RASSF1A methylation analysis in minimally invasive samples from lung cancer patients and individuals with risk factors

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Ras association domain family isoform A (*RASSF1A*) is a tumour suppressor gene. *RASSF1A* exerts its functions through its scaffolding properties, allowing the assembly of complexes involved in various signaling pathways. The RASSF1A protein contains three major domains: the C1/DAG domain, the Ras association domain (RA) and a Sav/RASSF/Hpo interaction domain (SARAH). The RA domain mediates the interaction of RASSF1A with members of the Ras GTPase families, inhibiting their oncogenic function and thereby affecting the processes of cell proliferation, differentiation, morphogenesis and apoptosis in response to extracellular signals. *RASSF1A* inactivation is common in several human cancers. The main mechanism associated with *RASSF1A* inactivation is gene silencing through DNA methylation. In particular, in lung cancer, the most aggressive tumours with the worst prognosis are those in which K-RAS is mutated and the *RASSF1A* promoter is hypermethylated.

In this work, we aimed to analyze the *RASSF1A* methylation status in minimally invasive samples (blood plasma) from lung cancer patients and individuals with risk factors (smoking and chronic obstructive pulmonary disease, COPD). Samples were classified into four groups: 1) control group without risk factors (healthy); 2) smokers with risk factors (Smokers); 3) COPD risk factor group (COPD) and 4) lung cancer group (LuCa). DNA was extracted, bisulfite-modified, and quantitative methylation specific PCR (qMSP) was performed to determine the methylation status of the gene. In addition, we analyzed the *RASSF1A* methylation status in samples from lung cancer cell lines (A549, H23, PC9 and H292).
We detected *RASSF1A* methylation in plasma samples from 38% LuCa, 10% COPD, 12.5% Smokers and 12.5% healthy subjects. These findings were confirmed in lung cancer cell lines. These preliminary data suggest that methylation of *RASSF1A* may be a useful epigenetic biomarker for diagnosis of lung cancer using minimally invasive samples.

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