Prognostic Value of Malic Enzyme and ATP-Citrate Lyase in Non-Small Cell Lung Cancer of the Young and the Elderly

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Abstract

Background
Lung cancer is the leading cause of death among malignancies worldwide. Understanding its biology is therefore of pivotal importance to improve patient’s prognosis. In contrast to non-neoplastic tissues, cancer cells utilize glucose mainly for production of basic cellular modules (i.e. nucleotides, aminoacids, fatty acids). In cancer, Malic enzyme (ME) and ATP-citrate lyase (ACLY) are key enzymes linking aerobic glycolysis and fatty acid synthesis and may therefore be of biological and prognostic significance in non-small cell lung cancer (NSCLC).

Material and Methods
ME and ACLY expression was analyzed in 258 NSCLC in correlation with clinico-pathological parameters including patient’s survival.

Results
Though, overall expression of both enzymes correlated positively, ACLY was associated with local tumor stage, whereas ME correlated with occurrence of mediastinal lymph node metastases. Young patients overexpressing ACLY and/or ME had a significantly longer overall survival. This proved to be an independent prognostic factor. This contrasts older NSCLC patients, in whom overexpression of ACLY and/or ME appears to predict the opposite.
Conclusion

In NSCLC, ME and ACLY show different enzyme expressions relating to local and mediastinal spread. Most important, we detected an inverse prognostic impact of ACLY and/or ME overexpression in young and elderly patients. It can therefore be expected, that treatment of NSCLC especially, if targeting metabolic pathways, requires different strategies in different age groups.

Introduction

Lung cancer is the leading cause of malignancy related death worldwide [1]. Despite tremendous advances in medical therapies, prognosis of lung cancer patients remains poor with a 5-year survival rate ranging between 7.9% and 16.5% [1, 2].

By showing that patients benefit from different chemotherapy regimens dependent on histological subtype, Scagliotti abolished the dogma to treat non-small cell lung cancer (NSCLC) as an oncologically homogenous group [3]. Thus, the major NSCLC subgroups, adenocarcinoma (LAC), squamous cell carcinoma (SCC) and large cell carcinoma (LCC) do not only show different histological patterns, but present specific biological and molecular features, too. A better insight into these distinct characteristics will aid to direct personalized therapies. In this context, metabolic changes associated with malignant cellular transformation are of pivotal importance.

For decades, it is known that malignant tumors produce excessive lactate even in the presence of sufficient oxygen (Warburg effect) [4]. Yet, mechanisms behind this phenomenon are not fully understood. Malignant cells function with metabolic autonomy, and glucose, its metabolites as well as glutamine are not only energy sources. They also serve as basic building units via generation of key molecules needed for cellular and thus malignant tumor growth [5, 6].

As enzymes of glucose metabolism represent a common downstream endpoint for various tumor driver mutations, they could be promising targets for new chemotherapeutic agents, too [7]. Among these enzymes, ATP-citrate lyase (ACLY) and malic enzyme (ME) are two key players: ME serves as source of reductive equivalents in highly cataplerotic malignant cells. ACLY builds a physiological shunt between glucose metabolism and fatty acid synthesis [6].

We therefore analyzed the expression patterns of these two enzymes to elucidate their association with clinico-pathological features and their biological impact on patient’s survival in NSCLC. Our results clearly show that functional metabolic changes in NSCLC are complex, differ in histological subtypes and predict different outcomes depending on patient’s age.

Materials and Methods

Ethics statement

The study has been approved by the University Medical Center Freiburg (Ethics committee University Medical Center Freiburg, EK 10/12). Patient related data has been pseudonymized and results obtained by this study did not influence patient’s treatment. Archived material had been used at least three years after initial diagnosis. By signing the treatment contract with the University Medical Center Freiburg, each patient agrees that his/her pseudonymized tissue(s) may be suspect to retrospective research trials not interfering with or influencing current
treatment options. The ethics committee of the University Medical Center Freiburg thus approved that no individual study specific consent of each patient had to be obtained.

