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Research Article

Deadenylase Expression in Small Cell Lung Cancer Related To Clinical Characteristics and Survival

Kokkori1 I[*], Maragozidis P[2], Antonopoulos D[2,3], Kyritsis A[2,3], Balatsos NAA[2], Gourgoulianis KI[3].

[1]Department of Respiratory and Oncology, Theageneio Hospital, Thessaloniki, Greece

[2]Department of Biochemistry and Biotechnology, University of Thessaly, Greece

[3]Department of Respiratory Medicine, University of Thessaly, Greece

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2. Keywords

SCLC, Deadenylases, PARN, Survival, Biomarker 3

1. Abstract

1.1. Aim: Lung cancer is the second common malignancy and the most aggressive cancer worldwide with late diagnosis and poor prognosis. The search for biomarkers that promote early diagnosis and improve therapeutic strategies focuses to the understanding of the mechanisms underlying cancer development and progression. The deregulation of gene expression is one of the cancer hallmarks reflected to the stability of mRNAs. The latter is determined by the removal of their poly (A) tails, which is catalysed by deadenylases. Herein, we analysed the expression levels of deadenylases in Small Cell Lung Cancer (SCLC) Clinical Samples and correlated them with the Clinical Characteristics and the Survival of Patients.

1.2. Patients and Methods: Computational transcriptomic analysis was performed to examine the expression of deadenylases in SCLC. Bronchoscopy lung biopsies from 19 patients were collected and subsequently analysed with real-time Polymerase Chain Reaction (PCR) to determine the levels of deadenylases.

1.3. Results: PARN, CNOT6, CNOT7 and NOC deadenylases were expressed in both malignant and non-pathological lung sample tissues. They were differentially expressed in malignant tissue compared to matched non-pathological samples. Older patients had increased expression of PARN (68.5 $\pm$ 6.7 versus 59.7 $\pm$ 6.2 years). Increased PARN levels correlated to decreased overall survival of 180 days. Similar results were obtained for CNOT6 and CNOT7.

1.4. Conclusion: Deadenylases are relevant to the development of carcinogenesis in SCLC as their altered levels affect the gene expression. PARN represents a promising prognostic biomarker and therapeutic target in a generally unknown disease like SCLC.

3. Introduction

Worldwide, lung cancer is the most common cause of major cancer incidence and mortality, primarily because it is detected in an advanced stage for both sexes. Among lung cancers, SCLC is the most aggressive type and accounts for 13 to 15% of all lung cancer cases [1]. The pathogenesis of the disease is still complex and unclear. Thus, the search of biomarkers for diagnosis, prognosis and therapeutic purposes is of primary importance. In this direction, any strategy to improve prediction, treatment outcome, early recognition and biomarker search has to focus on the mechanisms of development and progression of the disease. As the deregulation of gene expression is a hallmark of cancer, these mechanisms may be hidden behind the well-established alterations in the expression of many oncogenes and tumor-suppressors. Such a mechanism is the degradation of mRNA, where several factors are involved in the process, including deadenylases and micro RNAs (mi RNAs). The stability of mRNA, which regulates gene expression is deregulated in malignancies, including lung cancer. Deadenylases are Mg (II)-dependent exoribonucleases that catalyse the shortening of

***Corresponding Author (s):** Ioanna Kokkori, Department of Respiratory and Oncology, Theageneio Hospital, Thessaloniki, Greece, E-mail: kouirouxis@hotmail.gr*

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Poly (A) tails of mRNAs, which is the first and rate-limiting step of mRNA decay [2,3]. The lifetime of mRNAs depends on changes in poly (A) tail length and consequently determines the levels of the produced cognate. Thus, deadenylases are key players in the expression of oncogene and tumor suppressor genes [4-6].

