Potential role of somatic mutations in *PABPC* family, *NOTCH1* and *MTOR* genes in Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)

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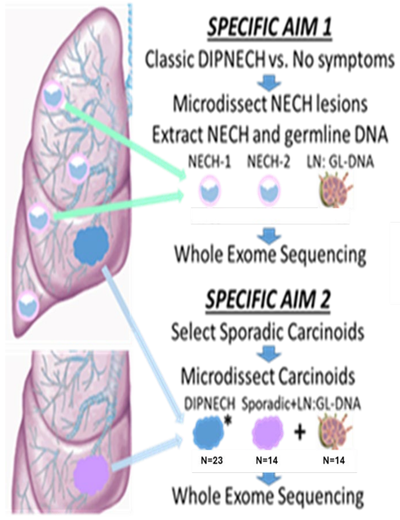
Background

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH) is a recognized pre-malignant condition which is characterized by generalized proliferation of pulmonary neuroendocrine cells (PNEC). These premalignant lesions lead to life-threatening complications including multifocal carcinoid tumors and severe airway obstruction with respiratory failure. Surgical resection and lung transplantation have shown limited efficacy.

Objectives

The identification of the common somatic mutations in DIPNECH will allow us to understand the molecular basis of the disease to discover vulnerabilities that can be exploited to interrupt the development and progression of neuroendocrine tumors and to discover diagnostic and prognostic markers that can guide treatment planning decisions.

Methods



Pathologic Features	≥ 3 NECHs
	Multiple carcinoid tumors or tumorlets
Clinical Features	Dyspnea
	Persistent cough
	Pulmonary function test abnormalities
Radiologic Features	Multiple nodules
	Parenchymal mosaicism
	Diffuse, bilateral changes

47 patients with pathologic features of DIPNECH & multiple NECH lesions

With clinical and/or radiologic features

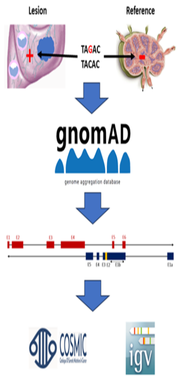
Strong evidence DIPNECH (N=36). 94 lesions for WES

No clinical and/or radiologic features

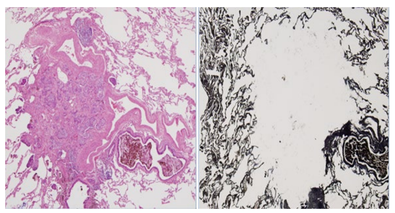
Weak/Intermediate evidence DIPNECH (N=11). 28 lesions for WES

Somatic Mutations in DIPNECH – SNV Selection

- Removal of Germline variants
- gnomAD MAX_AF populational frequency ≥ 0.005
- Mutation Impact score calculated by COSMIC inclusion & SIFT/PolyPhen-Potential tumorigenic
- Mutations affecting coding regions included
- IGV review – confirm



Lesion Type	Lesion Numbers (Strong DIPNECH N.)
NECH	87 (67)
DIPNECH related carcinoid tumors	23 (19)
DIPNECH related tumorlets	12 (7)
Sporadic carcinoid tumors	14 (Not Applicable)

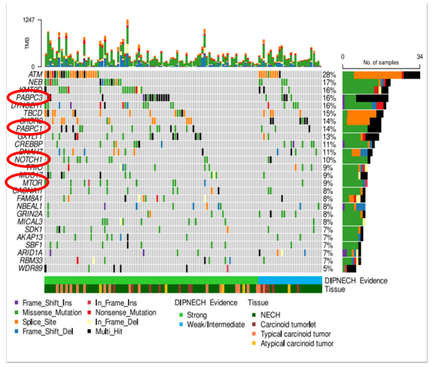


HE 4X Before Microdissection

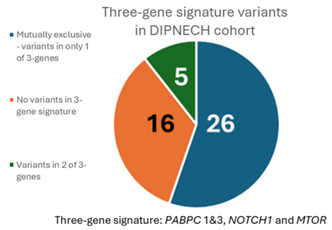
After Microdissection 4x, no staining

Results

Identification of DIPNECH Related 3 Gene Signature



The top 26 genes by mutation frequency in the DIPNECH cohort
Mean Impact Score > 2.25 for all variants within a gene

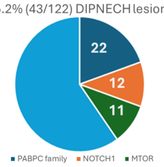


Three-gene signature: *PABPC* 1&3, *NOTCH1* and *MTOR*

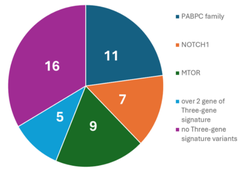
- Mutations in genes of the three gene signature group are nearly mutually exclusive across DIPNECH cases
- These genes appear to effect gene expression profiles that have been or maybe related to NE differentiation and proliferation

