RESEARCH ARTICLE

Distinguishing Lung Adenocarcinoma from Lung Squamous Cell Carcinoma by Two Hypomethylated and Three Hypermethylated Genes: A Meta-Analysis

Tao Huang1‡, Jinyun Li1‡, Cheng Zhang1, Qingxiao Hong1, Danjie Jiang1, Meng Ye2*, Shiwei Duan1*

1 Zhejiang Provincial Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, Zhejiang 315211, China, 2 The Affiliated Hospital, Ningbo University, Ningbo, Zhejiang 315000, China

‡ TH and JL are co-first authors on this work.
* duanshiwei@nbu.edu.cn (SD); yemeng@nbu.edu.cn (MY)

Abstract

Significant differences in the aberrant methylation of genes exist among various histological types of non-small cell lung cancer (NSCLC), which includes adenocarcinoma (AC) and squamous cell carcinoma (SCC). Different chemotherapeutic regimens should be administered to the two NSCLC subtypes due to their unique genetic and epigenetic profiles. The purpose of this meta-analysis was to generate a list of differentially methylated genes between AC and SCC. Our meta-analysis encompassed 151 studies on 108 genes among 12946 AC and 10243 SCC patients. Our results showed two hypomethylated genes (CDKN2A and MGMT) and three hypermethylated genes (CDH13, RUNX3 and APC) in ACs compared with SCCs. In addition, our results showed that the pooled specificity and sensitivity values of CDH13 and APC were higher than those of CDKN2A, MGMT and RUNX3. Our findings might provide an alternative method to distinguish between the two NSCLC subtypes.

Introduction

Lung cancer remains the main contributor to cancer-related mortality, with 224,210 new cases and 159,260 deaths in the United States in 2014, although the incidence rate of lung cancer has been declining since the middle of 2000s [1,2]. Non-small cell lung cancer (NSCLC), accounting for almost 84% of lung cancer, includes two histological subtypes adenocarcinoma (AC) and squamous cell carcinoma (SCC), which stem from epithelial cells that line the larger airways and the peripheral small airways, respectively [2].

Differential diagnosis between AC and SCC is of clinical significance. Chemotherapy regimens for AC and SCC are different according to the guidelines of National Comprehensive Cancer Network (NCCN) for NSCLC. For instance, pemetrexed is a multiple-enzyme inhibitor, which is utilized in AC patients rather than in SCC patients [3–5]. The current methods in the differential diagnosis often involve in immunohistochemical stainings of complete surgical...
resection specimens. The staining proteins consist of AC positive markers (TTF-1, CK7, Muci, and Napsin A) and SCC positive markers (CK5/6, HMWCK, NTRK1/2, and p63) [6]. The sensitivity of the most widely used TTF-1 is only 62%, suggesting a need to develop new markers for the differential diagnosis [6]. Moreover, almost 25% poorly differentiated NSCLC patients cannot be classified by TTF-1, suggesting that complimentary markers are needed to enhance the specificity [7–9].

Epigenetic modifications have been shown to be an important regulatory mechanism during the multistep development of human cancers [10]. Different epigenetic modifications [11] and different microRNA and gene expression profiles were found between AC and SCC [12], suggesting that there were distinct molecular signatures between the two subtypes [13,14]. Several studies have reported that the methylation rates of APC, CDH13, RARβ, LINE-1, RASSF1, and RUNX3 were significantly higher in AC than in SCC [15,16], while higher methylation frequencies of DAPK, TIMP3, TGIF and SFRP4 were more often observed in SCC compared to AC [17,18]. In addition, there were significantly different chemotherapeutic outcomes between AC and SCC [19].

Due to the increasing amount of evidence, it was necessary to establish a short list of methylated genes through a comprehensive literature review. Meta-analysis can overcome the limitation of small-size samples in single study, and achieve more reliable and completed consequences through the combination and quantitative assessment of various studies [20]. In this study, we systematically reviewed the recent methylation studies and summarized the differential gene methylation between AC and SCC, and aimed to provide a handful of epigenetic clues to elaborate the molecular biomarkers of the different histological subtypes of NSCLC.

Materials and Methods

Identification of relevant studies

All the relevant studies, updated until January 11, 2016, were systematically searched for in the PubMed, China National Knowledge Infrastructure and Wanfang literature databases. The keywords were as follows: "(histolog OR patholog OR clinic) AND lung cancer (methylation OR epigenes)". In addition, a manual search was performed to seek other potential studies in the references of the retrieved publications.

Inclusion and exclusion criteria

All the eligible studies should meet the following criteria: (1) the study should refer to the measurement of the gene methylation status in NSCLC patients rather than cancer cell lines; (2) the study should have sufficient methylation information on the relative genes; and (3) the study should provide detailed information on NSCLC, such as the pathological subtypes of NSCLC and the number of NSCLC subtypes. In addition, neither reviews nor abstracts were included in our analysis. Studies without detailed information on gene methylation or pathological types of NSCLC data were also excluded from the current study. The current meta-analysis was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (S1 PRISMA Checklist).