PARN, the best studied mammalian deadenylase, can act as a tumor suppressor because it is activated by the tumor–suppressor BARD1 [7] and it plays role in the degradation of IL-8, VEGF, c-Jun, UPA, c-FOS and TNF-alpha mRNAs, while it is involved in the maturation of regulatory RNAs, including SNO RNAs and telomeric RNA [8-11]. Studies about the deadenylase expression in various types of cancer are limited. Previous work has shown that their expression is altered in Acute Lymphoblastic Leukemias (ALL) and Acute Myeloid Leukemias (AML) with slightly up-regulated CNOT6 expression, down-regulated CNOT6L and CNOT7 and up-regulated PARN [12]. CNOT6L was present in human colon adenocarcinoma samples [13] and CNOT8 was up-regulated in primary colorectal carcinoma and metastatic regions compared to the normal mucosa [14]. In squamous cell carcinoma (a subtype of non-small cell lung cancer) was a shown differentially expressed deadenylase level in samples tissues [15]. In gastric cancer PARN expression has been studied in two cell lines of gastric cancer origin [16]. Despite the previous works, a study about deadenylases in SCLC is still pending. Based on the previous data, we investigate the relationship between the expression levels of deadenylases in both malignant and normal lung tissue from the same patient with SCLC. Further, we correlate this relationship with their clinical characteristics and survival. We hypothesize that if the expression levels of deadenylases are altered between malignant and normal lung tissues from the same patient, it provides us with preliminary information about their role and it may represent a useful prognostic biomarker in SCLC. It is worth noting though that the present study aims to investigate an 4 unknown field and thus offer a novel prognostic tool in addition to the current prognostic factors of the disease used by clinicians, as staging, Performance Status (PS) and age.

We intend to focus on the pathogenesis of SCLC and propably use a new biomarker like PARN on clinical practice hoping for new targeted therapies in the future.

4. Patients and Methods

4.1. Patients

Nineteen (19) patients of the study were over the age of 18 years old from both sexes. They had positive diagnosis of SCLC from bronchoscopy biopsy samples and they received no therapy until the time of diagnosis (Chemotherapy, Radiotherapy or Targeted Therapy). The individuals had no synchronous neoplasm except in situ neck cancer or skin cancer. They did not also suffer from other serious diseases including recent brain stroke, heart attack or congestive heart failure. After receiving the samples, the patients were treated with chemotherapy, radiotherapy, or both, and their response was accorded to RECIST criteria. Bronchoscopy samples of SCLC were collected from malignant and non-pathological tissues (matched samples) from the same patient (n = 19), as is summarized in (Table 1).

Samples were collected between 2010 and 2012 from University Hospital Larissa and Theageneio Cancer Hospital, under the approval of the Ethics Committees. The normal tissues were from the normal lung or from another lobe of the pathological lung of the same patient and the samples were reserved directly at -80^{o}C .

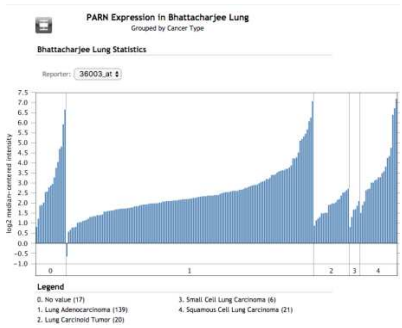
4.2. Methods

We performed the computational trascriptomic analysis in small cell lung cancer using microarray data from the Oncomine database [17, 18]. Subsequently total RNA was extracted from the clinical samples with the TRI Reagent Protocol (Sigma). RNA concentration was determined by spectrophotometry (BioPhotometer Plus, Eppendorf). Comparative quantification of deadenylase mRNAs was analysed by quantitative Real Time Polymerase Chain Reaction (Real-Time PCR). After RNA extraction, cDNA equivalent to 20 ng of total RNA was subjected to qPCR using the Stratagene Mx3005P Real-Time PCR System (Stratagene Agilent Technologies) following the manufacter's protocol (KAPA SYBR Fast Universal qPCR kit, KAPA Bio systems). PCR data and the relative expression results were analyzed by Mx3005P software (Stratagene) and mRNA levels samples were compaired to their paired ones and expressed as $\log_2$ fold-change (FC).

5. Results

5.1. Computational Transcriptomic Analysis of Deadenylase Expression in Small Cell Lung Cancer

We used computational transcriptomics in order to study the expression of deadenylases in SCLC in comparison with normal tissue serving as control. Microarray data from the Oncomine database17 showed that PARN expression was significantly reduced in 6 SCLC samples compared to 17 healthy controls (p=5.00) while the expression profiles of the deadenylases ANGEL1 (p=0.100), ANGEL2 (0.256), PAN2 (0.164) and CNOT8 (p=0.380) did not Differ significantly (Figure.1). The analysis [18] showed also that CNOT7 was significantly increased in 5 SCLC samples compared