Cohort

258 patients suffering from NSCLC were included in this study. Patients underwent surgical treatment between 1990 and 2007 (Department of Thoracic Surgery, University Medical Center Freiburg; S1 Dataset) and had not received neoadjuvant therapy. Fixation, grossing and paraffin embedding were performed according to routine protocols. All cancer cases were reclassified according to the current WHO classification [8], staging was reassessed in concordance with the latest UICC classification [9]. Tissue multi arrays (TMA) were constructed with a core diameter of 2 mm. From all specimens three TMA-cores were taken from different sites to avoid bias from intratumoral heterogeneity. A TMA of 36 corresponding non-neoplastic lung tissues served as control set. (S1 Table: summary of clinic-pathological data).

Immunohistochemistry and scoring

Heat-induced antigen retrieval was performed at pH 9.0 for ACLY and at pH 6.0 for ME. Primary antibody incubation time was 30 minutes (ACLY: 1:400, Cell Signaling Technologies 4331S; ME: 1:2000 dilution, Clone 3H5, Abnova Biozol). Visualization was performed by alkaline phosphatase with Fast Red-type chromogen (DAKO REAL K5005) and horseradish peroxidase with dianaminobenzidine based chromogen (DAKO FLEX EnVision) for ME and ACLY, respectively. Nuclear counterstaining was conducted with hematoxilin (Mayer’s acidic haemalaun, Waldeck, catalog no. 1A-552). The DAKO autostainer platform was used for staining procedures.

For both immunohistochemical stains, ACLY and ME, protocols were validated for specific staining by omission of the primary antibodies. These validation procedures did not show unspecific chromogen reactions.

Enzyme expression was considered positive, if specific cytoplasmic staining was detected. For ACLY, specific nuclear positivity was also assessed. Immunohistochemical scoring followed previously described protocols [10, 11] and was evaluated in analogy to internationally accepted scoring of predictive markers [12, 13]. Staining intensity was evaluated semi-quantitatively using a 4-tired scoring system (Fig 1). Percentage of positive tumor cells was determined by considering all positive tumor cells in relation to their absolute number. Percentage figures were rounded to the next decimal. Nuclear and cytoplasmic expressions of ACLY were evaluated separately.

Statistics

For all statistical analyses mean values of the three TMA-cores of each case were used. Differences in enzyme expression were evaluated by non-parametric tests.

Survival analysis included Kaplan-Meier curves and log-rank tests. For multivariate analyses, Cox-regression models were used. All statistical analyses were performed using the SPSS 21.0 software suite. Level of significance was set to 5% (i.e. \( p < 0.05 \)). The overall level of significance has been adjusted for multiple testing using the Benjamini-Hochberg method [14] (S2 Table).
Results

ACLY and ME are upregulated in NSCLC

ACLY expression in tumor tissue was detected in both, cytoplasm and nucleus (Fig 1), whereas ME was detectable only in the cytoplasm (Fig 1). Immunohistochemical enzyme expression was significantly higher in tumor cells (ACLY nucl.: 19.92 +/- 21.57; ACLY cytopl.: 22.23 +/- 21.57; ME: 52.49 +/- 37.86) than in corresponding non-neoplastic lung tissue (ACLY nucl.: 16.57 +/- 16.75; ACLY cytopl.: 11.43 +/- 20.97; ME: 9.53 +/- 10.50; \( p < 0.001 \))

ME but not ACLY is differentially expressed in histological NSCLC subtypes

As differentiation between LAC and SCC of the lung is of therapeutic importance, we analyzed expression patterns in relation to these two histological NSCLC subtypes. ME expression was higher in SCC compared to LAC (\( p < 0.001 \)). ACLY did not show a significant correlation with histological subtype. Furthermore, a significantly higher expression only of ME was found in smokers compared to non-smokers (\( p = 0.012 \)).

