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Title: Methylation of p16 in EBUS-TBNA samples as a predictor of advanced disease in lung cancer

Laura 28408 Millares lmillares@tauli.cat^{1,2}, Laia 28409 Setó setogort_laia@hotmail.com^{4,2}, Felipe 28410 Andreo fandreo@separ.es^{3,2}, Jose 28411 Sanz 34057jss@comb.cat^{3,2}, Mireia 28412 Serra mserraf@tauli.cat⁴, Concepcion 28413 Monton cmonton@tauli.cat⁴ and Eduard 28447 Monsó emonso@tauli.cat^{4,2}. ¹ Neumology, Fundació Parc Tauli, Sabadell, Spain ; ² CIBERES, ISCIII, Mallorca, Spain ; ³ Neumology, Germans Trias I Pujol Hospital, Badalona, Spain and ⁴ Neumology, Parc Tauli Hospital, Sabadell, Spain .

Body: Introduction Aberrant methylation of DNA results in gene silencing and is frequently observed in tumour samples from patients with lung cancer. Epigenetic changes significantly contribute to tumour progression allowing cancer cells to disseminate from the primary tumour and metastasize to and survive at a distant site. Thus, the detection of methylation in the draining lymph nodes may be important to assess the prognosis of the disease. Objective To determine the relationship between the methylation status of 5 genes in samples of lymph nodes obtained by endobronchial ultrasound with real-time guided transbronchial needle aspiration (EBUS-TBNA) and the presence of an advanced stage of lung cancer. Methods Nodal samples obtained with EBUS-TBNA were flash-frozen with N2. After DNA extraction, DNA was chemically modified and the methylation status of DAPK, p16, RASSF1, APC and CDH-13 genes was determined by methylation-sensitive high resolution melting Results Fifty four samples were obtained from patients with NSCLC (age 70 SD 8 years). Forty nodal samples show malignancy (23 adenocarcinoma, 13 squamous and 4 undifferentiated carcinoma) and 14 samples were non-metastatic nodes. P16 gene appeared methylated in significantly more samples from patients with advanced stage of lung cancer, 7/24 (29%) IIIb- V stage vs 2/30 (7%) I-IIIa stage, $p=0.027$, χ^2 test. The other 4 tested genes did not show statistical differences ($p>0.05$) between early and more advanced stage of cancer. Conclusion Methylation of p16 gene in cells obtained from lymph nodes accessible to EBUS-TBNA is a predictor of advanced disease in NSCLC and may be important in the management of patients with lung cancer Funded by FIS 09/01612 and FUCAP.