PARN Expression in Bhattacharjee Lung
Grouped by Cancer Type
Bhattacharjee Lung Statistics
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Legend
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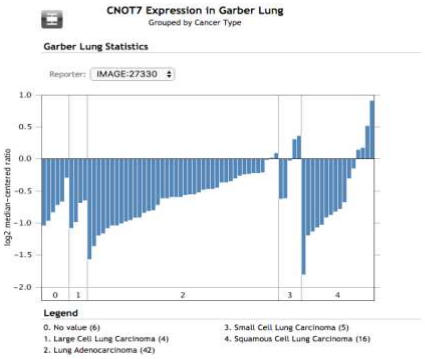
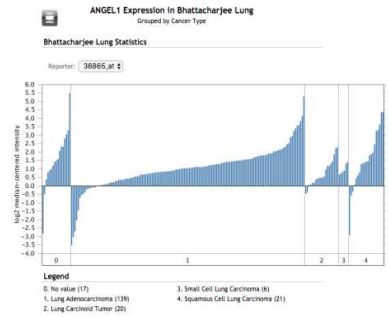
1. Lung Adenocarcinoma (139)
2. Lung Carcinoid Tumor (20)
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)


CNOT7 Expression in Garber Lung
Grouped by Cancer Type
Garber Lung Statistics
Reporter: IMAGE:27330
log2 median-centered ratio
Legend
0. No value (6)
1. Large Cell Lung Carcinoma (4)
2. Lung Adenocarcinoma (42)
3. Small Cell Lung Carcinoma (5)
4. Squamous Cell Lung Carcinoma (16)


ANGEL1 Expression in Bhattacharjee Lung
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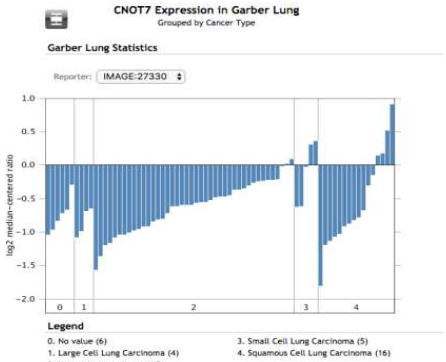
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)


CNOT7 Expression in Garber Lung
Grouped by Cancer Type
Garber Lung Statistics
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log2 median-centered ratio
Legend
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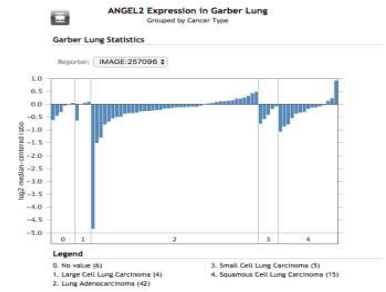
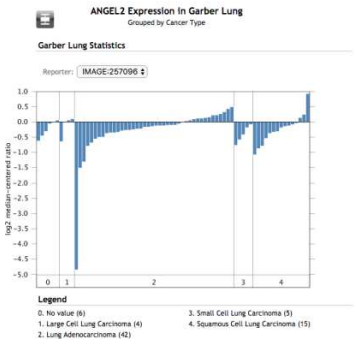
1. Large Cell Lung Carcinoma (4)
2. Lung Adenocarcinoma (42)
3. Small Cell Lung Carcinoma (5)
4. Squamous Cell Lung Carcinoma (16)


ANGEL2 Expression in Garber Lung
Grouped by Cancer Type
Garber Lung Statistics
Reporter: IMAGE:257096
log2 median-centered ratio
Legend
0. No value (6)
1. Large Cell Lung Carcinoma (4)
2. Lung Adenocarcinoma (42)
3. Small Cell Lung Carcinoma (5)
4. Squamous Cell Lung Carcinoma (15)


ANGEL2 Expression in Garber Lung
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Garber Lung Statistics
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log2 median-centered ratio
Legend
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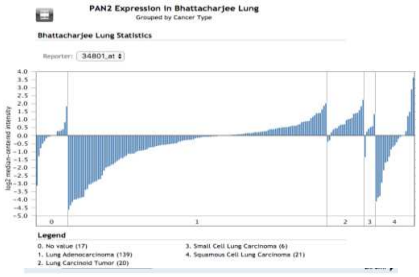
2. Lung Adenocarcinoma (42)
3. Small Cell Lung Carcinoma (5)
4. Squamous Cell Lung Carcinoma (15)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Bhattacharjee Lung Statistics
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log2 median-centered intensity
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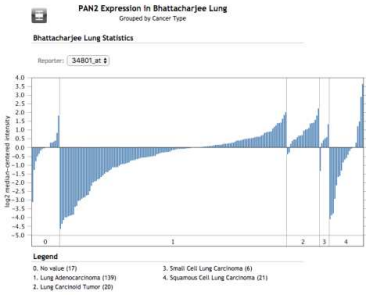
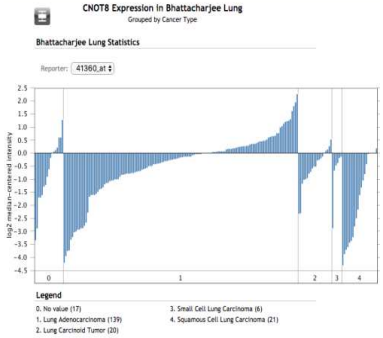
2. Lung Carcinoid Tumor (20)
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)


