An exploration of pathways involved in lung carcinoid progression using gene expression profiling

Carcinogenesis vol.00 no.00 p.1 of 12, 2013
doi:10.1093/carcin/bgt271
Advance Access publication August 8, 2013

An exploration of pathways involved in lung carcinoid progression using gene expression profiling

Dorian R.A.Swarts*,†, Leander Van Neste[1,†], Mieke E.R.Henfling, Ivo Eijkenboom, Paul P.Eijk[2], Marie-Louise van Velthuysen[3], Aryan Vink[4], Marco Volante[5], Bauke Ylstra[2], Wim Van Criekinge[6], Manon van Engeland[7], Frans C.S.Ramaekers and Ernst-Jan M.Speel[7]

Department of Molecular Cell Biology, GROW – School for Oncology & Developmental Biology, Maastricht University Medical Center, PO Box 616, 6200 MD Maastricht, The Netherlands, [1]MDxHealth, Irvine, CA, USA, [2]Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands, [3]Division of Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands, [4]Department of Pathology, University Medical Center, Utrecht, The Netherlands, [5]Division of Pathology, Department of Oncology, University of Turin at San Luigi Hospital, Orbassano, Turin, Italy, [6]Laboratory of Bioinformatics and Computational Genomics, Ghent University, Ghent, Belgium and [7]Department of Pathology, GROW – School for Oncology & Developmental Biology, Maastricht University Medical Center, PO Box 616, 6200 MD Maastricht, The Netherlands

*To whom correspondence should be addressed. Tel: +31 43 3882998; Fax: +31 43 3884151;
Email: d.swarts@maastrichtuniversity.nl

Pulmonary carcinoids comprise a well-differentiated subset of neuroendocrine tumors usually associated with a favorable prognosis, but mechanisms underlying disease progression are poorly understood. In an explorative approach to identify pathways associated with progression, we compared gene expression profiles of tumors from five patients with a favorable and five with a poor disease outcome. Differentially expressed genes were validated using quantitative real-time PCR on 65 carcinoid tumors, in combination with survival analysis. One of the identified pathways was further examined using immunohistochemistry. As compared with other chromosomal locations, a significantly higher number of genes downregulated in carcinoids with a poor prognosis were located at chromosome 11q ($P = 0.00017$), a region known to be frequently lost in carcinoids. In addition, a number of upregulated genes were found involved in the mitotic spindle checkpoint, the chromosomal passenger complex (CPC), mitotic kinase CDC2 activity and the BRCA-Fanconi anemia pathway. At the individual gene level, *BIRC5* (survivin), *BUB1*, *CD44*, *IL20RA*, *KLK12* and *OTP* were independent predictors of patient outcome. For survivin, the number of positive nuclei was also related to poor prognosis within the group of carcinoids. Aurora B kinase and survivin, major components of the CPC, were particularly upregulated in high-grade carcinomas and may therefore comprise therapeutic targets for these tumors. To our knowledge, this is the first expression profiling study focusing specifically on pulmonary carcinoids and progression. We have identified novel pathways underlying malignant progression and validated several genes as being strong prognostic indicators, some of which could serve as putative therapeutic targets.

Introduction

Pulmonary carcinoids are well-differentiated neuroendocrine tumors with little relation to cigarette smoking (1). In contrast to other lung neuroendocrine neoplasms (NENs), i.e. the poorly differentiated large cell neuroendocrine carcinoma and small cell lung cancer (SCLC), carcinoids are characterized by a low metastatic rate and a relatively favorable prognosis. According to the World Health Organization, based on histopathologic features, lung carcinoids are subclassified as typical carcinoid or atypical carcinoid (AC) (2), and the latter being characterized by a more aggressive clinical behavior and a lower 5 year survival (3). Pulmonary carcinoids are considered as separate entities and transitions from typical to atypical subtypes have not been reported (1). Metastases will usually develop in regional lymph nodes, but also at distant sites including liver, bone, brain, subcutaneous tissue and breast (1,3). Clinical management of metastatic disease remains difficult and a curative treatment strategy for these cases is not available (1,4). Metastatic lung carcinoids are usually subjected to SCLC treatment regimens, but their response rate is considerably lower (4).

Pulmonary carcinoids have been reported in the context of the multiple endocrine neoplasia type 1 syndrome and a number of sporadic tumors have a mutation of the *MEN1* gene (5,6). We have previously described a tentative model for the tumorigenesis of pulmonary carcinoids (1). However, although clinical and molecular parameters with prognostic value have been described previously (1,7–10), the processes underlying malignant progression of lung carcinoids, defined as extensive spread of the disease and/or distant metastasis, are poorly understood (1). We have previously found that deletion of chromosome 11q22.3–q25 is associated with ACs and a poor disease outcome (9). Furthermore, aggressive carcinoids may show a high Bcl-2/Bax ratio, indicating that apoptosis may be hampered (11). Ki-67 may or may not be enhanced in cases with a poor disease outcome (12,13), whereas CD44 may be lost in aggressive cases (10,14). To identify molecular parameters that distinguish tumors with a favorable outcome from those with a poor clinical outcome, genome-wide gene expression profiling has proven helpful (15). However, only few of such studies that included lung carcinoids have been published (16–20), whereas none of these studies focused specifically on carcinoid tumors and progression (1).

In the underlying study, differentially expressed genes and associated pathways involved in carcinoid progression were identified using high-resolution gene expression microarrays on carcinoids from patients with a very poor disease outcome on the one hand and those of patients with a favorable outcome on the other. These pathways, as well as the prognostic value of a number of candidate genes, were validated by quantitative real-time PCR (qRT–PCR) as well as immunohistochemistry. The findings were also related to the results from high-resolution array comparative genomic hybridization (array CGH) assays.

Materials and methods

For more information, see Supplementary Materials and methods, available at *Carcinogenesis* Online.

