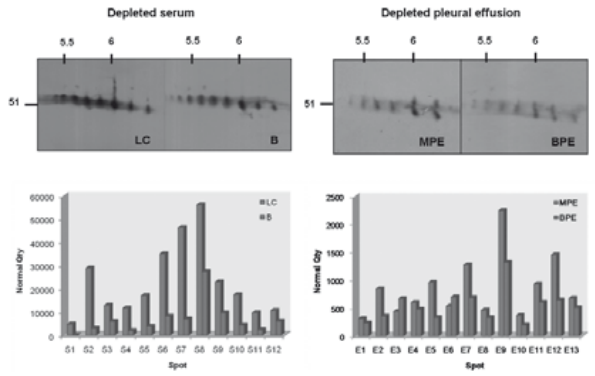

Figure 3. *2D immunodetection of PEDF. Both in depleted serum (left) and pleural effusion (right) several PEDF isoforms could be detected. Immunoquantification revealed different variations between benign- and malignant-origin samples, though most of the spots showed increased values in tumor-derived fluids. LC: lung cancer; B: benign; MPE: malignant pleural effusion; BPE: benign pleural effusion; Normal Qty: normalized quantity (by total density in each blot).*

Literature concerning PEDF expression on lung cancer is not conclusive, with a described reduction at the transcript level in lung tissues compared to normal specimens [3]. However, when protein level was examined the profile was reversed for adenocarcinoma type, the one we analised, with 18/33 specimens overexpressing PEDF. The overall increase showed by our work could be a response of PEDF anti-angiogenic capability to counteract VEGF induced vasopermeability.

A final step in the pathway of discovering biomarkers is to corroborate this discrimination by feasible and highly sensitive methods (commonly ELISAs) that could be implemented in the clinical setting. This requires that the differential expression observed in fractionated proteomes should hold a parallel comparison on crude samples, regarding different histological types.

References

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