Data extraction

For the eligible studies, we extracted the gene, the first author’s name, the published year, the race of the study subjects, the methylation assessment method, the number of cases of AC and SCC, and the frequency of gene methylation (S1 Table).
Statistical analysis

Review manager 5.2 software (Cochrane Collaboration, Oxford, UK) was used to calculate the combined odds ratios (ORs) and the corresponding 95% confidence intervals (95% CIs) to estimate the association in the meta-analysis. $\chi^2$ test was used to assess the significant heterogeneity across studies, and the result of $\chi^2$ test was expressed by $I^2$ metric. When $I^2$ metric was more than 50%, we considered that obvious heterogeneity existed in the involved studies, and a random-effect model was applied for the meta-analysis. Otherwise, a fixed-effect model was used. The aggregated sensitivity, specificity, area under the receiver operating characteristic curve (AUC) and their 95% CIs were calculated by STATA software (Stata Corporation, College Station, TX).

Results

As shown in Fig 1, a total of 2137 articles were initially retrieved from the literature databases. A filtration removed 115 duplicated publications, 1685 studies that were not human studies or full-text inaccessible studies, 77 studies without detailed information regarding pathological types of NSCLC, 51 studies without methylation frequency data, 24 studies only including AC methylation data, and 31 studies only including SCC methylation data as controls. Finally, a total of 154 eligible studies on 111 genes were included in the current meta-analysis. Among the identified genes, there were 75 genes reported by only one study, 20 genes involved in two studies, and 16 genes covered by at least three studies. The 16 genes reported by at least three studies were CDKN2A, RASSF1, MGMT, MLH1, CDH13, CDH1, DAPK, RUNX3, APC, FHIT, SFRP1, RARB, WIF1, DLEC1, IGFBP7 and TFPI2 (Table 1). The genes with fewer than 3 studies were listed in S2 Table.

According to our systematic review, there were 5 aberrantly methylated genes (including CDKN2A, MGMT, CDH13, RUNX3 and APC) associated with the pathological types of NSCLC, and the remaining 11 gene methylation events showed no significant difference between AC and SCC. As shown in Table 1, CDKN2A and MGMT were significantly less methylated in AC rather than in SCC, while CDH13, RUNX3 and APC genes were significantly more methylated in AC than in SCC.

As shown in Fig 2, the meta-analysis of CDKN2A methylation in 40 studies among 1609 ACs and 1392 SCCs revealed that CDKN2A methylation was less frequently observed in AC than in SCC (OR = 0.75, 95% CI = 0.63–0.89, $P = 0.0008$, $I^2 = 39\%$). Meta-analysis of 15 studies among 680 ACs and 710 SCCs showed that MGMT was significantly more methylated in SCC than in AC (OR = 0.66, 95% CI = 0.52–0.82, $P = 0.0003$, $I^2 = 0\%$).

CDH13, RUNX3 and APC genes were shown to have significantly higher methylation frequencies in AC. Specifically, our meta-analysis of 8 studies among 299 ACs and 211 SCCs revealed that CDH13 methylation was more frequently observed in AC than in SCC (OR = 2.60, 95% CI = 1.73–3.90, $P < 0.00001$, $I^2 = 0\%$). Meta-analysis of 7 studies among 286 ACs and 201 SCCs showed that RUNX3 was more often methylated in SCC than in AC (OR = 3.34, 95% CI = 2.10–5.31, $P < 0.00001$, $I^2 = 35\%$). The meta-analysis of APC in 7 studies among 157 ACs and 94 SCCs showed that APC methylation was more often methylated in AC than in SCC (OR = 2.82, 95% CI = 1.72–4.62, $P < 0.0001$, $I^2 = 18\%$, Fig 3).

As shown in Table 1, the methylation of 11 genes (including RASSF1, MLH1, CDH1, DAPK, FHIT, SFRP1, RARB, WIF1, DLEC1, IGFBP7 and TFPI2) could not distinguish between AC and SCC. And as demonstrated in Figs 2 and 3, the funnel plots of CDKN2A, MGMT, CDH13, RUNX3 and APC indicated no significant publication bias.

Subsequently, we performed sensitivity meta-analyses of the five significant genes (Table 2). Our results showed that the pooled specificity values as differential diagnostic markers between AC and SCC for CDH13, APC, CDKN2A, MGMT and RUNX3 were 0.74 (0.65–0.81), 0.65...
Some chemotherapeutic regimens were more effective in SCC, while other drugs were more effective in non-squamous histological types [3–5]. Thus, it is necessary to differentiate the two
major types of NSCLC (AC and SCC). Generally, well-differentiated AC can be identified according to the immunohistochemical staining results of TTF-1, napsin-A, and other markers [6]. However, some studies have reported that a minor fraction of poorly differentiated SCC still reacted with TTF-1 [7–9]. Our results showed that the pooled specificity and sensitivity values of CDH13 and APC were higher than those of CDKN2A, MGMT and RUNX3. The joint effect of these methylation markers is of interest to be explored in the future.