Tumor material and case selection

In order to discover novel genes associated with pulmonary carcinoid progression, 10 pulmonary carcinoids were subjected to microarray experiments. Frozen tissue (≥70% tumor cells) was selected from five tumors of carcinoid patients with a favorable prognosis (all ≥7 year disease-free survival without metastasis or recurrence) and five from patients with a very poor disease outcome (all distant metastasis or deceased ≤3 years after diagnosis) (Table I).

Frozen tissue material (≥70% tumor cells) from 55 additional carcinoids and 16 high-grade neuroendocrine carcinomas was collected for qRT–PCR as described previously (10). In addition, nine neuroendocrine cell lines and four normal tissues (adrenal gland, liver, lung and pancreas) were analyzed (10). Formalin-fixed paraffin-embedded material of 65 pulmonary NENs (50 carcinoids and 15 high-grade carcinomas) was included for immunohistochemistry.

Abbreviations: ABk, Aurora B kinase; AC, atypical carcinoid; CGH, comparative genomic hybridization; CIN, chromosomal instability; CPC, chromosomal passenger complex; FA, Fanconi anemia; mRNA, messenger RNA; MSC, mitotic spindle checkpoint; NEN, neuroendocrine neoplasm; NSCLC, non-small cell lung cancer; qRT–PCR, quantitative real-time PCR; SCLC, small cell lung cancer.

†These authors contributed equally to this work.

(Article begins on next page)

Table II. *Continued*

Gene abbreviation[b]	Median (poor prognosis)	Median (good prognosis)	Median fold change	Chromosomal location[c]	Gene name
SLC1A2	15.33	264.78	0.058	11p13–p12	Solute carrier family 1
ELMO1	65.12	1095.59	0.059	7p14.1	Engulfment and cell motility 1
MT1F	652.32	10 455.69	0.062	16q13	Metallothionein 1F
PGC	7.31	106.22	0.069	6p21.1	Progastricsin
RELN	3.54	47.69	0.074	7q22	Reelin
MT1M	27.31	291.10	0.094	16q13	Metallothionein 1M
MRAS	273.50	2805.97	0.097	3q22.3	Muscle RAS oncogene homolog
MCTP2	36.37	359.79	0.101	15q26.2	Multiple C2 domains, transmembrane 2
C10orf114	66.79	657.29	0.102	10p12.31	Overlapping with MiRNA1915
CA4	12.71	123.79	0.103	17q23	Carbonic anhydrase IV
GHSR	48.72	451.47	0.108	3q26.31	Growth hormone secretagogue receptor
EFCAB10	3.51	31.72	0.111	7q22.3	EFCAB10: EF-hand calcium-binding domain 10
DNAJC22	204.18	1739.58	0.117	12q13.12	DnaJ (Hsp40) homolog, subfamily C, member 22 (wurst)
PLAG1	125.83	1056.82	0.119	8q12	Pleiomorphic adenoma gene 1
SCTR	7.22	59.12	0.122	2q14.1	Secretin receptor
TSC22D1	51.99	400.74	0.130	13q14	TSC22 domain family, member 1
MPPED2	12.60	94.69	0.133	11p13	Metallophosphoesterase domain containing 2
LRRFIP1	62.69	459.87	0.136	2q37.3	Leucine rich repeat (in FLII) interacting protein 1
MT1G	1486.93	10 773.20	0.138	16q13	Metallothionein 1G
STOX1	51.59	368.36	0.140	10q22.1	Storkhead box 1, DNA-binding domain
VSTM2L	144.95	991.69	0.146	20q11.23	V-set and transmembrane domain containing 2 like
LRRFIP1	112.75	628.40	0.179	2q37.3	Leucine rich repeat (in FLII) interacting protein 1
SERPINE2	87.04	470.24	0.185	2q33–q35	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
FRMPD1	17.37	93.80	0.185	9p13.2	FERM and PDZ domain containing 1
MT1X	157.63	844.65	0.187	16q13	Metallothionein 1X
MAN2A1	93.32	468.95	0.199	5q21–q22	Mannosidase, alpha, class 2A, member 1

[a]The genes displayed here were selected from the 307 differentially expressed genes displayed in Supplementary Table S1, available at *Carcinogenesis* Online. Selected genes are annotated, display a median fold change difference in expression levels ≥5 and have an expression level ≥200 for at least one of the samples. When multiple probes and/or amplicons showed a significantly different expression, only the probe displaying the largest median fold change difference is listed.
[b]Gene abbreviations.
[c]Source of chromosomal locations shown in this table: UCSC genome browser (UCSC Genome Bioinformatics, version GRCh37/hg19, Santa Cruz, CA).

Of the 48 genes downregulated with a median fold change ≥5 in the poor prognosis group, 6 were located on chromosome 11q and 4 were members of the gene family of metallothioneins, i.e. *MT1F*, *MT1G*, *MT1M* and *MT1X* (Table II). Also the tumor suppressor genes *ADAMTS18*, *RELN* and *SOD3* were strongly downregulated in this group.

Validation of gene expression profiling results by qRT–PCR

To examine whether the results of the microarray study are reproducible, we performed qRT–PCR on the same 10 cases for a subset of up- and downregulated genes identified in the microarray. The downregulated genes *CD44* (10), *FOLR1*, *IL20RA*, *MT1F*, *OTP* (10) and *PCK1*, as well as the upregulated *BIRC5*, *BUB1*, *FANCA*, *KLK12* and *RET* (10) genes were selected from those listed in Table II based on their fold change in gene expression and/or their possible role(s) in disease progression. The microarray results could be reproduced for all genes tested, i.e. the expression trends were confirmed in the majority of the samples (Supplementary Table S3, available at *Carcinogenesis* Online). In addition, the prognostic value of the expression of the 11 selected genes was tested using qRT–PCR on an additional group of 55 carcinoid tumors. The qRT–PCR data were correlated with clinical patient follow-up data, when available. Using the Kaplan–Meier method, all genes described above were significantly associated with prognosis as shown in Figure 1 and as described previously for *CD44*, *OTP* and *RET* (10). In multivariate analyses, comparing the individual genes with clinical parameters (age at diagnosis, diameter, histopathology, sex), *BIRC5* ($P = 0.0066$), *BUB1* ($P = 0.0023$), *CD44* ($P = 0.012$), *IL20RA* ($P = 0.036$), *KLK12* ($P = 0.030$) and *OTP* ($P = 0.032$) were independent predictors of patient outcome. These findings were also validated at the protein level by immunohistochemical staining of CD44 and OTP, which were shown to be powerful prognostic indicators for lung carcinoids (10).

