Key Genes in Lung Cancer Translational Research: A Meta-Analysis

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Key Words

cDNA array • Lung cancer • Meta-analysis • Non-small cell lung cancer • Small cell lung cancer • Translational research

Abstract

In lung cancer, integrating translational data from various histologies obtained in different patients under different conditions can increase their robustness. This is a meta-analysis of cDNA array data obtained in 688 tumor patients (541 non-small cell lung cancer, 33 small cell lung cancer and 114 others) and 205 controls. 1,206 genes were found to be dysregulated in one of the 12 transcriptomics studies available. 748 results (62%) were obtained only once and might be questioned. 38% of observations could be reproduced twice or more. 346 genes were reported twice, 80 three times, 27 four and 5 five times. A common set of genes dysregulated in lung cancer was obtained, including BPA1, DUSP6, ASCL1, RNAS1 and S100P. p63 and CK 5/6 p63 are useful for differentiating adenocarcinoma and small cell lung cancer from squamous cell carcinoma. TFF-3 and MUC1 are over-expressed in adenocarcinoma. INSM1, SGNE1 and H2AFZ are typical for small cell lung cancer. Using a meta-analysis approach, it was possible to detect a robust set of genes differentially expressed in lung cancer and to determine a limited number of key genes linked to subtypes in lung cancer molecular pathology.

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Introduction

In 2006, lung cancer was the most common cause of death from cancer in Europe, with an estimated 334,800 mortalities (19.7% of the total attributable to cancer) [1]. Since no screening method is available to date, most lung cancers are of considerable size when detected, and about 60% are incurable as a result of local invasion or distant metastasis at time of diagnosis. It is estimated that only 10% of patients survive 5 years after diagnosis.

Beside anti-smoking campaigns aimed at reducing lung cancer incidence, these unfavorable figures underscore the urgent need for early diagnostic tools and better drug therapy. Against this framework, the appearance about 10 years ago of high-throughput, large-scale gene expression research tools, in particular cDNA arrays, has generated high expectations in translational lung cancer research.

These techniques have been used with great success for molecular profiling in oncology. However, with growing availability of data, it has become apparent that results obtained are highly variable. Translational studies in human patients are usually characterized by small size, various sample preparation techniques and the use of varying technologies. Not only it has become difficult for the individual researcher to get an overview of the hundreds of publications and terabytes of data available, but these results are also in part contradictory.

Several studies have shown the applicability and reproducibility of the microscopic classification of lung cancer derived by the scheme originally adopted by the WHO committee [2]. These categories are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), which mainly includes squamous cell carcinoma and adenocarcinoma. However, part of the difficulty in sharply separating lung carcinomas into this classification stems from the fact that up to two thirds of them show a combination of patterns [3]. Moreover, the problem of profiling these subtypes is worsened by the limited availability of clinical samples, in particular for SCLC.

Thus, to transfer translational research data from the bench to the bedside, there is an obvious need to integrate data from various histologies obtained in different patients under different conditions, in order to increase the robustness of these data. Indeed, to detect universal biomarkers, integrated translational research analysis based on large patient groups is required [4]. As in clinical medicine, meta-analysis methodology can improve the level of evidence and meet significance.

The aim of the present meta-analysis was to detect a robust set of genes differentially expressed in lung cancer and, if possible, to find out a limited number of key genes linked to subtypes in lung cancer molecular pathology.

Materials and Methods

Principles of Analysis

According to the above observations, we assumed that, for confirming gene expression results obtained in independent studies on lung cancer, it would be useful to compare and contrast the results obtained in these individual studies. For example, if a single author claims that the over-expression of secretory granule neuroendocrine protein 1 is a specific feature of SCLC, then this finding has to be confirmed in another independent study on SCLC. If this over-expression is not confirmed, then the hypothesis is rejected. If, however, it is regularly confirmed in slightly different but related contexts, then it can be assumed that this over-expression is a robust observation specific to SCLC.

In this respect, we do not consider it useful to compute the individual level of over- or under-expression of a given gene in a particular setting. There are several reasons to justify this choice:

- cDNA arrays experiments are necessitating a pre-amplification of genetic material so that the technology is only semi-quantitative;
- there is no internal standard between different experiments;
- phenotypically different types of cancers are compared;
- genotypic backgrounds vary among individuals;
- microscopic diagnosis has not been confirmed by a reference pathologist;

- no sample preparation (e.g. enrichment of epithelial cells) was performed, so a heterogeneous proportion of cancer cells in the specimen is to be expected;
- different array generations with different sets of genes have been used;
- chips were purchased from different manufacturers.