Epigenetic modifications have been shown to account for the mechanisms in the development of different histological subtypes of cancers [21]. Besides, other studies have identified genes with significantly different methylation between different subtypes, and the differentially methylated genes (including CDKN2A, APC, CDH13, THBS2 and ERG) have been utilized to distinguish these different histological subtypes of cancers [22,23]. Previous study has identified that CDKN2A, APC and CDH13 have significantly different methylation frequencies between AC and SCC [23]. Another study observed that RUNX3 methylation was significantly more often in AC than in SCC [15]. The above findings were also confirmed in the current meta-analyses. However, MGMT methylation frequency was not different between 77 AC and 38 SCC in the previous study [23], and this might be due to a lack of power [23]. In contrast,

| Gene   | Studies | Overall OR [95% CI] | I² | P Value | Median Methylation (AC/SCC, %) | 25% Methylation Quartile (AC/SCC, %) | 50% Methylation Quartile (AC/SCC, %) | 75% Methylation Quartile (AC/SCC, %) |
|--------|---------|---------------------|----|---------|--------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| CDH13  | 8       | 2.60 [1.73, 3.90]   | 0% | < 0.00001 | 40/25                          | 36/19                                | 44/25                                | 66/36                                |
| RUNX3  | 7       | 3.34 [2.10, 5.31]   | 35%| < 0.00001 | 36/11                          | 27/7                                 | 36/11                                | 41/26                                |
| APC    | 7       | 2.82 [1.72, 4.62]   | 18%| < 0.00001 | 62/37                          | 43/30                                | 63/37                                | 73/57                                |
| MGMT   | 15      | 0.66 [0.52, 0.82]   | 0% | 0.0003   | 32/36                          | 29/27                                | 32/36                                | 40/53                                |
| CDKN2A | 40      | 0.75 [0.63, 0.89]   | 39%| 0.0008   | 36/49                          | 23/33                                | 37/49                                | 58/57                                |
| WIFI   | 4       | 0.67 [0.43, 1.02]   | 0% | 0.06     | 32/39                          | 8/3                                  | 25/16                                | 35/30                                |
| RASSF1 | 19      | 1.15 [0.94, 1.40]   | 33%| 0.16     | 39/36                          | 14/5                                 | 17/15                                | 26/22                                |
| FHIT   | 6       | 0.82 [0.57, 1.17]   | 25%| 0.27     | 27/31                          | 7/10                                 | 14/18                                | 23/29                                |
| SFRP1  | 5       | 1.23 [0.81, 1.86]   | 0% | 0.33     | 37/31                          | 9/4                                  | 11/10                                | 36/19                                |
| DLEC1  | 4       | 0.80 [0.42, 1.55]   | 53%| 0.51     | 34/40                          | 8/16                                 | 12/19                                | 25/31                                |
| CDH1   | 8       | 1.06 [0.63, 1.78]   | 22%| 0.82     | 39/33                          | 4/3                                  | 5/5                                  | 13/6                                 |
| DAPK   | 8       | 1.02 [0.69, 1.51]   | 0% | 0.92     | 35/36                          | 7/6                                  | 12/9                                 | 16/12                                |
| MLH1   | 9       | 0.98 [0.53, 1.78]   | 63%| 0.94     | 57/55                          | 6/10                                 | 11/19                                | 33/36                                |
| TFPI2  | 3       | 0.99 [0.50, 1.94]   | 0% | 0.97     | 26/29                          | 2/6                                  | 15/7                                 | NA/NA                                |
| RARB   | 5       | 1.00 [0.40, 2.46]   | 82%| 0.99     | 50/49                          | 7/10                                 | 32/17                                | 45/55                                |
| IGFBP7 | 3       | 1.00 [0.50, 2.00]   | 0% | 0.99     | 47/47                          | 3/1                                  | 25/4                                 | NA/NA                                |

NA stands for not available. From the overall OR values, CDH13, RUNX3 and APC were significantly more methylated in AC than in SCC; MGMT and CDKN2A were significantly less methylated in AC than in SCC.

doi:10.1371/journal.pone.0149088.t001
our meta-analyses among 680 ACs and 710 SCCs found MGMT methylation was significantly less in AC than in SCC.

In the current study, we identified five differentially methylated genes between AC and SCC. These five methylated genes could also be found in many other cancers. Loss of CDH13 expression caused by promoter hypermethylation was observed in breast [24], lung [24], colorectal [25,26], prostate [27], and nasopharyngeal [28] cancers. Besides, Methylated CDH13 could serve as a potential diagnostic and prognostic biomarker in nasopharyngeal carcinoma [28] and cervical cancer [29], respectively. Aberrantly methylated levels of APC and MGMT were also observed in colorectal cancer tissues [30]. Methylated APC was shown to be associated with prognostic outcomes in gastric carcinomas [31], breast cancer [32], and hepatocellular carcinoma [33]. MGMT was a DNA-repair gene, which greatly contributed to the microsatellite instability (MSI) in colorectal cancer [34]. Studies demonstrated that MGMT methylation triggered the incidence of MSI [35,36].

CDKN2A was a well-established gene, which played a critical role in cancer progression [37]. The inactivation of CDKN2A by promoter hypermethylation was observed in leukemia [38], colorectal [39], gastric [40], esophageal [41], and lung cancers [42]. aberrantly methylated RUNX3 was found to be associated with the risk of multiple cancers, such as hepatocellular carcinoma [43], esophageal cancer [43], gastric carcinoma [44] and NSCLC [44].

