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Gene expression profiling of pulmonary neuroendocrine neoplasms: A comprehensive overview

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ABSTRACT

Neuroendocrine neoplasms (NENs) of the lung comprise a heterogeneous group, including small cell lung cancer (SCLC), large cell neuroendocrine carcinoma and pulmonary carcinoids. To unravel their molecular biology, microarray studies have been conducted that provided lists of differentially expressed genes between lung NENs on the one hand and normal tissue and/or non-SCLCs on the other. However, the majority of studies paid little attention to the functions of candidates and their potency as diagnostic markers and/or therapeutic targets. Furthermore, at a first glance, only limited overlap was seen amongst these individual studies concerning differentially expressed transcripts.

By combining all originally published gene expression profiling studies on lung NENs, and by re-evaluating differentially expressed genes, we were able to identify major factors involved in lung NEN carcinogenesis. Thirty-three genes were found to be frequently deregulated in multiple studies. Amongst these are neuroendocrine-specific factors, including *ASH1*, *INSM1*, and *ISL1* and genes involved in neuronal differentiation and neurite outgrowth such as *DCX* and *NCAM1*. Also, multiple factors were involved in cell cycle progression, including members of the mitotic spindle checkpoint complex, and the regulated secretory pathway, e.g. *CHGA* and *CHGB* and *CPE*. For several of these candidates we propose possible functions in lung NEN carcinogenesis as well as potential roles in diagnosis and as targets for novel therapies.

This review elucidates potential genes of interest in pulmonary NENs on basis of the present expression profiling literature. We advocate that a selection of the identified candidates should be examined in depth for their clinical application.

1. Introduction

Lung cancer is a common type of malignancy, with one of the highest mortality rates in the USA [1]. Approximately 20–25% of lung tumors are neuroendocrine in nature, with small cell lung cancer (SCLC; 15–20%) being the most common and malignant subtype, while large cell neuroendocrine carcinoma (LCNEC; 30%) and pulmonary carcinoids (1–2%) comprise rarer entities [2]. Pulmonary carcinoids are further subdivided into the low grade typical carcinoid tumors, and intermediate grade atypical carcinoids. While many well-characterized oncogenes and tumor suppressor genes have been extensively studied in lung neuroendocrine neoplasms (NENs), the mechanisms of carcinogenesis of these neoplasias, and in particular of the smoking-unrelated pulmonary carcinoids, remain poorly understood.

In the past 10 years, a shift in molecular approaches to elucidate mechanisms of oncogenesis has become evident. Candidate gene approaches have been increasingly complemented by genome-wide

Abbreviations: APC/C, anaphase-promoting complex/cyclosome; CgA, chromogranin A; CgB, chromogranin B; CGH, comparative genomic hybridization; HCC, hepatocellular carcinoma; HGNEC, high-grade neuroendocrine carcinoma; LCNEC, large cell neuroendocrine carcinoma; MCC, mitotic checkpoint complex; NEN, neuroendocrine neoplasm; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PNEC, pulmonary neuroendocrine cell; SAC, spindle assembly checkpoint; SCLC, small cell lung cancer

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techniques. Next to array comparative genomic hybridization (array CGH) and gene expression profiling studies genome-wide methylome profiling and several sequencing techniques have become available. Up till now, only a few studies using these novel techniques have been performed on pulmonary NENs. Kalari et al. carried out methylation profiling on 18 primary SCLCs and cell lines using the methylated-CpG island recovery assay and identified tumor-specific methylation of several transcription factors involved in the determination of neural cell-fate [3]. Integrative analyses of multiple genome-wide approaches were performed by Peifer et al., who demonstrated that Rb- and P53-alterations are almost universal in SCLCs and identified novel disease-driving factors, including *CREBBP* and *MLL* [4]. Using a similar approach Rudin et al. observed frequent amplification of the *SOX2* gene in SCLC [5]. Recently, Fernandez-Cuesta et al. showed using genome and exome sequencing that pulmonary carcinoids frequently exhibit mutations of chromatin-remodeling genes [6].