Moreover, such attempts to determine the median or mean level of gene expression throughout different experiments is not necessary for detecting potential genes of interest.

We believe that the simplest and the most robust method of assessment of differential gene expression is to classify the results into 3 categories:

- statistically significant over-expression;
- statistically significant under-expression;
- no differential expression but expression observed.

The third category is useful for discriminating between missing and identical results.

On the basis of this simple classification, it is possible to rank the genes between different studies, to detect the common set of genes in a given pathology, and also to contrast results between different histology. Only raw data are used, and only descriptive statistics provided, so that possible bias induced by statistical data manipulation can be excluded. In a previous publication, we have shown that the results obtained using this straight strategy are very close to sophisticated statistical methods [5] and allow us to discriminate the genes of interest.

This conservative, non-quantitative approach is supported by recent methodological data reporting that a number of genes show a small fold-change in one cDNA platform and a large fold-change in another platform, even though the correlations between platforms are high. Thirty percent of gene expressions of the samples showed inconsistent patterns across different platforms. A non-overlapping gene can be truly differentially expressed with a stringent cut, and an overlapping gene can be non-differentially expressed with non-stringent cutoff. There is no criterion to determine a cutoff so that the percent of overlapping genes is optimized [6].

Selection of the Publications

Rather than applying sophisticated statistical methods or simulation models, we spent much more time in selecting appropriate studies. It was a priority to set up a relatively large collection of studies that would allow us to draw robust conclusions. Using the key words '(cDNA array or oligonucleotide) AND lung cancer', 2,363 studies were downloaded from PubMed, representing terabytes of gene expression data. We applied the following selection criteria:

- only studies in human patients;
- only analysis of tissue samples;
- no studies on cell lines;
- comparison between tumor and non-tumor tissue;
- no prognostic studies comparing different outcomes.

After applying the selection criteria above, 12 studies remained available for further study. Eight of these studies used cDNA arrays, and 4 used oligonucleotide arrays. These studies are summarized in table 1. Altogether, 688 tumor patients and 205 controls were analyzed. The following histologies were considered:

- 541 NSCLC (361 adenocarcinoma, 141 squamous cell carcinoma and 39 not further specified);
- 33 SCLC;

Table 1. Summary of the studies used in this meta-analysis

Author [ref]	Year	Sample origin	Methods	Tumor patients	Reference samples
Spira et al. [37]	2007	tissue	oligonucleotide microarray	60	unknown
Chen et al. [38]	2007	tissue	microarray + RT-PCR	125	60
Woenckhaus et al. [39]	2006	tissue	oligonucleotide microarray	27	8
Taniwaki et al. [40]	2006	tissue	cDNA microarray	15	15
Bhattacharjee et al. [41]	2001	tissue	oligonucleotide microarray	186	17
Garber et al. [42]	2001	tissue	cDNA microarray	67	5 (+ 1 fetal lung)
McDoniels-Silvers et al. [43]	2002	tissue	competitive cDNA library screening	14	14
Beer et al. [44]	2002	tissue	oligonucleotide microarray	86	10
Amatschek et al. [45]	2004	tissue	cDNA subtraction + cDNA microarray	22	22
Wikman et al. [46]	2004	tissue	cDNA microarray	37	4
Heighway et al. [47]	2002	tissue	cDNA microarray	39	39
Nakamura et al. [48]	2003	tissue	cDNA microarray	10	10

- 54 various histology (carcinoids, adenosquamous cancer, large cell lung cancer, neuroendocrine tumors);
- 60 lung cancers without further characterization.

For constructing the database, we considered cDNA array experiments as a semi quantitative and not quantitative analysis. For analysis, results were ranked according to the number of citations in the different studies. The possibility of adding over- or under-expression scores (see above) was rejected because of the risk of mutual exclusion in case of contradictory results between studies or between different histology.

Results

1,206 genes have been reported to be dysregulated (up- and/or down-regulated) in human lung cancer, representing about 5% of the 20,000–25,000 human genes according to the Human Genome Project information [7].

The vast majority of the 1,206 dysregulated genes in human lung cancer were found to be differentially expressed in only one of the 12 transcriptomics studies available. Of course, the validity of these 748 results obtained in only a single observation (62% of total data) might be questioned.