### Table: Methylation Profiles of Different Subtypes of NSCLC

#### CDKN2A

| Study No. | Subtype | Events | Total | Weight | Odds Ratio | 95% CI |
|-----------|---------|--------|-------|--------|------------|-------|
| Jumars et al. | AC | 3 | 11 | 9 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Meng et al. | SCC | 3 | 11 | 8 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Jumars et al. | AC | 4 | 17 | 13 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Meng et al. | SCC | 5 | 17 | 11 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Liang et al. | AC | 2 | 7 | 2 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Meng et al. | SCC | 1 | 7 | 1 | 1.0 | 0.33 (0.07, 1.70) | 2001 |

#### MGMT

| Study No. | Subtype | Events | Total | Weight | Odds Ratio | 95% CI |
|-----------|---------|--------|-------|--------|------------|-------|
| Oden et al. | AC | 8 | 21 | 2 | 1.0 | 0.33 (0.07, 1.70) | 2001 |
| Meng et al. | SCC | 6 | 10 | 3 | 1.0 | 0.33 (0.07, 1.70) | 2001 |

#### CDKN2A

### Fig 2. Forest and funnel plots of CDKN2A and MGMT. The forest plots of CDKN2A and MGMT displayed the effect size and 95% CIs for the included studies. Funnel plots suggested no publication bias in the meta-analyses of CDKN2A and MGMT genes. Our results showed that the total ORs for CDKN2A and MGMT were less than 1, which demonstrated the methylation of CDKN2A and MGMT in AC were relatively higher than in SCC. Funnel plots of meta-analyses of CDH13, RUNX3 and APC demonstrated no publication biases in the included studies. In addition, M-H denotes Mantel-Haenszel statistical method to calculate the combined odds ratios (ORs) and the corresponding 95% confidence intervals (95% CIs). Weight denotes the weighted average of the intervention effect estimated in each study. SE denotes standard errors.

![Forest plots of CDKN2A and MGMT](doi:10.1371/journal.pone.0149088.g002)

![Funnel plots of CDKN2A and MGMT](doi:10.1371/journal.pone.0149088.g002)
Cyclin-dependent kinase inhibitor 2A (CDKN2A) is known to be an important tumor suppressor gene with regulatory roles affecting CDK4 and p53 in cell cycle G1 control. This gene is frequently mutated or deleted, as well as hypermethylated, in a wide variety of tumors including NSCLC [45–47]. Interestingly, previous studies reported that the methylation status of CDKN2A might correlate with the response to certain chemotherapeutic drugs in breast cancer [48]. Cell line studies demonstrated that the usage of demethylating agents could reactivate

### Table 2. Specificity and sensitivity of five differentially methylated genes between AC and SCC.

| Gene    | Specificity [95% CI] | Sensitivity [95% CI] | AUC [95% CI] |
|---------|----------------------|----------------------|--------------|
| CDH13   | 0.74 [0.65, 0.81]    | 0.49 [0.38, 0.59]    | 0.68 [0.64, 0.72] |
| APC     | 0.65 [0.55, 0.74]    | 0.60 [0.44, 0.74]    | 0.66 [0.62, 0.70] |
| CDKN2A  | 0.55 [0.47, 0.63]    | 0.37 [0.29, 0.45]    | 0.45 [0.41, 0.49] |
| MGMT    | 0.60 [0.52, 0.68]    | 0.32 [0.27, 0.37]    | 0.40 [0.35, 0.44] |
| RUNX3   | 0.86 [0.75, 0.92]    | 0.47 [0.42, 0.51]    | 0.47 [0.42, 0.51] |

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is known to be an important tumor suppressor gene with regulatory roles affecting CDK4 and p53 in cell cycle G1 control. This gene is frequently mutated or deleted, as well as hypermethylated, in a wide variety of tumors including NSCLC [45–47]. Interestingly, previous studies reported that the methylation status of CDKN2A might correlate with the response to certain chemotherapeutic drugs in breast cancer [48]. Cell line studies demonstrated that the usage of demethylating agents could reactivate
CDKN2A, which was able to be silenced by hypermethylation [49]. Other clinical studies reported that NSCLC patients who underwent epigenetic therapy tended to have improved overall survival with statistical significance [46]. Our systematic review concluded that the methylation of CDKN2A was significantly more common in SCC than in AC.

MGMT plays a key role in regulating DNA repair via removing a methyl group from mutagenic O6-methylguanine, which can lead to a transition mutation through DNA replication [50]. Thus, inactivation of the O6-methylguanine-DNA methyltransferase (MGMT) gene plays an important role in the progression of cancer characterized by the accumulation of genetic changes. In addition, the epigenetic silencing of MGMT was shown to play a pivotal role in DNA repair pathway that was associated with cisplatin sensitivity [51]. MGMT promoter methylation was shown to be inversely correlated with MGMT expression, and silenced MGMT by promoter hypermethylation was observed in NSCLC [52]. Our meta-analysis found that the hypermethylation of MGMT was more common in SCC than in AC.