Thus, the microarray results were confirmed by qRT–PCR both through the prognostic value of a selection of 11 differentially expressed genes and by the similar ranking of their expression levels for the 10 samples (Supplementary Table S3, available at *Carcinogenesis* Online).

Identification of signaling pathways underlying carcinoid progression

Key pathways involved in carcinoid progression were queried by linking the list of 307 differentially expressed genes (Supplementary Table S1, available at *Carcinogenesis* Online) to the KEGG pathway database (http://www.genome.jp/kegg) using the online DAVID annotation tool (http://david.abcc.ncifcrf.gov/). Cell cycle and DNA damage detection and repair pathways were significantly altered (data not

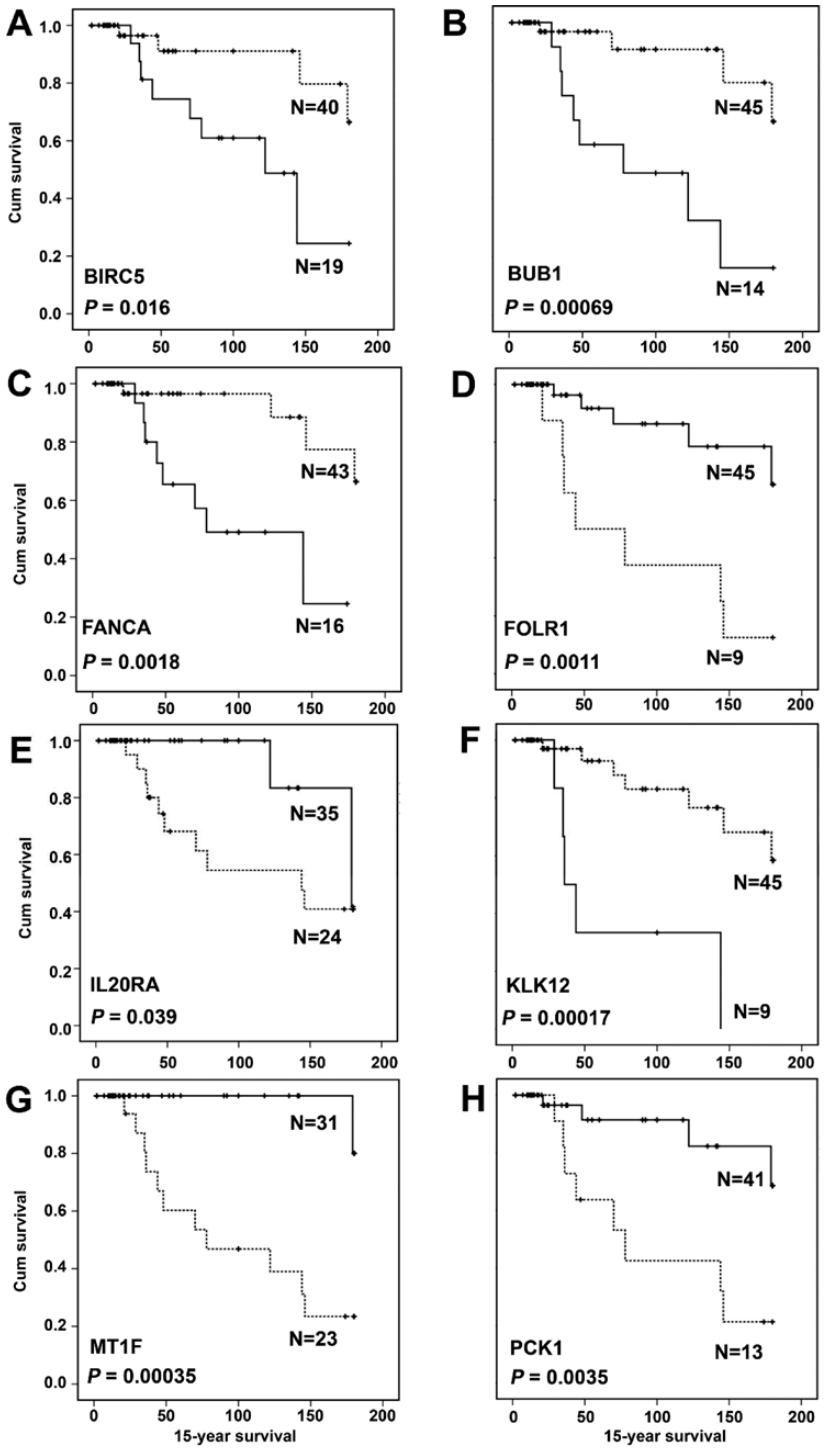

Fig. 1. Survival analyses of pulmonary carcinoids based on qRT–PCR of genes differentially expressed between tumors with a favorable and a poor prognosis. Kaplan–Meier survival analyses of pulmonary carcinoid tumors depicting the difference in 15 year overall survival for high (solid lines) or low (dotted lines) mRNA levels of a selection of differentially expressed genes relative to the geometric mean of *ACTB* and *CYPA*, and the sample with the highest expression (see Supplementary Materials and methods, available at *Carcinogenesis* Online). Optimal cutoff values were determined based on area under the receiver operating characteristic curve analyses. These cutoff points were 0.0020 for *BIRC5* (**A**), 0.0078 for *BUB1* (**B**), 0.012 for *FANCA* (**C**), 0.00080 for *FOLR1* (**D**), 0.15 for *IL20RA* (**E**), 0.0070 for *KLK12* (**F**), 0.018 for *MT1F* (**G**) and 0.0011 for *PCK1* (**H**).

