Prognostic and diagnostic significance of Cavin 2 in lung adenocarcinoma

Xianguo Wang[1], Diana Maria Cespedes Arcani[1], Jingping Zhao[1], Ming Xu[1], Xuefeng Zhou[1], Yibin Yang[2]

[1]Department of Thoracic and Cardiovascular Surgery, Zhongnan Hospital of Wuhan University, Wuhan City, Hubei, China
[2]Department of Respiratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan City, Hubei, China

Corresponding authors:
Xuefeng Zhou and Yibin Yang
169 Donghu Road
Wuchang District
Wuhan city 430000
Hubei, China
E-mail: zhou20180721@163.
com, zhou20180721@163.
com (Xuefeng Zhou)
1594559173@qq.com
(Yibin Yang)

Abstract

Introduction: Cavin 2 down-regulation is reported in several malignant tumors and is associated with tumor progression. However, the role of Cavin 2 in lung adenocarcinoma is unknown. This study aimed to investigate the prognostic and diagnostic significance of Cavin 2 in lung adenocarcinoma.
Material and methods: Cavin 2 expression levels were examined in 150 cases of lung adenocarcinoma and matched adjacent normal lung tissues using RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR), Western blotting and immunohistochemistry (IHC) assays. Then, the relationship of Cavin 2 expression with clinicopathological characteristics and patients' survival was further evaluated in lung adenocarcinoma.
Results: QPCR and Western blotting analysis indicated that Cavin 2 expression levels were significantly lower in lung adenocarcinoma tissues compared with those in adjacent normal lung tissues ($p < 0.0001$). The IHC results showed that positive expression of Cavin 2 was mainly located in cytoplasm as brown, but was hard to detect in lung adenocarcinoma tissues. The low-expression rates of Cavin 2 in lung adenocarcinoma and adjacent normal lung tissues were 62.0% and 20.0%, respectively, and the difference was significant ($p < 0.0001$). Lower expression of Cavin 2 was significantly associated with tumor size, TNM stage and lymph node metastasis ($p < 0.05$).
Conclusions: Cavin 2 has low expression in lung adenocarcinoma, which might be regarded as a potential prognostic and diagnostic biomarker.

Key words: Cavin 2, prognosis, lung cancer.

Introduction

Adenocarcinoma is the most common subtype of non-small cell lung cancer, which comprises more than 40% of lung cancers [1–3]. Surgical resection is the main treatment method for adenocarcinoma patients; however, patients' survival is still poor [4–6]. The overall survival rate of patients with lung adenocarcinoma is 13–58.3% [7]. The main reason is that lung adenocarcinoma tends to develop distant metastases at the early stage [8–11]. Identifying the potential molecular biomarkers in lung adenocarcinoma is essential for patients to select the best treatment strategy and improve outcomes.

Cavin 2, a membrane-bound phosphatidylserine-binding protein, is regarded as a critical organizer of caveolae and a regulator of angiogenesis [12, 13]. Cavin 2 is required for angiogenesis and plays a key

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role in cell proliferation, migration and invasion [14, 15]. Cavin 2 is expressed in heart and lung, but is hard to detect in pancreas, brain, kidney, skeletal muscle, liver and placenta [16]. A recent study showed that Cavin 2 is mostly expressed in the well-differentiated liposarcoma and correlates with the differentiation and proliferation of tumor cells [17]. Cavin 2 is significantly down-regulated in oral squamous cell carcinoma and associated with tumor progression [18]. However, the role of Cavin 2 in lung adenocarcinoma remains unclear.

In this study, we detected the expression levels of Cavin 2 in 150 cases of lung adenocarcinoma and matched adjacent normal lung tissues, and also evaluated the relationship of Cavin 2 with clinicopathological features and patients' survival.

