Gene expression profiling of large cell neuroendocrine carcinoma (LCNEC)

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Introduction: Large cell neuroendocrine carcinoma (LCNEC) is a diagnostic challenge for both pathologists and clinicians. We sought to determine the significance of overexpression of ASCL1 in LCNEC by gene expression profiling compared to AD.

Methods: We performed gene expression profiling of 100 lung adenocarcinoma (AD) and 6 LCNEC of lung for which the diagnosis was established based on detailed histologic review and immunohistochemistry (IHC). We performed quantitative real-time PCR (qRT-PCR) of frozen and formalin-fixed, paraffin-embedded (FFPE) samples and IHC of TMA to evaluate ASCL1 expression in LCNEC. Patients’ clinical data was collected from chart. IHC for common neuroendocrine markers (synaptophysin, CD56, chromogranin A), Ki67, caspase-3 and p53 was examined using TMA. Association between histology and gene expression, qRT-PCR, or IHC was examined by Fisher exact test, chi-square test or Kruskal Wallis test. Patients’ outcome was analyzed by Kaplan Meier survival analysis.

Results: By unsupervised hierarchical clustering of expression data, four different subgroups were defined, and most LCNEC (5/6) were clustered in the same branch of the dendrogram. Patients in this genetic subgroup were older, with heavier smoking history and shorter survival; tumors were larger, with high proliferation and apoptotic activity (labeling index of Ki67 and caspase-3) compared to other subgroups. LCNEC itself also showed higher proliferation and apoptotic activity and shorter survival than AD. Supervised analysis of the gene expression data identified many genes that were strongly differentially expressed between LCNEC and AD. ASCL1, a proneural basic helix-loop-helix transcription factor, was highly expressed in LCNEC relative to AD. Quantitative RT-PCR analysis of RNA from frozen and formalin fixed samples confirmed that ASCL1 expression was significantly higher in LCNEC and small cell lung cancer (SCLC) than AD and non-neoplastic lung (NL) (p<0.001, Kruskal Wallis test). By IHC, diffuse or focal immunoreactivity of ASCL1 was observed in the nuclei of most LCNEC (81%) and SCLC cases (73%), although the intensity was variable. ASCL1 immunoreactivity showed significantly greater correlation with histologic subtype (p=0.001) and was determined to have the best discrimination of all IHC assays tested based on receiver operator characteristics (AUC, ASCL1= 0.848, KLK11= 0.550, synaptophysin= 0.817, CD56 = 0.737, chromogranin A = 0.488). In addition, p53 immunoreactivity was positive in significantly more LCNEC (5/6) than AD (1/99) (p<0.001).

Conclusions: We conclude that LCNEC is biologically and clinically distinct from AD of the lung and should be distinguished. ASCL1 is a useful marker to aid accurate diagnosis of LCNEC.

High ERCC1 expression correlates with poor survival in lung adenocarcinoma

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Background: Excision repair cross-complementation group 1 (ERCC1) is an enzyme that functions in the process of nucleotide excision repair by removal of DNA adducts induced by cisplatin. In non-small cell lung cancer (NSCLC) lack of ERCC1 expression has been reported to correlate with benefit from cisplatin-based adjuvant chemotherapy. In NSCLC patients who did not receive chemotherapy lack of ERCC1 staining correlated with a more favorable prognosis. We sought to determine if ERCC1 expression correlated with clinical features and survival in both NSCLC and pulmonary neuroendocrine tumors including small cell lung carcinoma (SCLC), large cell neuroendocrine carcinoma (LCNEC), typical carcinoid (TC) and atypical carcinoid (AC).

Study Design: We immunohistochemically stained tissue microarrays with 283 NSCLC including 140 adenocarcinomas (ADC) and 71 squamous cell carcinomas (SCC) as well as 59 SCLC, 40 TC, 26 AC, and 21 LCNEC using the Neomarker, mouse, clone 8F1 antibody to ERCC1. A score for each case was made based on distribution and intensity of staining and positive thresholds set according to clinical/molecular correlations. Followup was available in 230 NSCLC and 133 NE tumors. Kaplan Meier survival analysis and chi-square statistics were made using SPSS v 13.

Result: For NSCLC a high level of ERCC1 staining was seen in 30/253 cases (11%) with 14/140 (10%) ADC and 16/143 (11.2%) squamous carcinomas. Five year survival was significantly reduced in patients with ERCC1 positive (87%) vs negative (39.8%) tumors (p=0.002). This was driven by the five year survival for adenocarcinoma that was significantly reduced in patients with ERCC1 positive (0.83%) vs negative (43.8%) (p=0.002). There was no significant difference in survival by ERCC1 for squamous cell carcinomas. For NE tumors, ERCC1 was positive in 5/40 TC (12.5%), 1/26 AC (3.8%) and 2/21 LCNEC (9.5%) with significantly more staining in 16/59 (27.1%) of SCLC (p=0.033). No correlations were found with age, sex or survival in the NE tumors.

Conclusion: Our finding that patients with ERCC1 positive tumors had a significantly worse outcome is different from a previous report that a control population of patients who did not receive chemotherapy who had with ERCC1 positive tumors had a better overall survival than patients with ERCC1 negative tumors. Because of the lack of clinical information regarding chemotherapy we were not able to address the issue whether ERCC1 correlated with response to chemotherapy or not.

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