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Purpose: Claudins are the main constituents of tight junctions. Little is known about their expression and localization in the normal bronchial epithelium and in lung cancer.

Patients and methods: 104 lung cancer tissue blocks were studied including 46 adenocarcinomas (ADC), 30 squamous cell carcinomas (SCC), 15 small cell lung cancers (SCLC), 8 typical and 5 atypical carcinoids. All slides contained normal bronchial mucosa as well. Immunohistochemistry using antibodies against claudins-1,-2,-3,-4, and -7 proteins, as well as semi-quantitative estimation were performed. RT-PCR analysis was also carried out in 22 mmunohistochemically representative tumor samples.

Results: Normal bronchial epithelial cells expressed all the examined claudin proteins. When compared, SCLCs and carcinoids showed striking differences in regard to claudins-1,-3, and -4 expressions ($p<0.0001$, $p<0.0001$, and $p<0.0004$, respectively), whereas ADCs and SCCs revealed significant differences in claudins-3,-4, and -7 expressions ($p<0.0001$, $p<0.0001$, and $p<0.0053$, respectively). However, comparison of ADCs with SCLCs revealed significant difference only in claudin-2 expression ($p<0.0002$). The comparison of ADCs and carcinoids resulted in significant differences regarding claudins-1,-3, and -4 expressions ($p<0.0006$, $p<0.0001$, and $p<0.0001$, respectively). SCCs and SCLCs varied in respect to claudins-2,-3, and -4 expressions ($p<0.0009$, $p<0.0001$, and $p<0.0019$, respectively), whereas SCCs and carcinoids showed different claudin-1 and -4 expressions ($p<0.0076$, and $p<0.0045$, respectively). RT-PCR analysis revealed parallel changes in the mRNA and protein expression of certain claudins.

Conclusions: The observed distinct claudin expression profile within the non-small cell lung cancer group, further, the marked differences between SCLCs and carcinoids may have differential diagnostic impact, and the overexpression of certain claudins might have therapeutic implications.

P3-013 **BSTB: Molecular Pathology Posters, Wed, Sept 5 – Thur, Sept 6**

K-ras codon 12 mutations in Non Small Cell Lung Cancer: high incidence of mutations in endobronchial tissue

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Lung cancer is a main cause of cancer death worldwide. In Colombia, the incidence and mortality of lung cancer are high. On 2002, it occupied the third place of incidence in men (20.2 by each 100000) and the fifth place in women (10.1 by each 100000). In the last years an increase of incidence in non-small-cell lung cancers (NSCLC) has been observed, particularly of adenocarcinoma. A proportion of NSCLC contains mutated K-ras oncogene. Some risk factors as cigarette smoking and gender have been associated also with the development of this cancer. The aim of this study was to determine the incidence of K-ras mutations (codon 12) in patients diagnosed with NSCLC who attended at the Pneumology and Thoracic Surgery Services of the National Cancer Institute.

7 samples of endobronchial tissue, 11 samples of bronchoalveolar lavage (BAL) and 52 samples of paraffin-embedded tissue were obtained of 66 patients before receive treatment. For the analysis of K-ras mutations (codon 12) it was developed an enriched PCR technique with modifications done according to the kind of samples.

We identified 66 patients with non-small-cell lung cancer (adenocarcinomas: n=38; squamous cell carcinoma: n=26; large cell carcinoma: n=2; TNM stages I-IV) of them 18 were women and 48 were men (rank of age 35-84 years). 18 mutations were detected in 70 samples (25.7%), distributed in 5/7 endobronchial tissue (71.4%), 3/11 BAL (27.2%) and 10/52 paraffin-embedded tissue (19.2%). Of these 18 mutations, 14 (77.7%) belong to adenocarcinoma type (14/38, 36.8%), 3 (16.6%) to squamous cell carcinoma type (3/26, 11.5%) and 1 (5.5%) to large cell carcinoma (1/2, 50%). Of the detected mutations, 15 were found in men (mean age of 68 years) and all of them were smokers. The 3 additional mutations were detected in women (mean age of 66 years) that were non smokers. All samples that had the K-ras mutations belong to stages III or IV.

The high incidence of K-ras mutations at codon 12 was observed in endobronchial tissue, due to the high amount of tumor cells found in the biopsy. In addition these mutations were more common in adenocarcinomas than in squamous cell carcinoma, suggesting a possible association between the presence of the mutation and the adenocarcinoma type. These results are similar to other reports for the carrying population of mutations K-ras at codon 12. This research contributes to our understanding of the role that these mutations play as risk factors of Non Small Cell Lung Cancer in our population.

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The role of calcium influx in ET-1-induced proliferation of human lung adenocarcinoma cells SPC-A1

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Aim: Endothelin-1 (ET-1) is a potent mitogen involved in tumor cell growth. The aim of this study is to explore the role of calcium influx in ET-1-induced proliferation of human lung adenocarcinoma cells SPC-A1.

Methods: Cell number was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) assay. Ca2+ concentration was measured by Fura-2/AM fluorescent assay.

Result: ET-1 (10-15 mol/L-10-8 mol/L) enhanced SPC-A1 cell growth and increase [Ca2+]i in a dose-dependent manner($p<0.05$) in vitro. Effect of ET-1 (10-10 mol/L) on the proliferation of SPC-A1 cells and the increase of [Ca2+]i was blocked by BQ123 (10-7 mol/L), a highly selective endothelin receptor A (ETA) antagonist ($p<0.05$), not by BQ788 (10-7 mol/L) ($p>0.05$), a highly selective endothelin receptor B(ETB) antagonist. Deletion of extracellular Ca2+ with Ethylene Diamine Tetraacetic Acid (EDTA,0.4mmol/L) or blockade of voltage dependent calcium channel with nifedipine (1umol/L) significantly reduce ET-1-induced increase of [Ca2+]i and proliferation of cell growth.

Conclusion: endothelin-1 enhances both the proliferation and the increase of [Ca2+]i by the activation of ETA receptor in SPC-A1 cells. Ca2+ influx from voltage dependent calcium channel may play a great role in this precess.