Although these recent studies have yielded important novel insights into the molecular biology of lung NENs, the large majority of genome-wide studies published so far consists of array CGH and gene expression profiling data. In a previous review [7], we have performed a meta-analysis of array CGH studies on pulmonary NENs and briefly discussed the main findings of a number of gene expression profiling studies. This included the important finding that LCNECs and SCLCs cluster either separately or together, but in general more closely towards normal bronchial epithelium, whereas carcinoids grouped always together in a different cluster, which is more closely related to brain tumors than to high-grade neuroendocrine carcinomas (HGNECs) [7]. These studies provided also lists of differentially expressed genes between lung NENs on the one hand and normal tissue and/or NSCLCs on the other, which will be the focus of the current review. Since pulmonary carcinoids and LCNECs comprise relatively rare entities, and SCLCs are only seldom resected [7], relatively few gene expression profiling studies have been undertaken to these entities, rendering them understudied as compared to NSCLCs. This makes a review of available gene expression profiling studies valuable. We noticed that for individual lung NEN expression profiling studies few top-candidates overlap, which is a common problem when comparing microarray studies [8]. This is not surprising, since these studies had different aims, not all of them focusing on lung NENs, and different platforms and control tissues were used (Table 1). Moreover, even when reported by multiple studies, often little attention has been given to putative role(s) of candidate genes in lung NEN oncogenesis. Here, we review candidates reported in at least two different lung NEN gene expression profiling studies. We will discuss the biological functions of these candidates in general and their putative role within lung NEN development and progression, and their potential value for diagnosis and therapy.

2. Approach

We have selected all original research papers (1999–2014) available through PubMed that included gene expression profiling data on pulmonary NENs, using the following keywords: ("expression profiles" OR "expression profiling" OR "cDNA microarray") AND ("pulmonary neuroendocrine tumors" OR "small cell" OR "lung carcinomas" OR "lung carcinoids" OR "pulmonary carcinoids" OR "SCLC" OR "LCNEC" OR "Large Cell Neuroendocrine Carcinoma"). From these, only the studies describing gene expression profiling on primary lung NENs were included (Table 1) [9–21]. Studies not reporting on differentially expressed genes in lung NENs were excluded [18], as well as studies only reporting genes differentially expressed between lung NEN subgroups [21]. We have combined gene lists (limited to the top-100 genes) as well as genes discussed elsewhere in the text body [13] of these individual studies. Subsequently, we have selected candidate genes described in at least two different studies to be differentially expressed in carcinoids, LCNECs and/or SCLCs as compared amongst each other [12] or to normal (lung) tissue and/or NSCLCs [9–11,13–17,22] (Table 2). With respect to our own gene expression profiling study [19], we have extracted the genes that are differentially expressed between carcinoid tumors and the normal reference (see Supplementary Table 1).

Table 1
Gene expression profiling studies performed on neuroendocrine lung tumors.

Year	Number of carcinoids	Number of LCNECs	Number of SCLCs	Platform	Reference material	Author/reference
1999	2	0	2	18 K cDNA microarrays (Genome Systems)	Normal lung tissue and the other NE lung cancer subtypes	Anbazhagan et al. [9]
2001	20	0	6	63 K U95A oligonucleotide probe arrays (Affymetrix)	Normal lung tissue and other lung cancers	Bhattacharjee et al. [10]
2001	0	0	5	23 K cDNA microarrays	Normal lung tissue and other lung cancers	Garber et al. [11]
2002	3	0	9	8 K microarrays	Normal lung tissue and other lung cancers	Virtanen et al. [16]
2004	11	2	3	9 K cDNA microarrays	The other NE lung cancer subtypes	He et al. [12]
2004[a]	13	8	17	40 K cDNA microarrays	Normal lung tissue and other lung cancers	Jones et al. [13]
2004	0	2	7	1 K cDNA microarrays (Human Cancer Gene Filter 1.2, Clontech)	Normal lung tissue and other lung cancers	Wikman et al. [17]
2006[b]	0	2	0	22 K oligonucleotide microarrays (Agilent)	Pool of cell lines	Takeuchi et al. [18]
2006	0	0	15	32 K cDNA microarrays	Normal lung tissue and NSCLC	Taniwaki et al. [15]
2008	0	0	9	9 K h6-Focus GeneChips (Affymetrix)	Normal lung tissue and NSCLC	Rohrbeck et al. [14]
2013	10	0	0	44 K v2 microarrays (Agilent)	Pool of cell lines	Swarts et al. [19]
2013	0	4	4	60 K single channel arrays (Agilent)	Normal lung tissue	Bari et al. [20]
2014	13	0	0	55 K HG-U133 Plus 2.0 oligonucleotide microarrays (Affymetrix)	None[c]	Toffalorio et al. [21]

Abbreviations used: LCNEC, large cell neuroendocrine carcinoma; NE, neuroendocrine; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer

[a] Genes taken for this review were described in the text body.
[b] No list of genes for the two LCNECs was reported. Therefore, this publication was not further analyzed for this review.
[c] Only a comparison was provided between typical and atypical carcinoids. Therefore, this publication was not further analyzed for this review.