PAN2 Expression in Bhattacharjee Lung
Grouped by Cancer Type
Bhattacharjee Lung Statistics
Reporter: 34801_at
log2 median-centered intensity
Legend
0. No value (17)
1. Lung Adenocarcinoma (139)
2. Lung Carcinoid Tumor (20)
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)


CNOT8 Expression in Bhattacharjee Lung
Grouped by Cancer Type
Bhattacharjee Lung Statistics
Reporter: 41360_at
log2 median-centered intensity
Legend
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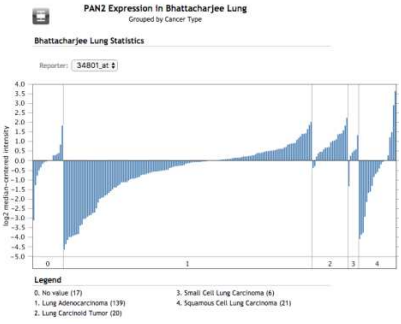
2. Lung Carcinoid Tumor (20)
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)


PAN2 Expression in Bhattacharjee Lung
Grouped by Cancer Type
Bhattacharjee Lung Statistics
Reporter: 34801_at
log2 median-centered intensity
Legend
0. No value (17)
1. Lung Adenocarcinoma (139)
2. Lung Carcinoid Tumor (20)
3. Small Cell Lung Carcinoma (6)
4. Squamous Cell Lung Carcinoma (21)

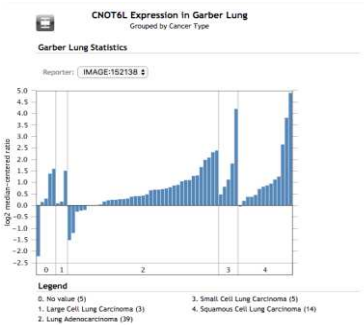


Figure1: Transcriptomics analysis of expression of deadenylases in Small Cell Lung Cancer (SCLC) from the Oncomine database. Differential mRNA expression of deadenylases in SCLC. PARN is significantly decreased in Bhattacharjee Lung study (p=5.00). CNOT7 is significantly increased in Garber Lung study (p=0.037). The expression of ANGEL1, ANGEL2, PAN2, CNOT8, CNOT6 and CNOT6L was not significantly altered in SCLC. In both cases the sample size very small to interpret with caution.

to 6 healthy controls (p=0.037) while CNOT6 (p=0.173), CNOT6L (p=0.096), ANGEL1 (p=0.126), ANGEL2 (p=0.156) and PAN2 (p=0.146) were not significantly different between SCLC and normal control samples (Figure 1).

5.2. Differential expression of deadenylases in SCLC

The differential mRNA expression of deadenylases in the computational transcriptomic analysis increased our interest in samples from patients with SCLC, so we examined their expression in malignant and non-pathological samples from the same patient.

We analyzed the mRNA levels of deadenylases by quantitative RT-PCR in SCLC specimens against their matched normal tissues. We collected clinical data of patients like age, sex, smoking history and staging of disease and correlated the expression levels of deadenylases with clinical data above and with their survival.

The expression of ANGEL1, ANGEL2, PARNL, CNOT8 and PAN2 deadenylases had not differences between the matched samples. PARN and CNOT7, belonging to DEDD nucleases, and CNOT6 and NOC, belonging to EEP nucleases, altered their expression profile significantly. As shown in (Figure 2) in 13 SCLC patients

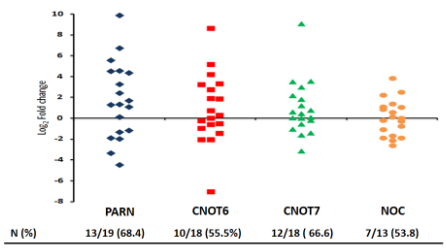


Figure 2: Comparative quantification of deadenylase expression in SCLC. PARN (rhombus), CNOT6 (square), CNOT7 (triangle) and NOC (circle) mRNA levels with real -time PCR in SCLC lung biopsies and expressed as log2 fold-change. Each point corresponds to the relative expression of the deadenylase in malignant tissue compared to the corresponding adjacent non-malignant tissue. The expression of the non-malignant tissue was set as 0. p<0.05.