shown). These pathways included well-known cancer-related genes, such as *CDK1/2*, cyclins and *TP53*, which were upregulated in carcinoids with a poor prognosis (Supplementary Table S1, available at *Carcinogenesis* Online). Strikingly, except for *BUB1*, none of the 71 top candidates shown in Table II were annotated to these pathways. Therefore, we performed an extensive manual literature search in combination with the online BioGRID tool (http://thebiogrid.org/) for protein interactions. As a result, a network of four interconnected pathways related to mitotic control mechanisms could be inferred from upregulated genes (Figure 2). This network included genes involved in (i) the mitotic spindle checkpoint (MSC); (ii) the CPC; (iii) mitotic kinase CDC2 activity and (iv) the BRCA-Fanconi

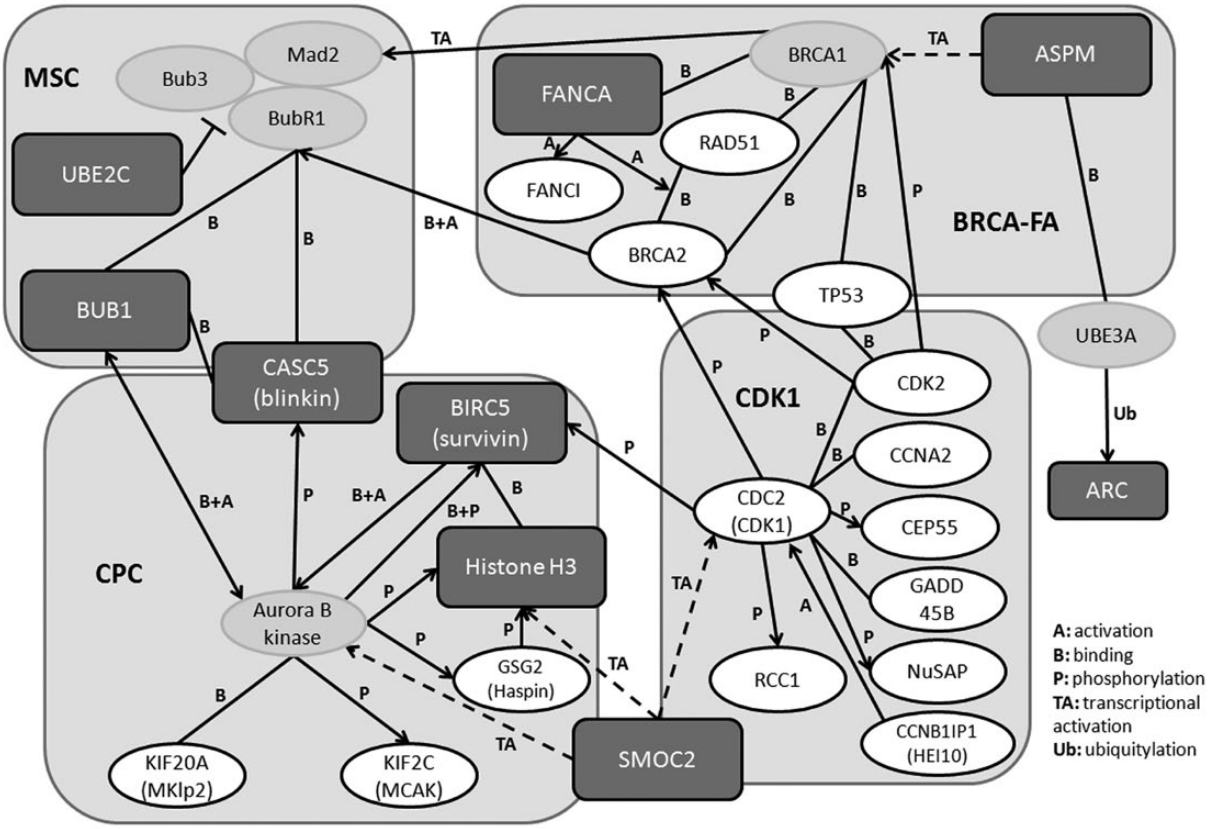

Fig. 2. Pathways related to lung carcinoid progression. Relationships between proteins encoded by upregulated genes in carcinoid tumors with an adverse disease outcome were compiled from data in the literature. Note that only relevant parts of the pathways are shown. The genes are taken from the lists in Table II (≥5-fold higher expressed in carcinoids with a poor prognosis; depicted as dark boxes) and Supplementary Table S1, available at *Carcinogenesis* Online (white circles). Genes in gray circles were not upregulated in our microarray but are related to multiple upregulated genes. B (for binding) indicates physical interactions between proteins. When these interactions lead to a phosphorylation event, this is indicated by P. TA indicates activation at the transcriptional (mRNA) level and A indicates posttranscriptional activation, whereas a bar-headed line indicates repression. UB indicates that the factor referred to is targeted to proteasomal degradation by the other factor, e.g. by ubiquitylation. The identified interactions between the depicted factors are summarized in the Supplementary Figure S2, available at *Carcinogenesis* Online.

anemia (FA) pathway. The identified interactions between the individually upregulated factors involved in these pathways are depicted in Figure 2 and justified in Supplementary Figure S2, available at *Carcinogenesis* Online.

Some of the genes from these pathways, i.e. *BIRC5*, *BUB1* and *FANCA*, were validated by qRT–PCR as described above and found to be upregulated in poor prognostic cases (see Figure 1).

