International Agency for Research on Cancer

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Molecular characterisation of pulmonary supra-carcinoids through integration of multi-omics data

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Introduction to pulmonary carcinoids

- Pulmonary carcinoids (PCA), comprising typical and atypical carcinoids, are a group of low-grade lung neuroendocrine neoplasms that, unlike their high-grade counterparts, have relatively good prognosis and no known risk factors.
- Little is known about the recently described supra-carcinoid subtype[1,2]. Supra-carcinoids are:
  - Morphologically similar to atypical carcinoids, with clinical and molecular features similar to high grade large cell neuroendocrine carcinoma (LCNEC).
  - Display higher expression of immune checkpoint genes and *MKI67* than PCA
- Given their rarity, characterisation of these tumours has been limited.

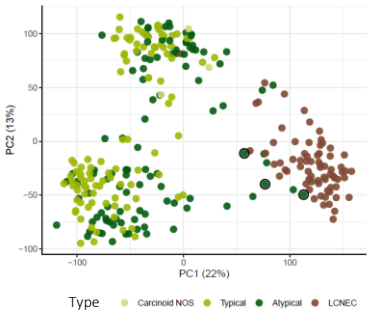
Primary aim: perform the first comprehensive multi-omic molecular, morphological and clinical characterisation of supra-carcinoids

Methods for identification of supra-carcinoids

- Established a new cohort of 91 PCA with clinical and morphological data, as well as whole-genome sequencing (WGS), RNA sequencing, and DNA methylation array data.
- Identify potential instances of supra-carcinoids by examining molecular clusters and tumour histology
  - Principal components analysis and consensus clustering of gene expression and methylation data from PCA and LCNEC (addition of published datasets[1,3,4,5,6]) → identifies PCA with LCNEC-like molecular features.
  - Analysis of tumour histology: mitoses, necrosis, KI-67, and protein expression of immune checkpoint genes → identifies PCA with LCNEC-like histology

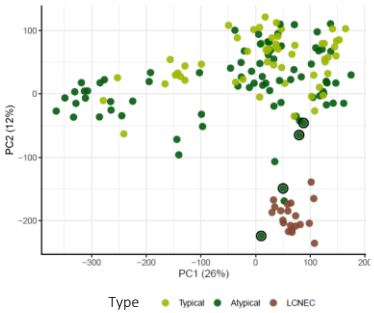
Gene expression and DNA methylation profiles identify new instances of supra-carcinoids

Gene expression



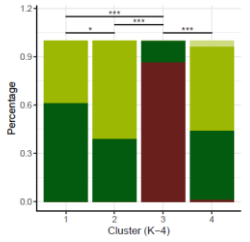
278 tumours plotted by their position along principal components 1 and 2 calculated from expression of the 5,000 most variable genes. Tumours coloured by type, n = 3 (carcinoid NOS), n = 100 (typical carcinoid), n = 104 (atypical carcinoid), n = 71 (LCNEC). Previously identified supra-carcinoids circled in black.

DNA methylation



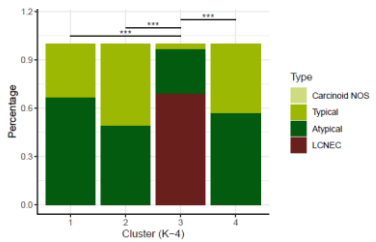
157 tumours plotted by their position along principal components 1 and 2 calculated from methylation level of 15,000 probes (5,000 most variable promoter, enhancer and gene body probes combined). Tumours coloured by type, n = 56 (typical carcinoid), n = 81 (atypical carcinoid), n = 20 (LCNEC). Previously identified supra-carcinoids circled in black.

Gene expression clusters



Consensus clustering of gene expression data identified four clusters, one (3) significantly enriched for LCNEC. Ten atypical carcinoids clustered in the LCNEC-enriched group.
*p-value < 0.05, ** < 0.01, *** < 0.001

DNA methylation clusters



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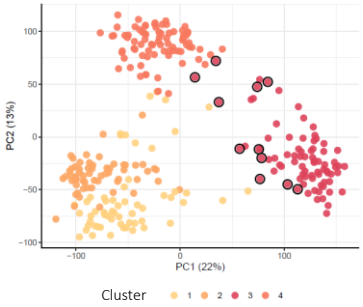
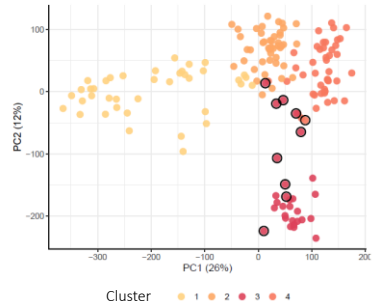
Gene expression



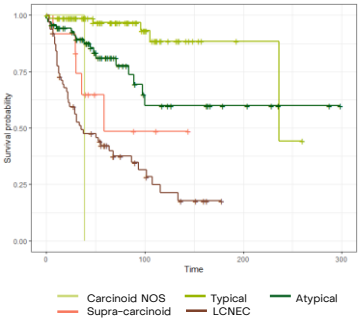
As above, tumours coloured by expression consensus clustering group. Non-LCNEC tumours clustering in group 3 (LCNEC enriched) classified as potential supra-carcinoids, are circled in black.

DNA methylation



As above, tumours coloured by DNA methylation consensus clustering group. Non-LCNEC tumours clustering in group 3 (LCNEC enriched) classified as potential supra-carcinoids, are circled in black.

Survival of lung neuroendocrine neoplasms



Kaplan-Meier curve depicting probability of overall survival over time (months). n = 1 (carcinoid NOS), n = 81 (typical), n = 71 (atypical), n = 12 (supra-carcinoid), n = 63 (LCNEC).

Supra-carcinoids had significantly shorter survival than typical carcinoid (p-value < 0.001).
Typical carcinoids had significantly better survival than atypical (p-value = 0.0023).
Atypical significantly better than LCNEC (p-value < 0.001)

- 11 new potential supra-carcinoids were identified in addition to the six previously described based on their gene expression and/or DNA methylation profile
- These patients had significantly poorer survival than typical carcinoids, and no difference in survival was observed between supra-carcinoids and LCNEC.

Next steps towards molecular, clinical and morphological characterisation of pulmonary supra-carcinoids

- Compare the results of molecular clustering data with histological analyses (including NanoString DSP analysis of immune checkpoint genes), to confirm supra-carcinoid cohort
- Integrate WGS data (copy number variants, small and structural variants) with gene expression and DNA methylation data using Multi-Omics Factor Analysis[7] to characterise the molecular profile of supra-carcinoids
- Correlate the molecular profiles with clinical and morphological features

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http://rarecancersgenomics.com/

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