Cadherin 13 (CDH13), also known as T-cadherin or H-cadherin (heart), is a unique member of the cadherin superfamily [53,54]. CDH13 proteins play important roles in cell differentiation and in anti-apoptosis [55]. However, CDH13 expression was generally down regulated by CDH13 promoter hypermethylation in human cancers [56,57]. CDH13 methylation was a common event in NSCLC, and it was also associated with its clinicopathological features. CDH13 hypermethylation was observed at higher frequency in AC than in SCC [23]. Patients with CDH13 hypermethylation tended to have lower survival [58], suggesting that CDH13 hypermethylation could serve as a prognostic biomarker in NSCLC. The current meta-analysis also confirmed this observation.

The RUNX3 proteins belong to the runt domain-containing family of transcription factors in the regulation of gene expression [59]. Transcriptional silencing of RUNX3 by hypermethylation was associated with various human cancers, including NSCLC [60–62]. Low RUNX3 mRNA expression level was found to be associated with RUNX3 promoter hypermethylation [62]. RUNX3 hypermethylation was mostly detected in AC [53]. Further studies demonstrated that patients with higher RUNX3 hypermethylation in AC had shorter survival even when undergoing positive treatment [63]. Our analysis indicated that RUNX3 hypermethylation might have the potential to predict treatment outcome as a differential diagnostic marker for NSCLC subtypes.

The tumor suppressor gene adenomatous polyposis coli (APC) is correlated with inhibition of the Wnt signaling pathway [64]. Mutation of APC was shown to be associated with the emergence of colorectal cancer [65]. Decreased expression of APC by its promoter hypermethylation was also often observed in NSCLC [66]. Aberrant epigenetic modification of APC was also observed in colorectal cancer as well as in NSCLC [66,67]. The current analysis revealed that APC hypermethylation was more frequent in AC than in SCC.

Although our meta-analyses were performed through carefully screening numerous relevant studies, several limitations should not be underestimated. Above all, conference abstracts and inaccessible full-text articles were excluded from our meta-analyses because we were unable to retrieve relevant data for the meta-analysis. Moreover, only reports in the English or Chinese languages were chosen, and this might introduce bias in the literature selection. Meanwhile, the majority of the harvested genes with only one or two studies were excluded from this analysis. It was possible that some of them were certain specific-histology genes. Thus, future analyses of these genes in larger sample sizes were needed to confirm our findings.

In summary, our meta-analysis provided a list of differently methylated genes between AC and SCC and identified two hypomethylated (CDKN2A and MGMT) and three hypermethylated genes (CDH13, RUNX3 and APC) that might help distinguish between AC and SCC.
Supporting Information

S1 PRISMA Checklist. The PRISMA checklist of our meta-analysis.

S1 Table. General characteristics of all the eligible studies in the current meta-analyses.

S2 Table. Genes with less than 3 methylation studies.