The other findings, representing 38% of observations, could be reproduced 2 or more times. This does not necessarily mean that the results have been obtained by 2 independent authors, since double observations might have been obtained by the same author in 2 different histological subtypes. 346 genes were reported twice, 80 three times, 27 four times and 5 five times. No gene was reported to be differentially expressed in 6 observations or more.

On the basis of this simple classification, it was possible to rank the genes between different studies, to detect the common set of genes in a given pathology, and also to contrast results between different histology.

Common Set of Genes in Lung Cancer

A core set of common genes regularly reported to be dysregulated in all human lung cancer histologies could be determined. These genes are summarized in table 2. Dystonin belongs to the plakin family of cytoskeleton linker proteins. These proteins are unique in their abilities to form bridges between different cytoskeleton elements through specialized modular domains, in particular actin and microtubule binding domains [8]. Moreover, patients suffering chronic lymphocytic leukemia develop an autoimmune vesiculobullous disease associated with the underlying malignancy, the so-called paraneoplastic pemphigus, and develop characteristic IgG auto antibodies against dystonin (reviewed in [9]). Dual specificity protein phosphatase 6 (DUSP6) is a strong tumor suppressor gene candidate in cancer and an inhibitor of phospho-ERK1/2. DUSP6 is up-regulated in a majority of dysplastic cells in pancreatic ducts but down-regulated in invasive carcinoma cells [10]. In human bronchial epithelium, mutant EGFRs were associated with increased expression of DUSP6 [11]. The basic helix-loop-helix protein, achaete-scute complex-like 1/achaete-scute homologue 1 (ASH1), is expressed in normal fetal pulmonary neuroendocrine cells and lung cancers with neuroendocrine elements and is suggested to be involved in lung carcinogenesis. Recent experimental evidence has suggested that *ASH1*

Table 2. Genes reported 4 or more times to be deregulated in lung cancer, classified by subtype

Accession number	SwissProt	Entry name	Gene name	NSCLC				ADCA				
				Chen et al. [38]	Wikman et al. [46]	Heighway et al. [47]	Nakamura et al. [48]	Taniwaki et al. [40]	Bhattacharjee et al. [41]	Woenckhaus et al. [39]	Garber et al. [42]	McDoniels-Silvers et al. [43]
M69225	Q03001	BPA1_HUMAN	Bullous pemphigoid antigen 1 (dystonin)									-1
BT006895	Q16828	DUS6_HUMAN	Dual specificity protein phosphatase 6	0						1		1
BC002341	P50553	ASCL1_HUMAN	Achaete-scute homolog-like 1			1		1				
BC005324	P07998	RNAS1_HUMAN	Ribonuclease pancreatic			-1				1	-1	
AA131149	P25815	S100P_HUMAN	S100 calcium-binding protein P			1		1	1	1		1
J05582	P15941	MUC1_HUMAN	Mucin 1, epithelial			-1		1	1			
AF111167	P01100	FOS_HUMAN	Proto-oncogene protein c-fos			-1					-1	
BC002532	P61916	NPC2_HUMAN	Epididymal secretory protein (19.5 kDa)							1	-1	
BC002829	P29034	S10A2_HUMAN	S100 calcium-binding protein A2			1						
BC002539	P08727	K1C19_HUMAN	Keratin 19		1	1	1					1
L24203	Q14134	TRI29_HUMAN	Tripartite motif-containing protein 29			1						
BC014152	P02538	K2C6A_HUMAN	Keratin 6A			1						1
K01144	P04233	HG2A_HUMAN	CD74 antigen			-1		1			-1	
BC015642	P01009	A1AT_HUMAN	Alpha-1-antitrypsin			-1				1		
AF111856	Q95436	NPT2B_HUMAN	Solute carrier family 34, member 2			-1				1		1
U49184	Q16625	OCLN_HUMAN	Occludin							1	-1	
BC093053	P05408	7B2_HUMAN	Secretory granule, neuroendocrine protein 1					1				
J02761	Q81WL2	SFTA1_HUMAN	Pulmonary surfactant-associated protein A1			-1		1			-1	
N93656	O60895	RAMP2_HUMAN	Receptor activity modifying protein 2					1			-1	
J03464	P08123	CO1A2_HUMAN	Collagen, type I, 2			1	1					1
BM472056	P0C0S5	H2AZ_HUMAN	H2A histone family, member Z					1				1
AF432265	Q07654	TFF3_HUMAN	Trefoil-factor 3 (intestinal)						1	1		1
NM000442	P16284	PECA1_HUMAN	Platelet endothelial cell adhesion molecule			-1	-1				-1	
U19816	P43699	NKX21_HUMAN	Thyroid transcription factor 1						1	1		
X58288	P28827	PTPRM_HUMAN	Protein tyrosine phosphatase, receptor type, M							1	-1	
J05013	P15328	FOLR1_HUMAN	Folate receptor 1 (adult)			-1		1	1			
AB009010	P62988	UBIQ_HUMAN	Ubiquitin			1					-1	-1
M57230	P40189	IL6RB_HUMAN	Interleukin 6 signal transducer					1		1		
L07916	P52566	GDIR2_HUMAN	Rho GDP dissociation inhibitor beta							1	1	
L39945	P00167	CYB5_HUMAN	Cytochrome b5 (CYB5) gene			-1		1	1			
NM001964	P18146	EGR1_HUMAN	Early growth response protein 1				-1				-1	
U78095	O43291	SPIT2_HUMAN	Hepatocyte growth factor activator inhibitor type 2				-1				1	