ACLY but not ME expression is associated with local tumor stage

Analyzing immunohistological patterns of ACLY and ME in correlation with local tumor stages, i.e. \( pT \), we observed that ACLY expression was significantly lower in advanced \( pT \)-stages. Though a significant positive overall correlation of ME and ACLY was found (nuclear ACLY—ME: correlation coefficient = 0.179, \( p < 0.001 \); cytoplasmic ACLY—ME: correlation coefficient = 0.155, \( p = 0.001 \)), ME expression did not show a correlation with local tumor stage.
stages. Analog results were observed for ACLY expression in correlation with metric tumor size (ACLY cytoplasmic: correlation coefficient -0.135, \( p = 0.003 \); ACLY nuclear: correlation coefficient: -0.144, \( p = 0.001 \); ME: correlation coefficient = 0.006, \( p = 0.887 \)). While infiltration of the visceral pleura also contributes to local tumor stage, no statistically significant correlation was observed regarding pleural invasion (ACLY cytoplasmic: \( p = 0.186 \); ACLY nuclear: \( p = 0.532 \); ME: \( p = 0.971 \)).

**ME but not ACLY expression is associated with mediastinal metastatic events**

To investigate the relationship of ME and ACLY expression with systemic tumor expansion, we separately analyzed NSCLC of nodal positive patients in correlation with location of lymph node metastases. The presence of mediastinal lymph node metastases was significantly correlated with higher expression of ME in primary tumor tissue (\( p = 0.041 \)) but not of ACLY (cytoplasmic: \( p = 0.511 \); nuclear: \( p = 0.446 \)). This association was particularly strong in LAC (ME: \( p = 0.030 \)).

**Overexpression of either ME, ACLY or both is an independent prognostic factor**

To avoid statistical bias, mean values of ME and ACLY expression were used for dichotomization. In the overall analysis no significant correlation of ME and ACLY overexpression with patient’s survival was found. Similar results were obtained in subgroup analyses according to smoking habits, sex, histological grading, pT- or pN-stages. Age stratification was performed by using the median age (65 years) at NSCLC diagnosis. No significant correlation between age and pT, pN or overall UICC stage was detected. In young patients, nuclear ACLY overexpression proved to be associated with a favorable overall survival (\( p = 0.029 \)), while this was not the case in patients older than 65 years (\( p = 0.626 \)). ME overexpression in these two age subgroups only showed a statistical trend in older patients (\( p = 0.093 \)). Young patients with overexpression of ME or nuclear ACLY or both in their tumors, had a significantly longer overall survival compared to those without overexpression of these enzymes (\( p = 0.007 \); Fig 2). Multivariate analysis, which included UICC stage, the only additional prognosticator in this patient group, proved this to be an independent prognostic factor (\( p = 0.002 \); Table 1). On the other hand, overexpression of either or both of the two enzymes resulted in shorter overall survival in older patients (Fig 2, \( p = 0.058 \)).

**Discussion**

Due to its high incidence and mortality, lung cancer still remains one of the major health burdens, worldwide. It is therefore of pivotal importance to better understand its biology in order to develop new suitable treatment options.

The metabolic switch of tumor cells to aerobic glycolysis is a well-known event. On the one hand, it serves to facilitate the uptake and incorporation of nutrients into basic cellular building blocks. On the other hand, it results in the production of lactate, which facilitates metastasis formation and therapy resistance [15, 16].

In several malignant tumors it has been shown that ACLY is not only elementary for de-novo fatty acid synthase [17], but also its rate limiting step [18–21]. As one of the key enzymes of de-novo fatty acid synthesis, ACLY generates cytosolic acetyl-coenzyme A (acyt-