Upregulation of mitotic genes in relation to chromosomal instability

Alterations of the MSC and CPC proteins have been reported to provoke chromosomal instability (CIN) (24,25). To identify chromosomal alterations in the 10 cases subjected to gene expression profiling, we performed high-resolution array CGH. The five carcinoid cases with a good prognosis generally exhibited low numbers of chromosomal alterations (≥10 Mb; mean 3, range 0–11; Supplementary Figure S1A, available at *Carcinogenesis* Online), whereas the five poor prognosis cases displayed a higher variability in the numbers of alterations (mean 12, range 0–27) (Table IC). Unexpectedly, one of the aggressive tumors (case 2) did not show major chromosomal alterations (Supplementary Figure S1B, available at *Carcinogenesis* Online), whereas two other poor prognosis cases (cases 1 and 4) displayed CIN, defined as described previously (9) (Supplementary Figure S1C, available at *Carcinogenesis* Online). These two cases exhibited higher expression of a number of mitotic genes than the other samples (without CIN), most notably *ASPM* and *UBE2C* (Supplementary Table S1, available at *Carcinogenesis* Online). Together, these data suggest a possible relationship between the upregulation of mitotic genes and CIN.

Amplifications (high copy number gains) and homozygous deletions of chromosomal regions were rare in the carcinoid cases. Five different amplifications and two homozygous deletions (present in at least two cases) could be identified (Table IC), including 8p11.2 that was homozygously deleted in four cases.

Upregulation of CPC components in relation to prognosis

As described above and shown in Figure 2, several of the upregulated genes, including *BIRC5* (survivin), could be linked to the CPC, with its major enzymatic component being ABk. This component was in itself not differentially expressed by the five cases with a poor outcome.

To further examine the role of the CPC in carcinoid progression, the messenger RNA (mRNA) expression levels of *AURKB* and *BIRC5* were analyzed in 65 carcinoid tumors and found to be strongly correlated with each other ($P < 1e^{-36}$, Pearson's correlation; Figure 3A and B) and with prognosis (see Figure 1A for *BIRC5* and Figure 3C for *AURKB*). In general, low expression levels were evident for both genes in carcinoids, whereas considerably higher expression levels were only present in a few ACs (Figure 3A and B), as well as in the carcinoid cell lines H720 and H727 (Figure 3D and E).

Protein expression levels of ABk and survivin were assessed in 50 carcinoids by immunohistochemical staining (Figure 4A–C and Supplementary Table S4, available at *Carcinogenesis* Online) and again, a strong correlation between the expression of both proteins was seen ($P = 7.2e^{-15}$, Pearson's correlation). ABk as well as survivin could exhibit nuclear and/or cytoplasmic reactivity. Nuclear ABk expression was present in ≤5% of nuclei of