Acknowledgments

The research was supported by the grants from the National Natural Science Foundation of China (31100919 and 81371469), the Natural Science Foundation of Zhejiang Province (LR13H020003), the K. C. Wong Magna Fund in Ningbo University, the Zhejiang Provincial Natural Science Foundation of China (LY16H160005), Project of Scientific Innovation Team of Ningbo (2015B11050) and the Ningbo Natural Science Foundation (2014A610235). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: TH JL MY SD. Performed the experiments: TH JL CZ DJ QH. Analyzed the data: DJ. Contributed reagents/materials/analysis tools: TH JL DJ. Wrote the paper: JL SD.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: A Cancer Journal for Clinicians. 2014; 64 (1):9–29. doi: 10.3322/caac.21208
2. America Cancer Society. Cancer Facts and Figures 2014. American Cancer Society. 2014. Available: http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/index
3. Hirsch FR, Spreatico A, Novello S, Wood MD, Simms L, Papotti M. The prognostic and predictive role of histology in advanced non-small cell lung cancer: a literature review. Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer. 2008; 3(12):1468–81. doi: 10.1097/JTO.0b013e318189f551 PMID: 19057275.
4. Scagliotti G, Brodowicz T, Shepherd FA, Zielinski C, Vansteenkiste J, Manegold C, et al. Treatment-by-histology interaction analyses in three phase III trials show superiority of pemetrexed in nonsquamous non-small cell lung cancer. Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer. 2011; 6(1):64–70. doi:10.1097/JTO.0b013e3181f7c6d4 PMID: 21119545.
5. Scagliotti G, Hanna N, Fossella F, Sugarman K, Blatter J, Peterson P, et al. The differential efficacy of pemetrexed according to NSCLC histology: a review of two Phase III studies. The oncologist. 2009; 14 (3):253–63. doi: 10.1634/theoncologist.2008-0232 PMID: 19221167.
6. Terry J, Leung S, Laskin J, Leslie KO, Gown AM, Ionescu DN. Optimal immunohistochemical markers for distinguishing lung adenocarcinomas from squamous cell carcinomas in small tumor samples. Am J Surg Pathol. 2010; 34(12):1805–11. doi: 10.1097/PAS.0b013e3181f8cc7f PMID: 21107086.
7. Fabbro D, DiLoreto C, Stamerra O, Beltrami CA, Lonigro R, Damante G. TTF-1 gene expression in human lung tumours. Eur J Cancer. 1996; 32A(3):512–7. PMID: 8814700.
8. Pelosi G, Fraggetta F, Pasini F, Maisonneuve P, Sonzogni A, Iannucci A, et al. Immunoreactivity for thyroid transcription factor-1 in stage I non-small cell carcinomas of the lung. Am J Surg Pathol. 2001; 25 (3):369–72. PMID: 11224607.
9. Tan D, Li Q, Deeb G, Ramnath N, Slocum HK, Brooks J, et al. Thyroid transcription factor-1 expression prevalence and its clinical implications in non-small cell lung cancer: a high-throughput tissue microarray and immunohistochemistry study. Hum Pathol. 2003; 34(6):597–604. PMID: 12827614.
23. Toyooka S, Toyooka KO, Maruyama R, Virmani AK, Girard L, Miyajima K, et al. DNA methylation pro-
10. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. Nature reviews Genetics. 2006; 7(1):21–33. doi: 10.1038/nrg1748 PMID: 16369569.
11. Liu J, Yang XY, Shi WJ. Identifying differentially expressed genes and pathways in two types of non-
small cell lung cancer: adenocarcinoma and squamous cell carcinoma. Genetics and molecular research: GMR. 2014; 13(1):95–102. doi: 10.4238/2014.January.8.8 PMID: 24446291.
12. Hsiung CA, Lan Q, Hong YC, Chen CJ, Hosgood HD, Chang IS, et al. The 5p15.33 locus is associated with risk of lung adenocarcinoma in never-smoking females in Asia. PLoS genetics. 2010; 6(8). doi: 10.1371/journal.pgen.1001051 PMID: 20700438; PubMed Central PMCID: PMC2916850.
13. Daraselia N, Wang Y, Budoff A, Lituev A, Potapova O, Vansant G, et al. Molecular signature and path-
tologic type and geography-related differences in the methylation profiles of non-small cell lung cancer. International journal of cancer Journal international du cancer. 2003; 103(2):153–60. doi: 10.1002/ijc.10787 PMID: 12455028.
14. Lockwood WW, Wilson IM, Coe BP, Chari R, Pikor LA, Thu KL, et al. Divergent genomic and epige-
nomic landscapes of lung cancer subtypes underscore the selection of different oncogenic pathways during tumor development. PloS one. 2012; 7(5):e37775. doi: 10.1371/journal.pone.0037775 PMID: 22629454; PubMed Central PMCID: PMC3357406.
15. Jin M, Kawakami K, Fukui Y, Tsukioka S, Oda M, Watanabe G, et al. Different histological types of non-
small cell lung cancer have distinct folate and DNA methylation levels. Cancer research. 2009; 100(12):2325–30. doi: 10.1111/j.1349-7006.2009.01321.x PMID: 19764999.
16. Toyooka S, Maruyama R, Toyooka KO, McLerran D, Feng Z, Fukuyama Y, et al. Smoke exposure, histor-
tic type and geography-related differences in the methylation profiles of non-small cell lung cancer. International journal of cancer Journal international du cancer. 2003; 103(2):153–60. doi: 10.1002/ijc.10787 PMID: 12455028.
17. Castro M, Grau L, Puerta P, Gimenez L, Venditti J, Quadrelli S, et al. Multiplexed methylation profiles of tumor suppressor genes and clinical outcome in lung cancer. Journal of translational medicine. 2010; 8:66. doi: 10.1186/1479-5876-8-86 PMID: 20849603; PubMed Central PMCID: PMC2955578.
18. Niklinska W, Naumnik W, Sulewska A, Kozlowski M, Pankiewicz W, Milewski R. Prognostic signifi-
cance of DAPK and RASSF1A promoter hypermethylation in non-small cell lung cancer (NSCLC). Folia histochemica et cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cyto-
chemical Society. 2009; 47(2):275–80. doi: 10.2478/v10042-009-0091-2 PMID: 19926549.
19. Hou J, Aerts J, den Hamer B, van Ijcken W, den Bakker M, Riegman P, et al. Gene expression-based classification of non-small cell lung carcinomas and survival prediction. PloS one. 2010; 5(4):e10312. doi: 10.1371/journal.pone.0010312 PMID: 20421987; PubMed Central PMCID: PMC2858668.
20. Lipsey MW, Wilson DB. Practical meta-analysis. Thousand Oaks, Calif.: Sage Publications; 2001. ix, 247 p. p.
21. Feinberg AP. Epigenetics at the epicenter of modern medicine. Jama. 2008; 299(11):1345–50. doi: 10.1001/jama.299.11.1345 PMID: 18349095.
22. Kelemen LE, Kobel M, Chan A, Taghaddos S, Dinu I. Differentially methylated loci distinguish ovarian carcinoma histological types: evaluation of a DNA methylation assay in FFPE tissue. BioMed research international. 2013; 2013:815894. doi: 10.1155/2013/815894 PMID: 24175302; PubMed Central PMCID: PMC3794544.
23. Toyooka S, Toyooka KO, Maruyama R, Virmani AK, Girard L, Miyajima K, et al. DNA methylation pro-
files of lung tumors. Molecular cancer therapeutics. 2001; 6(12):3382–90. doi: 10.1016/j.bjcp.2001.11.007 PMID: 11839671.
24. Hibi K, Nakayama H, Kodera Y, Ito K, Akiyama S, Nakao A. CDH13 promoter region is specifically methylated in poorly differentiated colorectal cancer. British journal of cancer. 2004; 90(5):4556–60. doi: 10.1038/sj.bjc.6601647 PMID: 14997203; PubMed Central PMCID: PMC2409627.
25. Maruyama R, Toyooka S, Toyooka KO, Zochbauer-Muller S, Farinas AJ, et al. Aberrant methylation of the CDH13 (H-cadherin) promoter region in colorectal cancers and adenomas. Cancer research. 2002; 62(12):3382–6. PMID: 12067979.
26. Sun D, Zhang Z, Van do N, Huang G, Emmberg I, Hu L. Aberrant methylation of CDH13 gene in nasopa-
ryngeal carcinoma could serve as a potential diagnostic biomarker. Oral oncology. 2007; 43(1):82–7. doi: 10.1016/j.oraloncology.2006.01.007 PMID: 16807071.
29. Widschwendter A, Ivarsson L, Blas Archig A, Muller H, Wiedemair A, et al. CDH1 and CDH13 methylation in serum is an independent prognostic marker in cervical cancer patients. International journal of cancer Journal international du cancer. 2004; 109(2):163–6. doi: 10.1002/ijc.1706 PMID: 14750164.