(68.4%) PARN mRNA levels were elevated (log_2 Fold Change (FC)>0), while in 6 SCLC patients (31.6%) they were reduced (log2 FC<0). CNOT6 levels were elevated in 10 patients (55.5%) and they were reduced in 8 (44.5%). CNOT7 levels were elevated in 12 (66.65%) patients and in 6 (33.4%) were reduced. NOC was over expressed in 7 (53.8%) patients and under expressed in 6 (46.2%). 6

5.3. Deadenylase Expression Levels and Clinical Characteristics

The correlation between the deadenylase expression with the clinical characteristics and the survival of patients were further analyzed after having divided the patients in two groups according to the expression levels of deadenylase (increased or decreased) like in previous work in SCC15. We investigated characteristics like smoking history and age.

We found no correlation with the smoking history of the patients. Older patients had increased PARN levels than younger ones ±(68.56.7 vs. 59.7±6.2 years, p=0.014) as shown in (Figure 3).

5.4. Deadenylase expression levels and survival

The relationship between PARN, CNOT6 and CNOT7 expression with survival is described by means of Kaplan-Meier curves. Patients with increased PARN levels had lower survival of 180 days (234 vs. 414 days, p=0.016). Similar results were obtained for CNOT6 (224 vs. 377 days, p=0.035) and CNOT7 (231 vs. 419 days, p=0.021) as shown in (Figure 4).

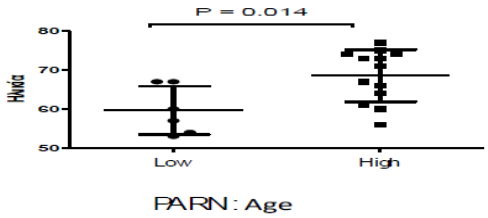


Figure 3: Association of age and expression levels of PARN in SCLC samples. Old patients overexpress PARN (68.5□6.7 vs 59.7□6.2 years), p=0.014.

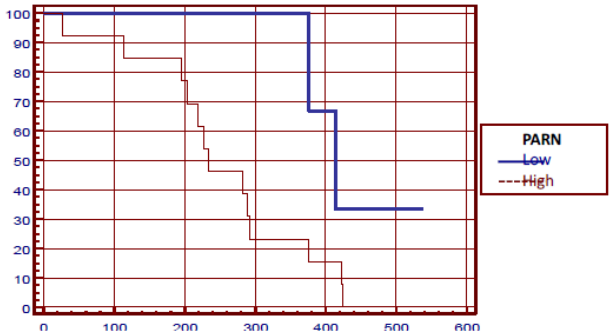


Figure 4: Kaplan-Meier survival curves show the correlation between expression levels of PARN and survival. Samples were divided in two groups, where deadenylase expression levels are either increased or reduced, between the malignant sample and the matched non- pathological one.
The red line shows the overall survival in patients overexpressing PARN which is lower by 6 months than in patients who underexpress PARN (blue line), p=0.016.

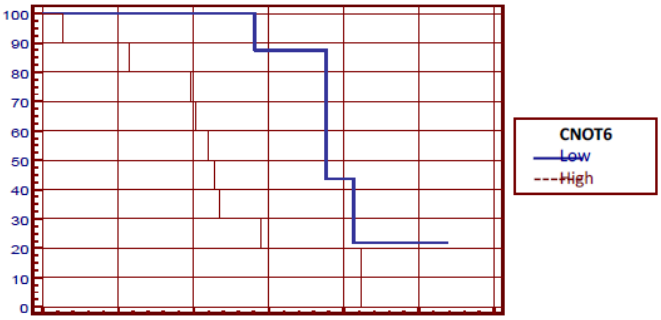


Figure 4: Association of expression levels of CNOT6 and survival. The red line shows the overall survival in patients overexpressing CNOT6 which is lower by 153 days than in patients who underexpress CNOT6 (blue line), p=0.035.

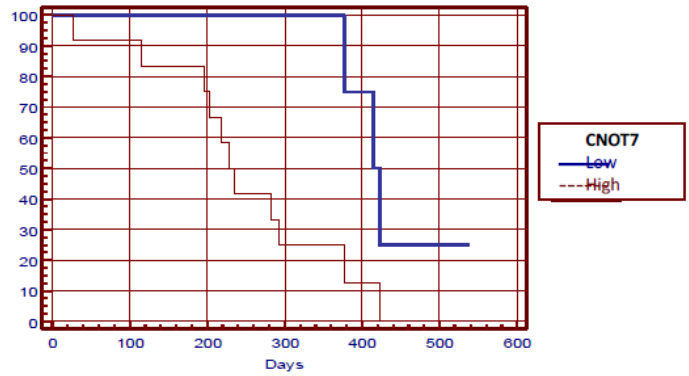


Figure 4: Association of expression levels of CNOT7 and survival. For patients who overexpress the enzyme (red line), overall survival is 188 days lower compared to patients that underexpress it (blue line), p=0.021.