Features of DIPNECH Related 3-Gene Signature

Three-gene signature mutations in 35.2% (43/122) DIPNECH lesions



Three-gene signature mutations in 66% (31/47) DIPNECH patients



NOTCH1 mutations (Impact Score=2.31) inactivating mutations promote neuroendocrine cell differentiation

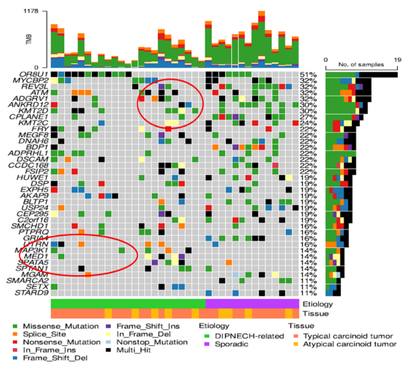
MTOR mutations (Impact Score = 2.45) – activating mutations promote cell proliferation and metabolism that contributes to tumor initiation and progression

PABPC (Poly-A Binding Protein C) family mutations (Impact Score = 2.58) – Affect binding affinity to Poly A and therefore impact the translational efficiency of groups of mRNAs.

HYPOTHESIS: These somatic mutations in *NOTCH1*, *MTOR* and *PABPC1/3* initiate and promote the progression of premalignant NECHs in DIPNECH due to gene expression changes that promote neuroendocrine differentiation and oncogenic outgrowth and that characterization of these changes will inform identification of potential targets for therapeutic interventions.

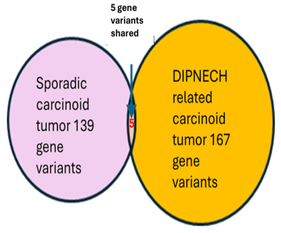
Mutational Profiles in DINPECH vs. Sporadic Carcinoid Tumors

DIPNECH vs Sporadic Carcinoid Tumors



The gene mutation profile of DIPNECH related vs Sporadic NETs are dissimilar indicating potential different pathogenesis

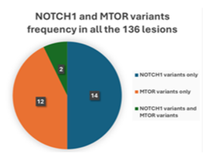
Unique mutations in tumorigenesis related gene



Top mutated genes with Impact Factor ≥ 2.5

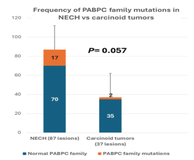
Results

3-genes signature mutation frequency in DIPNECH cohort and Sporadic carcinoid tumors



Mutually exclusive NOTCH1 and MTOR mutations suggest important roles of these gene alterations in tumorigenesis, which are commonly seen in NSCLC

PABPC gene family mutations are more common in NECH vs. Invasive lesions: Potential role in early DIPNECH tumorigenesis



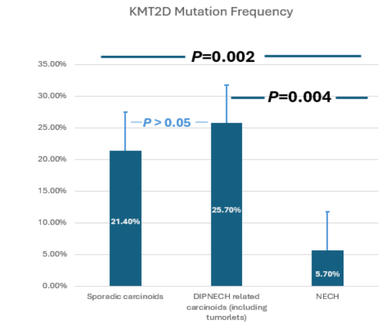
Carcinoid tumors include all the DIPNECH related and Sporadic carcinoid tumors

Within Patient Lesion Inter-Relatedness Analysis

- Used all variants including non-coding
- Gene variants had to be identical in respect to base change in two or more lesions
- all the variants identified by FreeBayes, Mutect 2 and Strelka were included in the analysis, with all microsatellites associated variants excluded due to the high numbers of artifact prone
- Compare "Strong" to "Weak/Intermediate" evidence DIPNECH

CONCLUSION: A trend toward higher frequency of shared variants in strong evidence case suggests possible clonal expansion as important mechanism in DIPNECH pathogenesis

Chromatin-remodeling gene KMT2D mutation frequency in DIPNECH cohort and Sporadic carcinoids



KMT2D significantly highly mutated in invasive carcinoid tumors than in benign NECH lesions

Conclusion

- *PABPC1/3, NOTCH1* and *MTOR* have been identified as potential critical alterations in the pathogenesis and disease progression of DIPNECH.
- DIPNECH cases show frequent and mutually exclusive mutations in the three gene signature.
- Different mutational profiles are seen between DIPNECH carcinoid tumor and Sporadic carcinoid tumor indicating possible different pathogenesis.
- Shared gene variants in lesions within DIPNECH individuals suggests possible clonal spread underlying the diffuse lesions seen in DIPNECH.
- MTOR inhibitor maybe a potential therapy for the treatment of DIPNECH.

Future research

1. Compare gene expression profiles of *NOTCH1, MTOR* and *PABPC1/3* mutated NECH and carcinoid tumor to the matched bronchial epithelium to identify differentially expressed genes that promote neoplastic neuroendocrine cell (NE) change.
2. Assess the impact of mutationally defined DIPNECH lesions on neuroendocrine oncogenicity and potential drug susceptibility.

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