30. Michailidis C, Theocharis S, Tsouroullis G, Plets V, Kourakis G, Patsouris E, et al. Expression and promoter methylation status of MLY1, MGMT, APC, and CDH1 genes in patients with colon adenocarcinoma. Experimental biology and medicine. 2015; 240(12):1599–605. doi: 10.1177/1535370115583800 PMID: 25908636.

31. Balkoumardiou I, Matthaios D, Karayiannakis A, Balanaki H, Michailidis P, Xenidis N, et al. Prognostic role of APC and RASSF1A promoter methylation status in cell free circulating DNA of operable gastric cancer patients. Mutation research. 2015; 778:46–51. doi: 10.1016/j.mrfmmm.2015.05.002 PMID: 26073472.

32. Muller HM, Widschwendter A, Fieg H, Ivarsson L, Goebel G, Perkmann E, et al. DNA methylation in serum of breast cancer patients: an independent prognostic marker. Cancer research. 2003; 63 (22):7641–5. PMID: 14633683.

33. Calvisi DF, Lado S, Gorden A, Farina M, Lee JS, Conner EA, et al. Mechanistic and prognostic significance of aberrant methylation in the molecular pathogenesis of human hepatocellular carcinoma. The Journal of clinical investigation. 2007; 117(9):2713–22. doi: 10.1172/JCI31457 PMID: 17717605; PubMed Central PMCID: PMC1950459.

34. Kohonen-Corish MR, Daniel JJ, Chan C, Lin BP, Kwun SY, Dent OF, et al. Low microsatellite instability is associated with poor prognosis in stage C colon cancer. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2005; 23(10):2318–24. doi: 10.1200/JCO.2005.00.109 PMID: 15800322.

35. Ogin S, Hazra A, Tranah GJ, Kirkner GJ, Kawasaki T, Nosho K, et al. MGMT germline polymorphism is associated with somatic MGMT promoter methylation and gene silencing in colorectal cancer. Carcinogenesis. 2007; 28(9):1985–90. doi: 10.1093/carcin/bgm160 PMID: 17621591.

36. Whitehall VL, Walsh MD, Young J, Leggett BA, Jass JR. Methylation of O-6-methylguanine DNA methyltransferase characterizes a subset of colorectal cancer with low-level DNA microsatellite instability. Cancer research. 2001; 61(3):827–30. PMID: 11221863.

37. Foulkes WD, Flanders TY, Pollock PM, Hayward NK. The CDKN2A (p16) gene and human cancer. Molecular medicine. 1997; 3(1):5–20. PMID: 9132280; PubMed Central PMCID: PMC2230107.

38. Nosaka K, Maeda M, Tamiya S, Sakai H, Matsuoka M. Increasing methylation of the CDKN2A and RARB genes. Cancer genetics and cytogenetics. 2005; 162(1):10 –21. doi: 10.1016/j.cancergen.2005.03.008 PMID: 16157195.

39. Shima K, Nosho K, Baba Y, Cantor M, Meyerhardt JA, Giovannucci EL, et al. Prognostic significance of the CDKN2A (p16) promoter methylation and loss of expression in 902 colorectal cancers: Cohort study and literature review. International journal of cancer Journal international du cancer. 2011; 128(5):1080–94. doi: 10.1002/ijc.25432 PMID: 20749320; PubMed Central PMCID: PMC2958235.

40. Peng D, Zhang H, Sun G. The relationship between P16 gene promoter methylation and gastric cancer: a meta-analysis based on Chinese patients. Journal of cancer research and therapy. 2014; 10(7):598–607. doi: 10.1158/2159-8290.CD-11-0214 PMID: 22586682; PubMed Central PMCID: PMC3353724.
47. Narayan G, Arias-Pulido H, Koul S, Vargas H, Zhang FF, Villella J, et al. Frequent promoter methylation of CDH1, DAPK1, RARB, and HIC1 genes in carcinoma of cervix uteri: its relationship to clinical outcome. Molecular cancer. 2003; 2(2):24. PMID: 12773202; PubMed Central PMCID: PMC156646.