The graphs above indicate the different correlation between the expression levels of deadenylases PARN, CNOT6 and CNOT7 and patient's survival. As we can see, high expression of PARN is correlated with lowest survival curve, which could play the role of a corestone biomarker in patients with SCLC. In addition to the expression levels of CNOT6 and CNOT7 were detected as high to the patients with lower survival. This result could indicate that the high expression of deadenylases may interfere as a possible cancer biomarker in future clinical diagnostics in SCLC.

6. Discussion

As the study on the detection of deadenylase levels in SCLC is still pending, the gene expression profile has to be compared with studies on other types of cancer. Our search in computational transcriptomic analysis showed that PARN expression was Significantly reduced18 while CNOT7 expression was significantly increased [18] but in both cases the sample size is very small, so interpret should be with caution. The results in our study suggest that PARN, CNOT6, CNOT7 and NOC levels are altered in SCLC compared to healthy samples. The expression of ANGEL1, ANGEL2, PARNL, CNOT8 and PAN2 was not significantly altered. PARN over expression was observed in 68.4% of patients, while 7 older patients had increased PARN levels than the younger ones (68.5±6.7 vs. 59.7±6.2 years). PARN over expression was observed in patients with decreased overall survival by 180 days (234 versus 414 days for PARN over expressed vs. under expressed, respectively; p=0.016). Similar results were obtained for CNOT6 and CNOT7.

Work from our team showed that the expression of several deadenylases is altered in SCC [15]. PARN, CNOT6, CNOT7 and NOC were differentially expressed in SCC clinical samples compared to healthy controls, while PAN2 and CNOT8 were barely detectable. PARN over expression was correlated with younger patients and CNOT6 over expression with non-metastatic tumors. The increased levels of PARN and NOC were observed in patients with increased overall survival. These two types of lung cancer are characterized by differences in molecular and clinical level although both appear to heavy smokers [19, 20].

The results described in this work on SCLC suggest that the expression of ANGEL1, ANGEL2, PARNL, CNOT8 and PAN2 deadenylases showed no differences between the malignant and normal tissue samples. PARN, CNOT6, CNOT7 and NOC mRNA expression didn't vary between normal and cancer lung tissues. Furthermore, no correlation between smoking history of patients and lung cancer was found, but it was observed that older patients had increased PARN levels compared to younger patients. Finally, it was observed how the expression levels of deadenylases PARN, CNOT6 and CNOT7 affected patient's survival curves. It came as a conclusion that high expression levels of PARN, CNOT6 and CNOT7, may lead to lower patients' survival, naming them as possible cancer biomarker and a clinical tool of possible low viability.

It is interesting that PARN, CNOT6, CNOT7 and NOC expression levels are altered in SCC and SCLC malignant samples compared to healthy controls and especially with over expression of PARN and NOC (Table 2). PARN over expression is associated with longer survival in SCC but with decreased in SCLC as seen in (Table 3).

Our knowledge on the role of deadenylases in SCLC is limited. There is growing interest to elucidate the underlying protein profiles in SCLC. The study of proteomics in SCLC showed that

Table 1. Patient characteristics

Individuals,n	19
Age (median)	65.73
Sex (male/female)	18 / 1
Pys (median)	68
Smokers/ex smokers	17 / 2
Staging (extensive/limited)	16 / 3
Survival (median)	9.8 months

Table 2. Comparison of the expression levels of deadenylases between SCLC and SCC.

	PARN High a (%)	CNOT6 High a (%)	CNOT7 High a (%)	NOC High a (%)
SCLC	68.4	55.5	66.6	53.8
SCC	52	48	48	56

Table 3. Comparison of the association between PARN and NOC expression levels with clinical outcome in SCLC and SCC

	PARNa	NOCa
SCLC	6 MONTHSb	0 MONTHSc
SCC	7 MONTHSd	7.9 MONTHSd

KHSRP, which belongs to RNA-binding proteins, is involved in degradation of inherently unstable mRNAs that contain AU-rich elements (AREs) in their 3-UTR, possibly by recruiting degradation machinery to ARE-containing mRNAs by recruiting PARN [21]. This protein is overproduced by chemo resistant SCLC cells GCLC1-M13 compared to GCLC1 cells. The first cell line is a sub-clone of GLC1 obtained through selection of cells cultivated in serum starved medium and limited dilution [22].