CoA) [22, 23] and oxaloacetate. The latter is reduced to malate by malate dehydrogenase. The cytosolic isoform of malic enzyme converts malate into pyruvate [24]. Pyruvate that is not shuttled into...
the mitochondrion to generate oxaloacetate, is further converted into lactate [6]. ACLY and ME expression may therefore be altered in malignant tumor cells compared to non-neoplastic tissues. Comparing non-neoplastic lung and NSCLC tissue, we found a statistically significant increase of both, ACLY and ME expression within neoplastic cells. This is in concordance with other findings concerning altered carbohydrate metabolism in cancer [10, 11, 25–27]. In our cohort, ME and ACLY expression, nuclear as well as cytoplasmic, showed a positive correlation. The fact that correlation coefficients are rather small may reflect complex interrelations between several metabolic enzymes as well as high histomorphological heterogeneity of NSCLC. Comparison of immunohistochemical expression patterns of ME and ACLY further supports this statement as only ME-expression significantly differed between LAC and SCC. This kind of differential expression patterns in NSCLC subtypes has also been shown for other enzymes related to altered cellular cancer metabolism [10, 11, 28].

Since SCC is often found in heavy smokers and LAC are so called typical non-smoker carcinomas, significant higher expression of ME in tumors of patients with smoking history is not surprising. This can be due to different hypoxic states of tumors and/or patients leading to different metabolic states in NSCLC and most probably in SCC compared to LAC, too. The majority of smoking associated NSCLC possess p53 mutations and thus, the frequency of p53 mutations in SCC is higher compared to LAC [29]. Recently, Jiang could verify that ME expression is regulated by p53. According to their findings, p53 is responsible for down regulating ME expression [30]. These findings are in good concordance with ours, that ME is not only overexpressed in NSCLC compared to tumor free lung tissue but also, that ME expression is higher in SCC compared to LAC and in smokers compared to non-smokers.

Striking to us was, that ACLY and ME revealed different expression patterns dependent on local or mediastinal tumor spread. While ACLY was negatively correlated with local tumor extension measured by pT-stage, as well as metric tumor size, ME only showed a significant correlation with mediastinal metastatic events in comparison to hilar lymph node metastases (pN1 vs pN2/pN3). Changes in tumor metabolism, therefore, seem to be complex and may be different not only in histological subtypes but also according to local or systemic tumor spread.

Furthermore, in recent research results, additional functions of ACLY beside involvement in glucose metabolism have been detected: ACLY is also a key-player in histone acetylation. These findings suggest a link between growth factor changes in cancer metabolism and gene expression.
expression which is realized by ACLY [31]. In analogy to Wellen, Londono Gentile just recently published that ACLY at least in part regulates DNA methyltransferase-1 (DNMT1) [32]. Different localization of ACLY may therefore reflect different activities within the cell, i.e. cytoplasmic ACLY is predominantly involved in cancer cell metabolism, while nuclear ACLY is predominantly involved in regulation of gene expression [31, 33]. For this reason, we assessed different localizations of ACLY, i.e. nuclear and cytoplasmic, separately. In concordance with our results, Migita showed that ACLY was significantly higher expressed in LAC compared to non-neoplastic lung tissue [22]. In their publication, ACLY was also a prognostic factor, while they showed that high levels of ACLY were associated with a poorer outcome [22]. We could not reproduce this finding of Migita. But in contrast to their results, nuclear ACLY overexpression was of significant benefit in young patients of our cohort. Compared to Migita, we included not only LAC but also SCC as well as LCC and immunohistochemical analysis of ACLY expression was assessed not only for staining intensity but also according to the fraction of positive cells and with regard to subcellular localization of ACLY. In addition to these analytical differences, Migita investigated the expression of phosphorylated ACLY, whereas our antibody is directed against ACLY regardless its phosphorylation status.

Furthermore, in our detailed subgroup analyses, we could show that patient’s age may also influence NSCLC biology. While high levels of either ACLY and/or ME were a good prognostic factor in patients younger than 65 years, a statistical trend to the opposite was detected in elderly patients. This may indicate different changes in tumor metabolism and enzyme function. Several metabolic changes are implicated with older age, such as increasing hypoxia and higher incidence of diabetes mellitus type 2. In a large cohort study, type 2 diabetes mellitus was associated with a higher risk to develop several types of cancer, including lung carcinoma [34]. In this context, both, ACLY and ME have been shown to be important for glucose related insulin secretion [24]. By the fact, that incidence of type 2 diabetes as well as generalized hypoxia due to lung function impairment is higher in older patients, our findings of changing impact of ACLY and ME enzymatic fitting in NSCLC can be functionally supported. Diversity of NSCLC in different age populations is known for genetic aberrations and incidences of driver mutations [35, 36]. Thus, our findings, that changes of metabolic enzymes can be of different influence on cancer biology in NSCLC, further support that lung cancer arising in young patients may be biologically and genetically different from those of older patients.

