MicroRNA prognostic signature identifies critical oncogenic pathways in neuroendocrine neoplasias (NENs)

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Background: Neuroendocrine neoplasias (NENs) are a group of very heterogeneous malignancies whose incidence has increased in the last years. Current available biomarkers have low specificity and sensitivity so its usefulness in clinical practice is scarce. MicroRNAs (miRNAs) are a class of short non-coding RNAs that regulate many biological processes including the hallmarks of cancer. They have been identified as potential biomarkers for cancer diagnosis, prognosis and as therapeutic targets, but few studies have assessed their role in NENs. The aim of this study was to characterize the miRNA profile in a large cohort of NENs and assess their correlation with clinical-pathological features and prognostic value in this setting.

Methods: 84 cancer-related miRNAs were analyzed by *PCR Array* technology. All samples were normalized using 5 housekeeping RNA (SNORD61, SNORD68, SNORD95, SNORD96A, RNU6B). For each patient, the relative quantification of normalized miRNA's expression was obtained by $2^{-\Delta\Delta Ct}$ method (tumor and paired-normal samples). Clinical and pathological variable distribution was assessed using parametric or non-parametric tests as appropriate. Kaplan-Meier method and long-rank test were used to assess the prognostic value of categorical variables. Multivariate Cox regression model was performed to evaluate the independent prognostic value of selected miRNAs, adjusted for age, primary tumor site, grade and stage. MiRNAs that showed independent prognostic value including multi-test adjustment by false discovery rate method (FDR) were selected. Hierarchical clustering using miRNA expression and euclidean distance of the selected miRNAs was performed. Three public databases (*TargetScan Release*, *miRDB* and *Diana-Tarbase*) were used to predict miRNA's biological targets. An *in silico* functional analysis (*R clusterProfiler*) was then performed to identify enriched pathways using *Hallmarks* and *KEGG* gene set collections from MsigDB.

Results: 86 NEN samples (51 Lung, 35 gastroenteropancreatic (GEP)) were analyzed: 51% grade 1 (G1) or typical carcinoids (TC), 19% grade 2 (G2) or atypical carcinoids (AC) and 30% grade 3 (G3) or neuroendocrine carcinomas (NEC). There were not well-differentiated grade 3 neuroendocrine tumors (NET G3). 40 miRNAs were significantly associated with different clinical and pathological features. Among them, we identified a signature of 8 miRNAs (hsa-miR-A, -B, -C, -D, -E, -F, -G, -H) with independent prognostic value ($P < 0,05$). Our signature identified 3 distinct prognostic groups, with 5-year overall survival (OS) of 97%, 64% and 39%, respectively. In silico analysis unraveled oncogenic

pathways regulated by these miRNAs including NFKβ, RAS, WNT/β-catenin, TGFβ, PI3K-AKT-mTOR and P53 and their implication in endocrine processes, cellular architecture, cell cycle and cell damage.

Conclusions: Our study has identified a novel 8-miRNAs signature able to predict OS of GEP and lung NENs, independent of other well established prognostic factors. In silico predicted pathways regulated by our firm suggest that it could contribute to regulate the classical hallmarks of cancer in NENs. These miRNAs regulate critical oncogenic pathways that could potentially be explored as novel targets for therapy.