0 = No differential expression, but expression observed; 1 = statistically significant over-expression; -1 = statistically significant under-expression.

NSCLC = Non-small cell lung cancer; ADCA = adenocarcinoma; SQCA = squamous cell carcinoma; LCLC = large cell lung cancer; SCLC = small cell lung cancer; HGNEC = high-grade neuroendocrine carcinoma; LC = lung cancer.

gene is a specific therapeutic target for lung cancers with neuroendocrine features [12]. Pancreatic ribonuclease (RNase A) is down-regulated in lung cancer. Ranpirinase, a cytotoxic ribonuclease from the frog *Rana pipiens*, has been developed as a novel chemotherapeutic agent. In combination with doxorubicin, it is used in

clinical trials for the treatment of unresectable malignant mesothelioma and other cancers. The putative mechanism for ranpirinase-mediated cytotoxicity involves binding to anionic components of the extracellular membrane, cytosolic internalization and degradation of transfer RNA leading to apoptosis [13]. S100 cal-

Table 3. Most cited up-regulated (1), down-regulated (–1), or mentioned (0) genes in human squamous cell carcinoma, sorted by expression level

Accession number	SwissProt	Entry name	Gene name	Bhattacharjee et al. [41]	Woenckhaus et al. [39]	Garber et al. [42]	McDoniels-Silvers et al. [43]	Amatschek et al. [45]	Number	Total
M21389	P13647	K2C5_HUMAN	Keratin 5	1		1	1	3	3	
BT006794	O60218	AK1BA_HUMAN	Aldo-keto reductase family 1, member B10, B11		1		1	2	2	
L24203	Q14134	TRI29_HUMAN	Tripartite motif-containing protein 29	1		0	1	3	2	
X62571	Q04695	K1C17_HUMAN	Keratin 17	1		1		2	2	
BC002829	P29034	S10A2_HUMAN	S100 calcium-binding protein A2	1		0	1	3	2	
AB010153	Q9H3D4	P63_HUMAN	Tumor protein 63	1		0	1	3	2	
BC014152	P02538	K2C6A_HUMAN	Keratin 6A	1			1	2	2	
M76482	P32926	DSG3_HUMAN	Desmoglein 3	1			1	2	2	
M24350	P12272	PTHR_HUMAN	Parathyroid hormone-like hormone		1		1	2	2	
M69225	Q03001	BPA1_HUMAN	Bullous pemphigoid antigen 1 (dystonin)	1		0	–1	1	4	1
L07769	P47929	LEG7_HUMAN	Galectin-7	1		0		2	1	
L02870	Q02388	CO7A1_HUMAN	Collagen alpha-1(VII)	1		0		2	1	
BC002532	P61916	NPC2_HUMAN	Epididymal secretory protein (19.5 kDa)			–1	–1	2	–2	
BC005324	P07998	RNAS1_HUMAN	Ribonuclease, RNase A family, 1 (pancreatic)			–1	–1	2	–2	
L07916	P52566	GDIR2_HUMAN	Rho GDP dissociation inhibitor (GDI) beta			–1	–1	2	–2	
U49184	Q16625	OCLN_HUMAN	Occludin			–1	–1	2	–2	
X58288	P28827	PTPRM_HUMAN	Protein tyrosine phosphatase, receptor type, M			–1	–1	2	–2	
AF069668	P35754	GLRX1_HUMAN	Glutaredoxin		–1	–1		2	–2	

Number = Frequency of citation; total = expression level.

tological types allowed us to not only detect a common core set of genes (see above), but also to determine genes typically dysregulated in a single histological type, suggesting involvement in histology-specific biological processes.

