**LS-11-P-5830 Immunohistochemical selection of tumor antigens as potential targets for non-small cell lung cancer immunotherapy.**

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**Abstract**

Introduction. Lung carcinoma is the leading cause of cancer related mortality worldwide. For this reason, some research efforts are focusing in the evaluation of a variety of tumor-associated antigens (TAAs) for diagnosis, prognosis and therapy monitoring as well as for lung cancer immunotherapy. In line with this, adequate methods to identify which patients are most likely to benefit from the targeted drugs against the identified TAAs are needed.

Aims. To evaluate the immunohistochemical detection of the N-glycolyl GM3 ganglioside (NeuGcGM3), the epidermal growth factor receptor (EGFR) and its ligand (EGF) in non-small cell lung carcinoma (NSCLC) using four cuban monoclonal antibodies.

Materials and Methods. Five micrometer serial sections from 64 routinely processed, formalin-fixed and paraffin-embedded archival samples with diagnosis of NSCLC cancer were obtained. For EGFR and EGF antigens, the slides were pre-treated with 0.4% pepsin in 0.1N hydrochloric acid solution at 37°C for 30 minutes. The samples were incubated with ior egf/r3 (anti-EGFR), CB-EGF1, CB-EGF2 (anti-EGF) and 14F7 (anti-N-glycolyl GM3 ganglioside) Mabs followed by a peroxidise avidin-biotin system. All markers were evaluated for percentage of positive cells (0-100%) and the intensity of reaction (0-3+). The results in agreement with two observers were considered as final.

Results. The reactivity of 14F7 Mab was evidenced in 61/64 (95.3%) of NSCLC samples. The pattern of staining of this Mab was finely granular and was located on both cell membrane and cytoplasm of malignant cells (Figure 1). In one of positive cases (1.6%) an additional nuclear staining of 14F7 Mab was detected.

The immunodetection of EGFR by means of the ior egf/r3 Mab was evidenced in 37/59 (62.7%) of NSCLC samples. The pattern of staining of ior egf/r3 Mab was finely granular and was mainly located in the plasmatic membrane of malignant cells (Figure 2), although their cytoplasm was also decorated.

The expression of EGF was observed in about 70% of NSCLC samples, using CB-EGF1 or CB-EGF2 Mabs. The pattern of staining of these Mabs was finely granular and mainly located in cytoplasm; although a membrane staining was also observed (Figure 3). An additional extracellular staining was detected. A slight increase in the intensity of reaction was observed with CB-EGF1 Mab, although a significant correlation was detected when the reactivity of these two Mabs were compared (p<0.0001, rs=0.5429; Spearman test).

Conclusions. Our data permit to consider the development of diagnostic kits using ior egf/r3, CB-EGF1 and 14F7 Mabs in order to achieve a better selection of patients to specific therapies.

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Fig. 1: Figure 1. Hematoxylin and eosin staining of lung adenocarcinoma (A). Note: the intense reaction of 14F7 Mab located on both cell membrane and cytoplasm of malignant epithelial cells (B) (Brown color). Counterstained with Mayer’s Hematoxylin (Blue color). White bar = 50 µm.

Fig. 2: Figure 2. Hematoxylin and eosin staining of lung adenocarcinoma (A). Note: the intense reaction of ior egf/r3 Mab mainly located in cell membrane and also in the cytoplasm of malignant epithelial cells (B) (Brown color). Counterstained with Mayer’s Hematoxylin (Blue color). White bar = 50 µm.

Fig. 3: Figure 3. Hematoxylin and eosin staining of lung adenocarcinoma (A). Note: the intense immunostaining with CB-EGF1 Mab located on both cell membrane and cytoplasm of malignant epithelial cells (B) (Brown color). Counterstained with Mayer’s Hematoxylin (Blue color). White Black bar = 100 µm.