48. Klajic J, Busato F, Edwardsen H, Touleimat N, Fleischer T, Bukholm I, et al. DNA methylation status of key cell-cycle regulators such as CDKNA2/p16 and CCNA1 correlates with treatment response to doxorubicin and 5-fluorouracil in locally advanced breast tumors. Clinical cancer research: an official journal of the American Association for Cancer Research. 2014; 20(24):6357–66. doi: 10.1158/1078-0432.CCR-14-0297 PMID: 25294903.

49. Zhu WG, Dai Z, Ding H, Srinivasan K, Hall J, Duan W, et al. Increased expression of unmethylated CDKN2D by 5-aza-2′-deoxycytidine in human lung cancer cells. Oncogene. 2001; 20(53):7787–96. doi: 10.1038/sj.onc.1204970 PMID: 11753657.

50. Soejima H, Zhao W, Mukai T. Epigenetic silencing of the MGMT gene in cancer. Biochemistry and cell biology = Biochimie et biologie cellulaire. 2005; 83(4):429–37. doi: 10.1139/o05-140 PMID: 16094446.

51. Rosell R, Taron M, Arias-Aranda C, Mate JL, Reguart N, et al. Molecular predictors of response to chemotherapy in lung cancer. Seminars in oncology. 2004; 31(1 Suppl 1):20–7. PMID: 14981577.

52. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer research. 1999; 59(4):793–7. PMID: 10029064.

53. Angst BD, Marcozzi C, Magee Al. The cadherin superfamily: diversity in form and function. Journal of cell science. 2001; 114(Pt 4):629–41. PMID: 11171368.

54. Takeuchi T, Ohkami Y. Recent progress in T-cadherin (CDH13, H-cadherin) research. Histology and histopathology. 2001; 16(4):1287–93. PMID: 11642747.

55. Rubina K, Kalinina N, Potekhina A, Efimenko A, Semina E, Poliakov A, et al. T-cadherin suppresses angiogenesis in vivo by inhibiting migration of endothelial cells. Angiogenesis. 2007; 10(3):183–95. doi: 10.1002/sa1456-007-9072-2 PMID: 17486418.

56. Chan DW, Lee JM, Chan PC, Ng IO. Genetic and epigenetic inactivation of T-cadherin in human hepatocellular carcinoma cells. International journal of cancer Journal international du cancer. 2008; 123(5):1043–52. doi: 10.1002/ijc.23634 PMID: 18553387.

57. Ren JZ, Hu JR. Correlation between T-cadherin gene expression and aberrant methylation of T-cadherin promoter in human colon carcinoma cells. Med Oncol. 2012; 29(2):915–8. doi: 10.1007/s12032-011-9836-9 PMID: 21298366.

58. Xue R, Yang C, Zhao F, Li D. Prognostic significance of CDH13 hypermethylation and mRNA in NSCLC. OncoTargets and therapy. 2014; 7:1987–96. doi: 10.2147/OTT.S67355 PMID: 25382980; PubMed Central PMCID: PMC4222896.

59. Bangsow C, Rubins N, Glusman G, Bernstein Y, Negreanu V, Goldenberg D, et al. The RUNX3 gene—sequence, structure and regulated expression. Gene. 2001; 279(2):221–32. PMID: 11733147.

60. Kim TY, Lee HJ, Hwang KS, Lee M, Kim JW, Bang YJ, et al. Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma. Laboratory investigation; a journal of technical methods and pathology. 2004; 84(4):479–84. doi: 10.1038/labinvest.3700060 PMID: 14968123.

61. Kim WJ, Kim EJ, Jeong P, Quan C, Kim J, Li QL, et al. RUNX3 inactivation by point mutations and aberrant DNA methylation in bladder tumors. Cancer research. 2005; 65(20):9347–54. PMID: 16001190; PubMed Central PMCID: PMC4222896.

62. Li QL, Kim HR, Kim WJ, Choi JK, Lee YH, Kim HM, et al. Transcriptional silencing of the RUNX3 gene by CpG hypermethylation is associated with lung cancer. Biochemical and biophysical research communications. 2004; 314(1):223–8. PMID: 14715269.

63. Yanagawa N, Tamura G, Oizumi H, Kanauchi N, Endoh M, Sadahiro M, et al. Promoter hypermethylation of RASSF1A and RUNX3 genes as an independent prognostic prediction marker in surgically resected non-small cell lung cancers. Lung cancer. 2007; 58(1):131–8. doi: 10.1016/j.lungcan.2007.05.011 PMID: 17606310.

64. Groden J, Thilvisser A, Samowitz W, Carlson M, Gelbert L, Albertsen H, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell. 1991; 66(3):589–600. PMID: 1651174.

65. Miyaki M, Konishi M, Kikuchi-Yanoshita R, Igaki T, Tanaka K, et al. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. Cancer research. 1994; 54(11):3011–20. PMID: 817091.

66. Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, et al. Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. Cancer research. 2000; 60(16):4366–71. PMID: 10969779.
67. Usadel H, Brabender J, Danenberg KD, Jeronimo C, Harden S, Engles J, et al. Quantitative adenomatous polyposis coli promoter methylation analysis in tumor tissue, serum, and plasma DNA of patients with lung cancer. Cancer research. 2002; 62(2):371–5. PMID: 11809682.