Genes typically deregulated in squamous cell lung cancer are summarized in table 3. Tumor protein 63 (p63), cytokeratins 5 and 6 (CK 5/6), p63 and CK 5/6 seem to be useful for differentiating adenocarcinoma and SCLC from squamous cell carcinoma with 100% specificity and 82% sensitivity, and 89% specificity and 79% sensitivity, respectively [15]. The expression of tight junction proteins, including occludin, differs between human lung squamous cell carcinomas and adenocarcinomas: in squamous cell carcinomas, the mRNA level of occludin is down-regulated [16], or disappears in squamous cell carcinoma, large cell carcinoma, small cell carcinoma, and large cell neuroendocrine carcinoma [17]. Erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, has clinical activity in advanced lung cancer, but

mechanism of acquired resistance is unclear. We recommend that the biomedical significance in squamous cell lung cancer of other molecules such as dystonin or tripartite motif-containing protein 29 should be the object of further focused research.

Specific patterns of gene expression have also been reproduced in lung adenocarcinoma (table 4). Trefoil-factor 3, intestinal (TFF-3) is known to play key roles in mucosal protection through mucous-barrier formation, and also in mucosal repair through promotion of restitution after injury [18] and – in the present meta-analysis – has been found to be over-expressed in 3 studies on lung adenocarcinoma, but down-regulated in squamous cell cancer. TFFs are associated with mucin-secreting epithelial cells [19], and it is no surprise that mucin-1 (epithelial) has been found to be up-regulated in lung adenocarcinoma. The role of mucin in the biological underpinning [20] and in vaccination strategies [21] in lung adenocarcinoma has been extensively studied. Over-expression of fructose-bisphosphate aldolase A in lung can-

Table 4. Most cited up-regulated (1), down-regulated (–1) or mentioned (0) genes in human adenocarcinoma, sorted by expression level