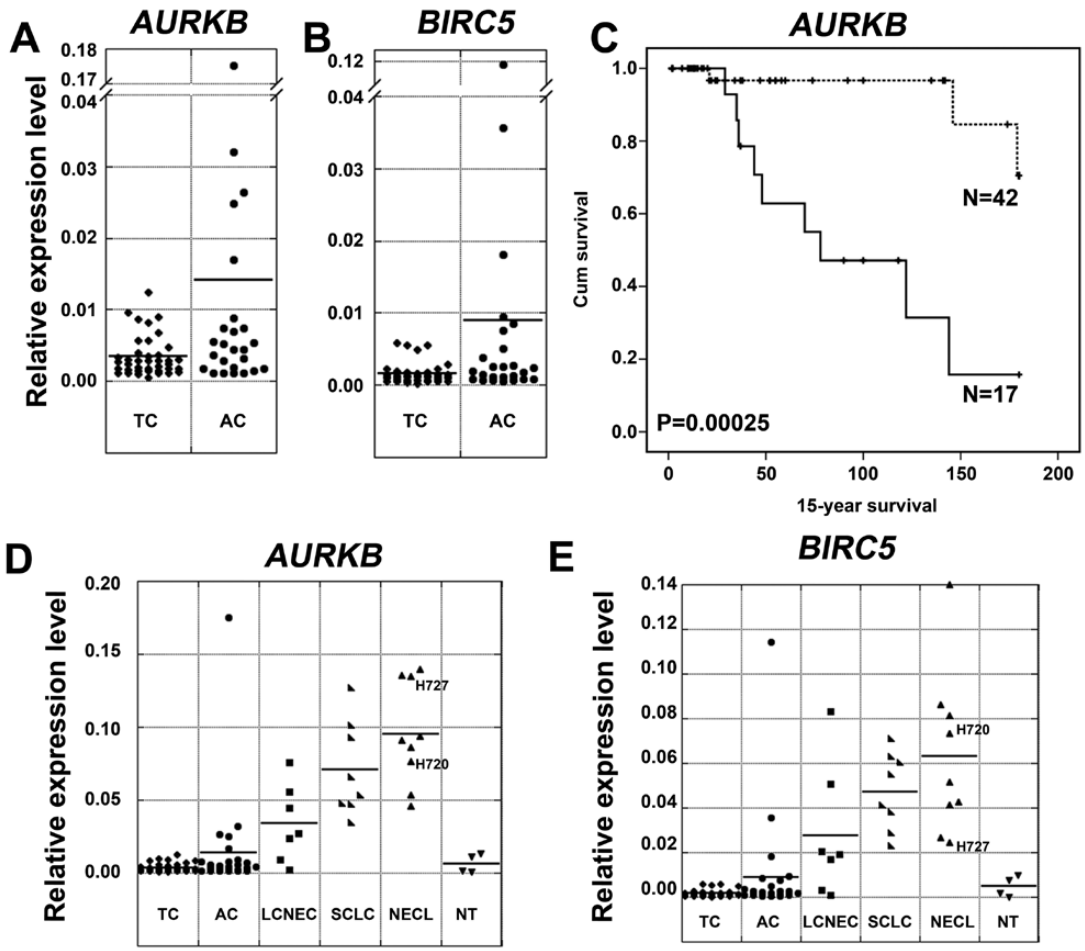

Fig. 3. Gene expression levels of *AURKB* and *BIRC5* in pulmonary NENs. (**A** and **B**) qRT–PCR results for *AURKB* and *BIRC5* in typical carcinoids (TC) and ACs. (**C**) Kaplan–Meier survival analysis plot depicting the differences in 15 year overall survival for high (solid line) or low (dotted line) *AURKB* gene expression levels within the group of pulmonary carcinoid tumors. Cutoff level based on area under the receiver operating characteristic curve: 0.0051. (**D** and **E**) qRT–PCR results for TCs, ACs, large cell neuroendocrine carcinomas, SCLC, neuroendocrine cell lines (NECL) and normal tissue (NT) for *AURKB* and *BIRC5*. mRNA expression levels are relative to the geometric mean of *ACTB* and *CYPA* and the sample with the highest expression (see Supplementary Materials and methods, available at *Carcinogenesis* Online). The mean values are indicated by the horizontal bars.

most carcinoid tumors (Figure 4A), with the exception of three cases (Figure 4B). Two of these three cases were included in the qRT–PCR and array CGH series and also exhibited higher mRNA levels, as well as CIN. The case with the highest mRNA expression, however, displayed strong cytoplasmic reactivity only (Figure 4C). Although the disease outcome for cases displaying ABk-positive cell frequencies >1% tended to be slightly worse (Figure 4D), the difference with cases without nuclear ABk reactivity was not significant.

The number of positive nuclei for survivin was on average higher as compared with ABk (3.6 and 1.7%, respectively), as could be expected from the microarray data. Only few cases showed expression in >10% of nuclei (Supplementary Table S4, available at *Carcinogenesis* Online and Figure 4B). A number of cases exhibited a prominent cytoplasmic staining reactivity for survivin (Supplementary Table S4, available at *Carcinogenesis* Online). Again, the case with the highest gene expression levels displayed strong cytoplasmic reactivity, but almost no nuclear expression (Figure 4D). Survivin protein expression in cases with >2.5% positive nuclei was also related to poor disease outcome (Figure 4D).

At the mRNA level, both *AURKB* (P = 0.00063, 95% confidence interval = 0.0279–0.0843) and *BIRC5* (P = 0.000049, 95% confidence interval = 0.0220–0.0505) were significantly higher expressed in neuroendocrine lung carcinomas as compared with the carcinoids (Figure 3D and E). Fifteen high-grade neuroendocrine carcinomas were analyzed for ABk and survivin protein expression using immunohistochemistry (Supplementary Table S4, available at *Carcinogenesis* Online). Although not all carcinomas showed staining for ABk, these tumors exhibited positivity in a much higher number of cases, and for the individual cases in a much higher frequency of nuclei (mean 25%, range 0–59%; Figure 4E). The same holds true for survivin (mean 40%, range 22–74%; Figure 4E).

Downregulation of 11q-located genes

Three out of five samples with a poor prognosis displayed deletion of chromosome 11q, reported previously as an indicator of adverse disease outcome in carcinoids (9). Significantly more downregulated genes are located on this chromosome arm as compared with other chromosome arms (P = 0.00017; Supplementary Table S5, available at *Carcinogenesis* Online). With the exception of *FOLR1*, the genes that were downregulated >5 times (Table II) displayed a tendency toward lower expression in the three cases combining a poor prognosis with a deletion of 11q, as compared with the two poor prognosis cases not containing this deletion (Supplementary Table S1, available at *Carcinogenesis* Online). This could indicate that the loss of one 11q chromosome arm is (partly) responsible for the further decrease in gene expression, although this needs to be confirmed on larger tumor series.

Supplementary material

Supplementary Materials and methods, Tables S1–S5 and Figures S1 and S2 can be found at http://carcin.oxfordjournals.org/

Funding

Jan Dekkerstichting & Ludgardine Bouwmanstichting (nr. 2010_010).

Acknowledgements

We thank Francois Rustenburg and Rinus Voorham, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands, for help with the gene expression arrays and the array CGH. Guido Roemen and Tina Pirens, Department of Pathology, Maastricht University Medical Center, Maastricht, The Netherlands, are acknowledged for performing *TP53* mutation analysis. Kim Smits, Department of Radiation Oncology (MAASTRO Clinic), Maastricht University Medical Center, Maastricht, The Netherlands, is acknowledged for assistance with statistical analyses. We furthermore thank Anne-Marie Dingemans, Department of Pulmonology, Maastricht University Medical Center, Maastricht, The Netherlands, for clinical patient follow-up data, and Robert-Jan van Suylen, Department of Pathology, Jeroen Bosch Hospital, 's-Hertogenbosch, and Erik Thunnissen, Department of Pathology, VU Medical Center, Amsterdam, The Netherlands, for tumor reclassifications.

Conflict of Interest Statement: W.V.C. is employed as CSO in MdxHealth. E.-J.M.S. has honoraria from Speakers Bureau of Pfizer and Lilly and is a consultant/advisory board member of Pfizer.