KHSRP is overproduced in SCLC compared to NSCLC, which has been studied in 3 SCLC cell lines (NCI-H69, NCI-H82 and NCI-H209) and 3 NSCLC cell lines (A549, NCI-H23 and NCI-H520) [23]. The role of KHSRP in SCLC is further investigated in the Oncoming database microarray. It is significantly over expressed in SCLC compared to normal lung tissue and other lung cancers in lung microarray data [18].

No significant interactions with any deadenylases were found in two studies of proteomics [24, 25]. The proteomic data in SCLC did not show increase of deadenylase production but increased production of RNA binding proteins, especially of KHSRP which directly interacts with PARN.

The role of PARN in cancer is currently under investigation. In SCC, PARN expression was increased in 88.8% of patients <65 years old. PARN was silenced in cells of squamous origin and the microarray analysis identified 233 up regulated mRNAs, probably direct targets of PARN. Lee and coworkers studied PARN in mouse my oblasts and reported genome-wide analyses following PARN knockdown. In particular, following PARN silencing factors responsible for cell migration and adhesion were stabilized [26, 27].

Recently, a feedback mechanism seemed to exist between phospholipase D (PLD2) and PARN which is deregulated in breast cancer cells and facilitates over expression of PLD2, protein highly relevant to cell migration and metastasis [28]. Taken together with previous observations in SCLC, SCC, acute leukemias, gastric cancer and breast cancer show that PARN could be used as a potential cancer biomarker.

Nocturnin (NOC) is the only deadenylase driven by the circadian clock and the metabolism of lipids and sugar [29]. The study of SCC revealed that NOC was also over expressed in 56% of patients and correlated with prolonged survival. In our study NOC was also over expressed in 7 patients (53.8%) but no correlation with survival was observed.

In this study we compared the deadenylase expression levels in samples of lung tissue from the same patient. Taken into consideration of previous observations on the role of deadenylases in cancer, our results show that deadenylases, and especially PARN may play an important role in SCLC carcinogenesis. PARN over expression was associated with worse survival (180 days) and it may be a prognostic marker of the disease. This might be of significant clinical importance in SCLC as PARN could be useful as a new biomarker in this aggressive disease and contribute to the better management of the disease. The idea and the need of new targeted therapies may be partially answered by PARN in daily clinical use.

7. Conclusion

We have shown that the expression of deadenylases is altered in SCLC. PARN, the best studied deadenylase, is associated with older patients and worse survival. PARN may be a promising prognostic marker for a particularly aggressive cancer, like SCLC.

References

1. Navada S, Lai P, Schwartz AG, Kalemkerian GP. Temporal trends in small cell lung cancer: analysis of the National Surveillance, Epidemiology, and End-Results database. J Clin Oncol. 2006; 24: 7082.

2. Goldstrohm AC, Wickes M. Multifunctional deadenylase complexes diversify mRNA control. Nat Rev Mol Cell Biol. 2008; 9: 337-344.

3. Zhang X, Virtanen A, Kleiman FE. To polyadenylate or to deadenylate. That is the question. Cell Cycle. 2010; 9: 4437-4449.

4. Kim WC, Lee CH. The role of mammalian ribonucleases (RNases) in cancer. Biochim Biophys Acta. 2009; 1796: 99-113.

5. Lau NC, Kolkman A, van Schaik FM, Mulder KW, Pijnappel WW, Heck AJ, et al. Human Ccr4-Not complexes contain variable deadenylase subunits. Bioche J. 2009; 422: 443-453.

6. Miyasaka T, Morita M, Ito K, Suzuki T, Fukuda H, Takeda S, et al. Interaction of antiproliferative protein Tob with the CCR4-NOT deadenylase complex. Cancer Sci. 2008; 99: 755-761.

7. Cevher MA, Zhang X, Fernandez S, Kim S, Baquero J, Nillson P, et al. Nuclear deadenylation / polyadenylation factors regulate 3 processing in response to DNA damage. EMBO J. 2010; 29; 1674-1687.

8. Chou CF, Mulky A, Maitra S, Lin WJ, Gherzi R, Kappes J, et al. Tethering KSRP, a decay-promoting AU-rich element-binding protein, to mRNAs elicits mRNAdecay. Mol Cell Biol. 2006; 26: 3695-3706.

9. Lai WS, Kennington EA, Blackshear PJ. Tristetraprolin and its family members can promote the cell-free deadenylation of AU-rich element-containing mRNAs by poly (A) ribonuclease. Mol Cell Biol. 2003; 23: 3798- 3812.