Accession number	SwissProt	Entry name	Gene name	Taniwaki et al. [40]	Bhattacharjee et al. [41]	Woenckhaus et al. [39]	Garber et al. [42]	McDoniels-Silvers et al. [43]	Beer et al. [44]	Amatschek et al. [45]	Number	Total
AA131149	P25815	S100P_HUMAN	S100 calcium-binding protein P	1	1			1		3	3	3
AF432265	Q07654	TFF3_HUMAN	Trefoil-factor 3 (intestinal)	1	1				1	3	3	3
J05582	P15941	MUC1_HUMAN	Mucin-1, epithelial	1	1					2	2	2
M11767	P04626	ERBB2_HUMAN	Tyrosine kinase-type cell surface receptor HER2			1		1		2	2	2
BT006895	Q16828	DUS6_HUMAN	Dual specificity protein phosphatase 6			1			1	2	2	2
L39945	P00167	CYB5_HUMAN	Cytochrome b5 (CYB5) gene	1	1					2	2	2
J05013	P15328	FOLR1_HUMAN	Folate receptor 1 (adult)	1	1					2	2	2
L07916	P52566	GDIR2_HUMAN	Rho GDP dissociation inhibitor (GDI) beta			1	1			2	2	2
AF111856	O95436	NPT2B_HUMAN	Solute carrier family 34, member 2			1			1	2	2	2
U46692	P04080	CYTB_HUMAN	Cystatin B (stefin B)						1	1	2	2
X05236	P04075	ALDOA_HUMAN	Fructose-bisphosphate aldolase A					1	1	2	2	2
K03195	P11166	GTR1_HUMAN	Solute carrier family 2, member 1						1	1	2	2
M57230	P40189	IL6RB_HUMAN	Interleukin 6 signal transducer	1		1				2	2	2
U19816	P43699	NKX21_HUMAN	Thyroid transcription factor 1		1	1				2	2	2
U21108	Q13115	DUS4_HUMAN	Dual specificity phosphatase 4		1	1				2	2	2
AB034951	P11142	HSP7C_HUMAN	Heat shock cognate 71-kDa protein			1		1		2	2	2
AY762101	P05783	K1C18_HUMAN	Keratin 18			1		1		2	2	2
X17094	P09958	FURIN_HUMAN	Furin (paired basic amino acid cleaving enzyme)			1		1		2	2	2
D29992	P48307	TFPI2_HUMAN	Tissue factor pathway inhibitor 2			1			1	2	2	2
Z93930	P17861	XBP1_HUMAN	X-box binding protein 1		1	1				2	2	2
BM472056	P0C0S5	H2AZ_HUMAN	H2A histone family, member Z	1					1	2	2	2
M33764	P11926	DCOR_HUMAN	Ornithine decarboxylase 1		1				1	2	2	2
NM001005	P23396	RS3_HUMAN	40S ribosomal protein S3					0	1	2	1	1
K01144	P04233	HG2A_HUMAN	CD74 antigen	1		–1				2	0	0
BC002532	P61916	NPC2_HUMAN	Epididymal secretory protein (19.5 kDa)			1	–1			2	0	0
BC005324	P07998	RNAS1_HUMAN	Ribonuclease, RNase A family, 1 (pancreatic)			1	–1			2	0	0
U49184	Q16625	OCLN_HUMAN	Occludin			1	–1			2	0	0
X58288	P28827	PTPRM_HUMAN	Protein tyrosine phosphatase, receptor type, M			1	–1			2	0	0
J02761	Q8IWL2	SFTA1_HUMAN	Pulmonary surfactant-associated protein A1		1		–1			2	0	0
N93656	O60895	RAMP2_HUMAN	Receptor (calcitonin) activity modifying protein 2	1			–1			2	0	0
L06623	P24530	EDNRB_HUMAN	Endothelin receptor type B			–1	1			2	0	0
AB029025	Q9UPQ0	LIMC1_HUMAN	LIM and calponin homology domains-containing protein 1			–1	1			2	0	0
BT007090	P62328	TYB4_HUMAN	Thymosin beta-4					0	–1	2	–1	–1
AB009010	P62988	UBIQ_HUMAN	Ubiquitin					–1	–1	2	–2	–2

Number = Frequency of citation; total = expression level.

cer has been known for two decades [22], and the presence of the enzyme in the blood of bronchial cancer patients even longer [23]. Lung adenocarcinomas showed higher pulmonary surfactant-associated proteins A1/surfactant-apoproteins A2 ratios than the tumor-free tissues and squamous cell cancer [24]. Also here, the bio-

logical significance of other genes typical of pulmonary adenocarcinoma deserves more attention.

Some genes of particular interest show differential behavior in lung adenocarcinoma and in squamous cell lung cancer. For example, Rho GDP dissociation inhibitor beta is up-regulated in lung adenocarcinoma (2 stud-

Table 5. Genes differentially expressed in NSCLC and SCLC

Accession number	SwissProt	Entry name	Gene name	ADCA				SQCA		SCLC			
				Taniwaki et al. [40]	Bhattacharjee et al. [41]	Garber et al. [42]	McDoniels-Silvers et al. [43]	Garber et al. [42]	McDoniels-Silvers et al. [43]	Taniwaki et al. [40]	Bhattacharjee et al. [41]	Number	Total
M57230	P40189	IL6RB_HUMAN	Interleukin 6 signal transducer	1		1		-1		1		4	2
U19816	P43699	NKX21_HUMAN	Thyroid transcription factor 1		1	1		-1		1		4	2
AF363368	Q8NFZ8	CADM4_HUMAN	Immunoglobulin superfamily, member 4 (cell adhesion molecule 4)			1		-1		1		3	1
N93656	O60895	RAMP2_HUMAN	Receptor (calcitonin) activity modifying protein 2	1			-1		-1	1		4	0
X02308	P04818	TYSY_HUMAN	Thymidylate synthase				0		-1		1	3	0
Details of each study cited are shown in table 1.													

ies) and down-regulated in squamous cell cancer (2 studies). Rho family GTPases play critical roles in maintaining the apical junctions of epithelial cells through reorganization of the actin cytoskeleton, and thus in a number of processes related to metastasis, and Rho GDP dissociation, inhibitors are regulating Rho family proteins [25].