In vitro and in vivo studies indicate that new pharmacological agents inhibiting ACLY can lead to significant decrease in cellular and tumor growth [22, 33, 37, 38]. Since our results show that overexpression of ACLY and/or ME in patients older than 65 years of age tend to have a poorer prognosis, they suppose that older patients may profit most from these ACLY inhibitors.

Concluding, expression patterns of the metabolic enzymes ACLY and ME are of different biological impact on survival in NSCLC patients. While in young patients overexpression of either ACLY or ME is indicative for a favorable overall survival, it tends to have the opposite effect in older patients. With the development of new inhibitory drugs directed against ACLY, our results support new treatment options with special focus on aged NSCLC patients.

Supporting Information

S1 Table. Summary of clinico-pathological data.
(DOCX)

S2 Table. Summary of the presented relevant statistical test results adjusted for multiple testing.
(DOCX)
S1 Dataset. Complete dataset.
(XLS)

Acknowledgments
We thank Nicola Bittermann for her technical support and advise. We acknowledge the support of this study by the Deutsche Forschungsgemeinschaft SFB850 and by the tumor bank of the Comprehensive Cancer Center Freiburg (CCCF).

Author Contributions
Conceived and designed the experiments: AC CK GK. Performed the experiments: AC CK MD. Analyzed the data: AC CK KA GK. Contributed reagents/materials/analysis tools: VG JR AP AzH SW MW. Wrote the paper: AC CK GK.

References
1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61(2):69–90. doi:10.3322/caac.20107 PubMed PMID:21296855.
2. Butler CA, Darragh KM, Currie GP, Anderson WJ. Variation in lung cancer survival rates between countries: do differences in data reporting contribute? Respir Med. 2006; 100(9):1642–6. doi: 10.1016/j.rmed.2005.12.006 PubMed PMID:16524710.
3. Scaglotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Maneogold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2008; 26(21):3543–51. doi: 10.1200/JCO.2007.15.0375 PubMed PMID:18506025.
4. Warburg O, Posener K, Negelein E. Über den Stoffwechsel der Carcinomzelle. Biochem Z. 1924;(152):309–44.
5. DeBerardinis RJ, Mancuso A, Daikinh E, Nissim I, Yudkoff M, Wehrli S, et al. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A. 2007; 104(49):19345–50. doi: 10.1073/pnas.0709747104 PubMed PMID:18032601; PubMed Central PMCID: PMC2148292.
6. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev. 2008; 18(1):51–61. doi: 10.1016/j.gde.2008.02.003 PubMed PMID:18387799; PubMed Central PMCID: PMC2476215.
7. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. Science. 2009; 324:1029–33. doi: 10.1126/science.1160809 PMID:19460998.
8. Travis WD, Brambilla E, Mueller-Hermelink HK, Harris CC, editors. Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARCPress; 2004.
9. Sobin LH, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours (UICC International Union Against Cancer). 7. Edition ed. Chichester: John Wiley & Sons; 2009.
10. Kayser G, Kassem A, Sielen W, Schulte-Uentrop L, Mattern D, Aumann K, et al. Lactate-dehydrogenase 5 is overexpressed in non-small cell lung cancer and correlates with the expression of the transketolase-like protein 1. Diagn Pathol. 2010; 5:22. doi: 10.1186/1746-1596-5-22 PubMed PMID:20385008; PubMed Central PMCID: PMC2861018.
11. Kayser G, Sielen W, Kubitz B, Mattern D, Stickeler E, Passlick B, et al. Poor outcome in primary non-small cell lung cancers is predicted by transketolase TKTL1 expression. Pathology. 2011; 43(7):719–24. doi: 10.1097/PAT.0b013e32834c352b PubMed PMID:22027741.
12. Ruschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, et al. HER2 testing in gastric cancer: a practical approach. Modern Pathol. 2012; 25(5):637–50. doi: 10.1038/modpathol.2011.198 PubMed PMID:WOS:000306174000001.
13. Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2013; 31(31):3997–4013. doi: 10.1200/JCO.2013.50.9984 PubMed PMID:24101049.
14. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistic Society. 1995; 57:289–300.