References

1. Swarts,D.R. *et al.* (2012) Molecular and cellular biology of neuroendocrine lung tumors: evidence for separate biological entities. *Biochim. Biophys. Acta*, **1826**, 255–271.
2. Beasley,M.B. *et al.* (2004) Carcinoid tumour. In Travis,W.D. *et al.* (eds) *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*. IARC Press, Lyon, pp. 59–62.
3. Travis,W.D. (2010) Advances in neuroendocrine lung tumors. *Ann. Oncol*., **21** (suppl. 7), vii65–vii71.
4. Detterbeck,F.C. (2010) Management of carcinoid tumors. *Ann. Thorac. Surg*., **89**, 998–1005.
5. Debelenko,L.V. *et al.* (1997) Identification of MEN1 gene mutations in sporadic carcinoid tumors of the lung. *Hum. Mol. Genet*., **6**, 2285–2290.
6. Srirajaskanthan,R. *et al.* (2009) Surgical management and palliative treatment in bronchial neuroendocrine tumours: a clinical study of 45 patients. *Lung Cancer*, **65**, 68–73.
7. Lim,E. *et al.* (2008) Proceedings of the IASLC International Workshop on Advances in Pulmonary Neuroendocrine Tumors 2007. *J. Thorac. Oncol*., **3**, 1194–1201.
8. Beasley,M.B. *et al.* (2000) Pulmonary atypical carcinoid: predictors of survival in 106 cases. *Hum. Pathol*., **31**, 1255–1265.
9. Swarts,D.R. *et al.* (2011) Deletions of 11q22.3-q25 are associated with atypical lung carcinoids and poor clinical outcome. *Am. J. Pathol*., **179**, 1129–1137.
10. Swarts,D.R. *et al.* (2013) CD44 and OTP are strong prognostic markers for pulmonary carcinoids. *Clin. Cancer Res*., **19**, 2197–2207.
11. Brambilla,E. *et al.* (1996) Apoptosis-related factors p53, Bcl2, and Bax in neuroendocrine lung tumors. *Am. J. Pathol*., **149**, 1941–1952.
12. Walts,A.E. *et al.* (2012) Limited role of Ki-67 proliferative index in predicting overall short-term survival in patients with typical and atypical pulmonary carcinoid tumors. *Mod. Pathol*., **25**, 1258–1264.
13. Zahel,T. *et al.* (2012) Phenotyping of pulmonary carcinoids and a Ki-67-based grading approach. *Virchows Arch*., **460**, 299–308.
14. Granberg,D. *et al.* (2000) Prognostic markers in patients with typical bronchial carcinoid tumors. *J. Clin. Endocrinol. Metab*., **85**, 3425–3430.
15. Van 't Veer,L.J. *et al.* (2002) Gene expression profiling predicts clinical outcome of breast cancer. *Nature*, **415**, 530–536.
16. Anbazhagan,R. *et al.* (1999) Classification of small cell lung cancer and pulmonary carcinoid by gene expression profiles. *Cancer Res*., **59**, 5119–5122.
17. Bhattacharjee,A. *et al.* (2001) Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc. Natl Acad. Sci. USA*, **98**, 13790–13795.
18. Jones,M.H. *et al.* (2004) Two prognostically significant subtypes of high-grade lung neuroendocrine tumours independent of small-cell and large-cell neuroendocrine carcinomas identified by gene expression profiles. *Lancet*, **363**, 775–781.
19. He,P. *et al.* (2004) Identification of carboxypeptidase E and gamma-glutamyl hydrolase as biomarkers for pulmonary neuroendocrine tumors by cDNA microarray. *Hum. Pathol*., **35**, 1196–1209.
20. Virtanen,C. *et al.* (2002) Integrated classification of lung tumors and cell lines by expression profiling. *Proc. Natl Acad. Sci. USA*, **99**, 12357–12362.
21. Van Neste,L. *et al.* (2012) The epigenetic promise for prostate cancer diagnosis. *Prostate*, **72**, 1248–1261.
22. den Bakker,M.A. *et al.* (2013) Neuroendocrine tumours--challenges in the diagnosis and classification of pulmonary neuroendocrine tumours. *J. Clin. Pathol*, in press.
23. Travis,W.D. *et al.* (1991) Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma. An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. *Am. J. Surg. Pathol*., **15**, 529–553.
24. Kops,G.J. *et al.* (2005) On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer*, **5**, 773–785.
25. Lens,S.M. *et al.* (2010) Shared and separate functions of polo-like kinases and aurora kinases in cancer. *Nat. Rev. Cancer*, **10**, 825–841.
26. Görtz,B. *et al.* (1999) Mutations and allelic deletions of the MEN1 gene are associated with a subset of sporadic endocrine pancreatic and neuroendocrine tumors and not restricted to foregut neoplasms. *Am. J. Pathol*., **154**, 429–436.
27. Swarts,D.R. *et al.* (2011) Reduced MEN1 gene expression in pulmonary carcinoids is associated with metastatic disease. *Neuroendocrinology*, **94**, 12.
28. Yuan,Y. *et al.* (2009) Expression of the folate receptor genes FOLR1 and FOLR3 differentiates ovarian carcinoma from breast carcinoma and malignant mesothelioma in serous effusions. *Hum. Pathol*., **40**, 1453–1460.
29. Iwakiri,S. *et al.* (2008) Expression status of folate receptor alpha is significantly correlated with prognosis in non-small-cell lung cancers. *Ann. Surg. Oncol*., **15**, 889–899.
30. Ventéo,S. *et al.* (2012) Regulation of the Na,K-ATPase gamma-subunit FXYD2 by Runx1 and Ret signaling in normal and injured non-peptidergic nociceptive sensory neurons. *PLoS One*, **7**, e29852.
31. Komori,T. *et al.* (2008) Expression of kin of irregular chiasm-like 3/mKirre in proprioceptive neurons of the dorsal root ganglia and its interaction with nephrin in muscle spindles. *J. Comp. Neurol*., **511**, 92–108.
32. Nishida,K. *et al.* (2011) Role of Neph2 in pontine nuclei formation in the developing hindbrain. *Mol. Cell. Neurosci*., **46**, 662–670.
33. Bunn,P.A. Jr *et al.* (1985) Small cell lung cancer, endocrine cells of the fetal bronchus, and other neuroendocrine cells express the Leu-7 antigenic determinant present on natural killer cells. *Blood*, **65**, 764–768.
34. Barrow,J. *et al.* (2011) Homozygous loss of ADAM3A revealed by genome-wide analysis of pediatric high-grade glioma and diffuse intrinsic pontine gliomas. *Neuro. Oncol*., **13**, 212–222.
35. Musacchio,A. *et al.* (2007) The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol*., **8**, 379–393.
36. Ruchaud,S. *et al.* (2007) Chromosomal passengers: conducting cell division. *Nat. Rev. Mol. Cell Biol*., **8**, 798–812.
37. Garner,E. *et al.* (2011) Ubiquitylation and the Fanconi anemia pathway. *FEBS Lett*., **585**, 2853–2860.
38. Venkitaraman,A.R. (2004) Tracing the network connecting BRCA and Fanconi anaemia proteins. *Nat. Rev. Cancer*, **4**, 266–276.
39. Malumbres,M. *et al.* (2009) Cell cycle, CDKs and cancer: a changing paradigm. *Nat. Rev. Cancer*, **9**, 153–166.
40. Takimoto,M. *et al.* (2002) Frequent expression of new cancer/testis gene D40/AF15q14 in lung cancers of smokers. *Br. J. Cancer*, **86**, 1757–1762.
41. Ricke,R.M. *et al.* (2011) Bub1 overexpression induces aneuploidy and tumor formation through Aurora B kinase hyperactivation. *J. Cell Biol*., **193**, 1049–1064.
42. Cahill,D.P. *et al.* (1998) Mutations of mitotic checkpoint genes in human cancers. *Nature*, **392**, 300–303.
43. van Ree,J.H. *et al.* (2010) Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. *J. Cell Biol*., **188**, 83–100.
44. Horvath,S. *et al.* (2006) Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. *Proc. Natl Acad. Sci. USA*, **103**, 17402–17407.
45. Rocnik,E.F. *et al.* (2006) The novel SPARC family member SMOC-2 potentiates angiogenic growth factor activity. *J. Biol. Chem*., **281**, 22855–22864.
46. Altieri,D.C. (2003) Validating survivin as a cancer therapeutic target. *Nat. Rev. Cancer*, **3**, 46–54.