SCLC accounts for 20–25% of all bronchogenic carcinoma and is associated with the poorest 5-year survival of all histological types. SCLC differs from NSCLC in its etiologic, pathologic, biologic and clinical features, so differences in molecular pathology are expected. Ultra-structurally, SCLC displays a number of neuroendocrine features, including dense core vesicles or neurosecretory granules. In the present meta-analysis, a limited set of genes are typically over-expressed in SCLC, namely insulinoma-associated protein 1, H2A histone family member Z and secretory granule neuroendocrine protein 1. The insulinoma-associated 1 (INSM1) gene is expressed during early embryonal development and in neuroendocrine tumors. The INSM1 promoter region shows very high levels of expression in most of the SCLC cell lines and expression is absent in cell lines of non-neuroendocrine origin so that it is considered a potential novel tool for transcriptionally targeted gene therapy for neuroendocrine tumors [26]. Secretory granule neuroendocrine protein 1 is a sensitive biochemical marker of neuroendocrine bronchial tumors in the human patient [27]. The role of H2A histone family member Z in lung cancer has been so far not been further characterized and deserves further research.

Of particular interest are genes differentially expressed in NSCLC and in SCLC. These genes are summarized in table 5. Thymidylate synthase (TS) is important for maintenance of the intracellular thymidine pool, which is crucial for DNA synthesis and repair. By means of both quantification by real-time PCR and immunohistochemistry, a higher TS expression in pulmonary SCLC and large cell neuroendocrine cancers compared with typical and atypical carcinoids was observed [28]. Intratumoral TS expression has been shown to be prognostic of outcome in stage I NSCLC [29]. TS is the main target of antifolate drugs, in particular of pemetrexed, a novel agent for the treatment of NSCLC, which is also currently investigated as a second-line therapy for SCLC [30]. Pemetrexed is rapidly converted to active polyglutamate derivatives in cells to achieve prolonged and potent inhibition of TS. Cyclooxygenase-2, activators of transcription 3 and IL-6 are elevated in NSCLC, affect numerous cellular pathways and may act in concert by cyclooxygenase-2-dependent activation of signal transducer and activator of transcription 3 by IL-6 [31]. Thyroid transcription factor-1 (TTF-1) immunostains are useful for diagnosing poorly differentiated squamous cell carcinoma from SCLC [32]. Receptor activity-modifying protein 2 mediates in a dose-dependent manner the promotion of tumor angiogenesis by adrenomedullin, in particular endothelial cell migration and invasion [33]. Adhesion enables and promotes cancer-defining biological processes like growth, survival, migration, extravasation, homing and metastasis. Furthermore, cell adhesion mediates drug resistance in SCLC and NSCLC [34]. All 5 classes of

cell adhesion molecules – integrins, cadherins, immunoglobulin-like cell adhesion molecules, selectins and CD44s – are characteristically dysregulated in human cancer. Cell adhesion molecule 4 is up-regulated in lung adenocarcinoma and SCLC but not in squamous cell cancer.

Discussion

Translational research in oncology is characterized by highly parallel array studies modeling the behavior of thousands of genes simultaneously. Due to the difficulty of accessing high-quality clinical samples, these studies are usually performed in a limited number of patients and controls. In the studies selected for the present meta-analysis, the mean number of tumor samples was 57 cancer patients versus 17 controls. So far, the number of studies available is also limited, with a total of 12 publications allowing comparison according to pre-determined inclusion and exclusion criteria.

In translational research in lung cancer, most exploratory studies have investigated differential expression between 2 different biological conditions in an independent, *de novo* approach. Hundreds of thousands of individual data were generated and these data were then mined to discover potential biomarkers. Of course, the level of confidence of these results can only be limited, due to heterogeneous groups of patients, no routine sample enrichment such as laser-capture microdissection or bead-based techniques, technical requirements such as pre-amplification of genetic material, and statistical considerations (number of patients, number of trials, size sample and power). There was no confirmatory study aimed at reproducing results in a second group of patients.

In lung cancer, the first meta-analyses of gene expression are now emerging and 2 published studies might be the first steps in the right direction. However, both studies available were designed using a bioinformatics approach rather than from the biological point of view [4, 35]. In the study of Fishel et al. [35], to obtain a homogeneous dataset only adenocarcinomas were included, so that only 3 data collections involving 266 lung cancer samples and 32 controls could be merged. In the second available meta-analysis [4], 186 lung cancer samples and 17 controls were compared with other cancers (breast, prostate, etc.), but no differentiation between lung cancer histologies was possible.