15. Walenta S, Mueller-Klieser WF. Lactate: mirror and motor of tumor malignancy. Semin Radiat Oncol. 2004; 14(3):267–74. doi: 10.1016/j.sram.2004.04.004 PubMed PMID: 15254870.

16. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. Cancer Res. 2011; 71(22):6921–5. Epub 2011/11/16. doi: 10.1158/0008-5472.CAN-11-1457 PubMed PMID: 22084445.

17. Menendez JA, Lupon R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007; 7(10):763–77. doi: 10.1038/nrc2222 PubMed PMID: 17882277.

18. Yancy HF, Mason JA, Peters S, Thompson CE 3rd, Littleton GK, Jett M, et al. Metastatic progression and gene expression between breast cancer cell lines from African American and Caucasian women. J Carcinog. 2007; 6:8. doi: 10.1186/1477-3163-6-8 PubMed PMID: 17472751; PubMed Central PMCID: PMC1876212.

19. Varis A, Wolf M, Monni O, Vakkari ML, Kokkola A, Moskaluk C, et al. Targets of gene amplification and overexpression at 17q in gastric cancer. Cancer Res. 2002; 62(9):2625–9. PubMed PMID: 11980659.

20. Torun J, Schlichtholz B, Dettlafl-Pokora A, Presler M, Goyke E, Matuszewski M, et al. Increased activity of glyceral 3-phosphate dehydrogenase and other lipogenic enzymes in human bladder cancer. Horm Metab Res. 2003; 35(10):565–9. doi: 10.1055/s-2003-43500 PubMed PMID: 14605988.

21. Halliday KR, Fenoglio-Preiser C, Sillerud LO. Differentiation of human tumors from nonmalignant tissue by natural-abundance 13C NMR spectroscopy. Magn Reson Med. 1988; 7(4):384–411. PubMed PMID: 2459580.

22. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuura M, et al. ATP citrate lyase: activation and therapeutic implications in non-small cell lung cancer. Cancer Res. 2008; 68(20):8547–54. doi: 10.1158/0008-5472.CAN-08-1235 PubMed PMID: 18922930.

23. Bauer DE, Hatzivassiliou G, Zhao F, Andreasis C, Thompson CB. ATP citrate lyase is an important component of cell growth and transformation. Oncogene. 2005; 24(41):6314–22. doi: 10.1038/sj.onc.1207773 PubMed PMID: 16007201.

24. Guay C, Madiraju SR, Aumais A, Joly E, Prentki M. A role for ATP-citrate lyase, malic enzyme, and pyruvate/citrate cycling in glucose-induced insulin secretion. J Biol Chem. 2007; 282(49):35657–65. doi: 10.1074/jbc.M707294200 PubMed PMID: 17928289.

25. Koukourakis MI, Giatromanolaki A, Bougioukas G, Sivridis E. Lung cancer: a comparative study of metabolism related protein expression in cancer cells and tumor associated stroma. Cancer biology & therapy. 2007; 6(9):1476–9. Epub 2007/09/21. PubMed PMID: 17881895.

26. Koukourakis MI, Giatromanolaki A, Sivridis E. Lactate dehydrogenase isoenzymes 1 and 5: differential expression by neoplastic and stromal cells in non-small cell lung cancer and other epithelial malignant tumors. Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine. 2003; 24(4):199–202. Epub 2003/12/05. 74430. PubMed PMID: 14654714.