47. Huang,L.N. *et al.* (2013) Expression of survivin and patients survival in non-small cell lung cancer: a meta-analysis of the published studies. *Mol. Biol. Rep*., **40**, 917–924.
48. Halasova,E. *et al.* (2013) Expression of Ki-67, Bcl-2, survivin and p53 proteins in patients with pulmonary carcinoma. *Adv. Exp. Med. Biol*., **756**, 15–21.
49. Belyanskaya,L.L. *et al.* (2005) Cisplatin activates Akt in small cell lung cancer cells and attenuates apoptosis by survivin upregulation. *Int. J. Cancer*, **117**, 755–763.
50. Smith,S.L. *et al.* (2005) Overexpression of aurora B kinase (AURKB) in primary non-small cell lung carcinoma is frequent, generally driven from one allele, and correlates with the level of genetic instability. *Br. J. Cancer*, **93**, 719–729.
51. Vischioni,B. *et al.* (2006) Frequent overexpression of aurora B kinase, a novel drug target, in non-small cell lung carcinoma patients. *Mol. Cancer Ther*., **5**, 2905–2913.
52. Guillon-Munos,A. *et al.* (2011) Kallikrein-related peptidase 12 hydrolyzes matricellular proteins of the CCN family and modifies interactions of CCN1 and CCN5 with growth factors. *J. Biol. Chem*., **286**, 25505–25518.
53. Lose,F. *et al.* (2013) Common variation in Kallikrein genes KLK5, KLK6, KLK12, and KLK13 and risk of prostate cancer and tumor aggressiveness. *Urol. Oncol.*, **31**, 635–643.
54. Zhao,E.H. *et al.* (2012) Clinical significance of human kallikrein 12 gene expression in gastric cancer. *World J. Gastroenterol*., **18**, 6597–6604.
55. Blumberg,H. *et al.* (2001) Interleukin 20: discovery, receptor identification, and role in epidermal function. *Cell*, **104**, 9–19.
56. Pedersen,M.O. *et al.* (2009) The role of metallothionein in oncogenesis and cancer prognosis. *Prog. Histochem. Cytochem*., **44**, 29–64.
57. Werynska,B. *et al.* (2013) Metallothionein 1F and 2A overexpression predicts poor outcome of non-small cell lung cancer patients. *Exp. Mol. Pathol*., **94**, 301–308.
58. Yan,D.W. *et al.* (2012) Downregulation of metallothionein 1F, a putative oncosuppressor, by loss of heterozygosity in colon cancer tissue. *Biochim. Biophys. Acta*, **1822**, 918–926.
59. Blouin,J.M. *et al.* (2010) Down-regulation of the phosphoenolpyruvate carboxykinase gene in human colon tumors and induction by omega-3 fatty acids. *Biochimie*, **92**, 1772–1777.
60. Gautschi,O. *et al.* (2008) Aurora kinases as anticancer drug targets. *Clin. Cancer Res*., **14**, 1639–1648.
61. Rohrbeck,A. *et al.* (2008) Gene expression profiling for molecular distinction and characterization of laser captured primary lung cancers. *J. Transl. Med*., **6**, 69.
62. Taniwaki,M. *et al.* (2006) Gene expression profiles of small-cell lung cancers: molecular signatures of lung cancer. *Int. J. Oncol*., **29**, 567–575.
63. Peifer,M. *et al.* (2012) Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nat. Genet*., **44**, 1104–1110.
64. Hook,K.E. *et al.* (2012) An integrated genomic approach to identify predictive biomarkers of response to the aurora kinase inhibitor PF-03814735. *Mol. Cancer Ther*., **11**, 710–719.
65. Sos,M.L. *et al.* (2012) A framework for identification of actionable cancer genome dependencies in small cell lung cancer. *Proc. Natl Acad. Sci. USA*, **109**, 17034–17039.
66. Mita,A.C. *et al.* (2008) Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clin. Cancer Res*., **14**, 5000–5005.

Received April 3, 2013; revised July 13, 2013; accepted August 1, 2013