Altogether, using stringent inclusion and exclusion criteria, translational research results from 12 studies ob-

tained in 688 tumor patients and 205 controls were merged in the present meta-analysis, resulting in the largest collection of gene expression data available to date in lung cancer. Moreover, this dataset allows contrasting results obtained in different lung cancer histologies.

The main result obtained is a core set of common genes that are dysregulated in lung cancer when compared with 'normal' tissue. These genes belong to various functional categories such as cytoskeleton, cell signaling, cell cycle, growth factors, receptors, cellular metabolism, transcription and translation. Of course, the biological significance of these results can be questioned, since many differences might not be cancer-specific. To enhance the interpretability of these findings, the meta-analysis should be extended to other organs and diseases to ensure their specificity. However, substantial publications (above) suggest that these similarity-driving genes and their products are significant in carcinogenesis, diagnosis, prognosis or even therapy, so that the proof of concept for the power of the meta-analytic approach is given. Many of the proteins/genes identified were already known and used in immunohistochemical stains, such as surfactant-related proteins, or S100-protein related factors. Other proteins (neuroendocrine secretory granule protein1, Achaete-scute homolog-like1) clearly relating to neuroendocrine differentiation have also been found in NSCLC, raising again the question of a correct tumor diagnosis. MUC genes have been previously investigated at the protein level, so the finding of MUC1 is not a surprise. Other molecules such as CK 5/6, p63, TTF1, surfactant apoproteins and precursors have been used quite a long time for differential diagnosis in pulmonary pathology.

The second important result is the determination of molecular differences between various cancer types, which was directly given by the negative picture of the determination of the similarities among gene lists. Differences were particularly evident between NSCLC and SCLC (such as insulinoma-associated 1 gene or secretory granule neuroendocrine protein 1; see above), between adenocarcinoma and squamous cell cancer (such as trefoil-factor 3 or mucin-1) and some hits concern molecules routinely applied in pathology laboratories for immunostaining of lung cancer, such as TTF-1 (above). In fact TTF-1 is not only useful to separate poorly differentiated squamous cell carcinoma from SCLC, but mainly squamous cell carcinoma from adenocarcinoma. However, the lists of potential biomarkers are rather long, and further focused research on possible clinical applications is already ongoing.

The third finding is to quantify the variability between results. Because of the probability of random differences and false positive results, it is unsurprising that the evidence resulting from this large amount of data is rather weak, with a high level of variability between studies (only 32% of results were reproduced in at least 2 studies out of 12). Of course, if this statement is true for all studies together, then it will certainly apply to a single study taken individually. This discrepancy of results can be explained by several methodological problems highlighted in this meta-analysis. The first drawback is related to missing controls and absence of tissue verification by an experienced pathologist. In several studies (see table 1, numbers 1, 4–6, 9, 11 and 12) no pathological control is mentioned, suggesting that diagnosis was not confirmed by a pathological reviewer or co-author. Moreover, the authors having performed tissue selection were neither trained nor familiar with pathological entities. Therefore, the tumor sample was arbitrarily selected from a fresh tissue sample or formalin-fixed paraffin-embedded tissue block handed over to the investigators. A further problem of cDNA array studies is that over-expression of a particular mRNA does not automatically mean that the corresponding protein is over-expressed too, or that the downstream signaling cascade is dysreg-

ulated. Post-translational modification might be even more important than mRNAs expression by itself: miRNA might inactivate any over-expressed mRNA, splicing might elicit even completely opposite functional activities. Protein phosphorylation at different sites might again result in a wide variety of functional outcome [36].

This shows how crucial it is to summarize all the evidence obtained and to combine it, in order to confirm or to invalidate experimental results. We believe that meta-analytic approaches will allow increases in the level of evidence available, which is preferable to increasing the number of patients in a single study, which is barely achievable due to cost and time constraints. Possibly, the novel approach of comparing and contrasting results obtained in different, independent studies addressing the same research question under slightly different but related contexts will revolutionize biomarker discovery and validation, because this *in silico* research will result in the identification of common molecular features shared among different types of cancer histology – or allow molecular classification of these histological types. In a similar way, meta-analytic approaches will allow screening for translational patterns characterizing different clinical phenotypes, such as metastasis or therapy response.

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