10. Moraes KC, Wilusz CJ, Wilusz J. CUG-BP binds to RNA substrates and recruits PARN deadenylase. RNA. 2006; 12: 1084-1091.

11. Tran H, Maurer F, Nagamine Y. Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen –activated protein kinase-activated protein kinase 2. Mol Cell Biol. 2003; 23: 7177-7188.

12. Maragozidis P, Kanangeli M, Papaspyrou C, Labrou M, Dimoulou G, Papaspyrou K, et al. Alterations of Deadenylase Expression in Acute Leukemias: Evidence for Poly(A)-Specific Ribonuclease as a Potential Biomarker. Acta Haematol. 2012; 28: 39-46.

13. Tay Y, Kats L, Weiss D, Tan SM, Ala U, Karreth F, et al. Coding independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell. 2011; 147: 344-357.

14. Seiden-Long M, Brown KR, Shin W, Wigle DA, Radulovich N, Jurisica I, et al. Transcriptional target of hepatocyte growth factor signaling and Ki-ras oncogene activation in colorectal cancer. Oncogene. 2006; 25: 91-102.

15. Maragozidis P, Papanastasi E, Scultenic D, Totomi A, Kokkori I, Zarogiannis SG, et al. Poly (A) specific ribonuclease and Nocturnin in squamous cell lung cancer: prognostic value and impact on gene expression. Mol Cancer. 2015; 14: 187.

16. Zhang L, Yan Y. Depletion of poly (A)-specific ribonuclease (PARN) inhibits proliferation of human gastric cancer cells by blocking cell cycle progression. Biochim Biophys Acta. 2015; 1853: 522-534.

17. Bhattacharjee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proc Natl Acad Sci USA. 2001; 98: 13790-13795.

18. Garber ME, Troyanskaya OG, Schluens K. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci USA. 2001; 98: 13784-13789.

19. Burstein HJ, Schwartz RS. Molecular Origins of Cancer. N Engl J Med. 2008; 358: 527.

20. De Stefani E, Boffetta P, Ronco AL, Brennan P, Correa P, Deneo-Pellegrini H, et al. Squamous and small cell carcinomas of the lung: similarities and differences concerning the role of tobacco smoking. Lung Cancer. 2005; 47: 1-8.

21. Gherzi R, Lee KY, Brista P, Wegmuller D, Moroni C, Karin M, et al. A KH domain RNA binding protein, KSRP, promotes ARE-directed mRNA turnover by recruiting the degradation machinery. Mol Cell. 2004; 14: 571-83.

22. Poschmann G, Lendzian A, Uszkoreit J, Eisenacher M, Borght AV, Ramaekers FC, et al. A combination of two electrophoretical approaches for detailed proteome-based characterization of SCLC subtypes. Archives of Physiology and Biochemistry. 2013; 119: 114-125.

23. Sebahat O, Friedman DB, Chen H, Ausborn JA. Discovery of New Membrane-Associated Proteins Overexpressed in Small-Cell Lung Cancer. Lung Cancer. 2014; 9: 324-336.

24. Eriksson H, Lengqvist J, Hedlund J, Uhlen K, Orre LM. Quantitative membrane proteomics applying narrow range peptide isoelectric focusing for studies of small cell lung cancer resistance mechanisms. Proteomics. 2008; 8: 3008-3018.

25. Byers LA, Wang J, Nilsson MB, Fujimoto J, Saintigny P. Proteomic Profiling Identifies Pathways in Small Cell Cancer and Novel Therapeutic Targers Including PARP1. Cancer Discov. 2012; 2: 798-811.

26. Lee JE, Lee JY, Trembly J, Wilusz J, Tian B, Wilusz CJ. The PARN deadenylase targets a discrete set of mRNAs for decay and regulates cell motility in mouse myoblasts. PLoS Genet. 2012; 8: e1002901.

27. Lee JE, Lee JY, Wilusz J, Tian B, Wilusz CJ. Systematic analysis of cis-elements in unstable mrnas demonstrates that CUGBP1 is a key regulator of mRNA decay in muscle cells. PLoS One. 2010; 5: e11201.

28. Miller TE, Gomez-Cambronero J. A feedback mechanism between PLD and deadenylase PARN for the shortening of eucaryotic poly(A) mRNA tails that is deregulated in cancer cells. The company of biologists Ltd., 2017; 6: 176-186.

29. Green CB, Douris N, Kojima S, Strayer CA, Fogerty J, Lourim D, et al. Loss of Nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity. Proc Natl Acad Sci USA., 2007; 104: 9888-9893.