27. Koukourakis MI, Giatromanolaki A, Sivridis E, Gatter KC, Harris AL. Pyruvate dehydrogenase and pyruvate dehydrogenase kinase expression in non small cell lung cancer and tumor-associated stroma. Neoplasia. 2005; 7(1):1–6. Epub 2005/03/02. PubMed PMID: 15736311; PubMed Central PMCID: PMC1490315.

28. Meijer TW, Schuurbiens OC, Kaanders JH, Looijen-Salamon MG, de Geus-Oei LF, Verhagen AF, et al. Differences in metabolism between adeno- and squamous cell non-small cell lung carcinomas: spatial distribution and prognostic value of GLUT1 and MCT4. Lung Cancer. 2012; 76(3):316–22. Epub 2011/12/14. doi: 10.1016/j.lungcan.2011.11.006 PubMed PMID: 22153830.

29. Mogi A, Kuwano H. TP53 mutations in nonsmall cell lung cancer. Journal of biomedicine & biotechnology. 2011; 2011:583929. doi: 10.1155/2011/583929 PubMed PMID: 21331359; PubMed Central PMCID: PMC3035360.

30. Jiang P, Du W, Mancuso A, Wellen KE, Yang X. Reciprocal regulation of p53 and malic enzymes modulates metabolism and senescence. Nature. 2013; 493(7434):689–93. doi: 10.1038/nature11776 PubMed PMID: 23334421; PubMed Central PMCID: PMC3561500.

31. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science. 2009; 324(5930):1076–80. doi: 10.1126/science.1164097 PubMed PMID: 19461003; PubMed Central PMCID: PMC2746744.

32. Londondo Gentile T, Lu C, Lodato PM, Tse S, Olejniczak SH, Witze ES, et al. DNMT1 is regulated by ATP-citrate lyase and maintains methylation patterns during adipocyte differentiation. Molecular and cellular biology. 2013; 33(19):3864–78. doi: 10.1128/MCB.01495-12 PubMed PMID: 23897429; PubMed Central PMCID: PMC3811875.
33. Migita T, Okabe S, Ikeda K, Igarashi S, Sugawara S, Tomida A, et al. Inhibition of ATP citrate lyase induces triglyceride accumulation with altered fatty acid composition in cancer cells. International journal of cancer Journal international du cancer. 2013. doi: 10.1002/ijc.28652 PubMed PMID: 24310723.

34. Kajuter H, Geier AS, Wellmann I, Krieg V, Fricke R, Heidinger O, et al. [Cohort study of cancer incidence in patients with type 2 diabetes: record linkage of encrypted data from an external cohort with data from the epidemiological Cancer Registry of North Rhine-Westphalia]. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz. 2014; 57(1):52–9. doi: 10.1007/s00103-013-1880-5 PubMed PMID: 24357173.

35. Pao W, Girard N. New driver mutations in non-small-cell lung cancer. The lancet oncology. 2011; 12 (2):175–80. doi: 10.1016/S1470-2045(10)70087-5 PubMed PMID: 21277552.

36. Zhou JX, Yang H, Deng Q, Gu X, He P, Lin Y, et al. Oncogenic driver mutations in patients with non-small-cell lung cancer at various clinical stages. Annals of oncology: official journal of the European Society for Medical Oncology / ESMO. 2013; 24(5):1319–25. doi: 10.1093/annonc/mds626 PubMed PMID: 23277484.

37. Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, et al. ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell. 2005; 8(4):311–21. doi: 10.1016/j.ccr.2005.09.008 PubMed PMID: 16226706.

38. Migita T, Okabe S, Ikeda K, Igarashi S, Sugawara S, Tomida A, et al. Inhibition of ATP citrate lyase induces an anticancer effect via reactive oxygen species: AMPK as a predictive biomarker for therapeutic impact. The American journal of pathology. 2013; 182(5):1800–10. doi: 10.1016/j.ajpath.2013.01.048 PubMed PMID: 23506948.