Material and methods

Patients and samples

A total of 150 cases of lung adenocarcinoma and matched adjacent normal lung tissues were collected from Zhongnan Hospital of Wuhan University from 2009 to 2017. The age of patients ranged from 28 to 76 years (mean: 50 years). None of the patients were treated with radiotherapy or chemotherapy before the surgical operation. Adjacent normal lung tissues were collected more than 3 cm far away from the tumor. Clinicopathological features such as age, sex, smoking history, tumor size, tumor differentiation, lymph node metastasis and TNM stage (UICC, 2009) were obtained from hospital records. Pathological diagnosis was performed by two pathologists in Zhongnan Hospital of Wuhan University. Follow-up time was recorded on the surgery day and lasted from 7 to 80 months (mean: 38.5 months). This study is supported by the Ethnics Committee of Zhongnan Hospital of Wuhan University (No. Z201603322).

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

All tissues were collected and stored in liquid nitrogen. Total RNAs were isolated using RNAiso PLUS (Invitrogen, California, USA), and reverse transcribed into cDNA by the cDNA Synthesis Kit (TaKaRa Corp, Dalian, China). According to manufacturer's protocol, qPCR was performed using the 7500 SYBR Green Fast Real-Time PCR System (ABI Corp, USA). The reaction conditions were as follows: 95°C for 10 min, 95°C for 15 s in 40 cycles and 60°C for 60 s. The primer sequences of Cavin 2 were 5'-TTGTGAAGGAGCCAGTTCCC-3' and 5'-TCA-GAGGAGAGGTCCACGTT-3'. The primer sequences of GAPDH were 5'-CCCCGCTACTCCTCCTCCTAAG-3' and 5'-TCCACGACCAGTTGTCCATTCC-3'. Relative mRNA expression was analyzed by the $2^{-\Delta\Delta Ct}$ method [19, 20].

Western blotting

Using protease inhibitors, total proteins were extracted. 50 µg of protein was detached by 10% SDS-PAGE and transferred to the polyvinylidene fluoride membranes. Membranes were blocked by 5% fat-free milk and incubated with Cavin 2 antibody (1 : 2500, Abcam Corp, USA) and GAPDH antibodies (1 : 2000, Zhongshan Corp, Beijing, China) overnight at 4°C, respectively. Then, membranes were washed with PBST and incubated with a rabbit anti-goat horseradish peroxidase-conjugated secondary antibody for 30 min at room temperature. Protein blots were detected by enhanced chemiluminescence detector.

Immunohistochemistry (IHC)

Sections (2-µm-thick) were obtained and deparaffinized with xylene and gradient ethanol. Antigen retrieval was performed with 3% hydrogen peroxide and 0.01 mol/l sodium citrate buffer (pH 6.0) at room temperature for 10 min. Then, sections were washed with phosphate buffered saline (PBS) solution and incubated with Cavin 2 antibody (1 : 100, Abcam Corp, USA). Finally, sections were incubated with horseradish peroxidase-labeled goat anti-mouse/rabbit IgG polymer for 40 min at room temperature. Signals were detected by diaminobenzidine tetrahydrochloride (DAB).

Based on staining intensity and positive cell percentage, a semi-quantitated method was used to analyze IHC results. Staining intensity was scored as follows: blank (0), weak (1), moderate (2) and strong (3). Positive cell percentage was scored as follows: < 5% (0), ≥ 5% to < 25% (1), 25% to 50% (2) and > 50% (3). Total score of each section ranged from 0 to 9, which was computed by multiplying these two values. Based on ROC analysis, a score ≥ 4 was regarded as high Cavin 2 expression and others were low Cavin 2 expression (< 4).

Statistical analysis

Data were recorded as mean ± standard deviation (SD) and analyzed using SPSS software (version 19.0; SPSS, Chicago, IL, USA). As data did not have a normal distribution, the non-parametric Mann-Whitney U-test was used to compare the differences between two groups. The correlation between Cavin 2 expression and clinicopathological characters was analyzed by chi-square test (χ^2). Survival analysis was evaluated using the Kaplan-Meier method and Cox's proportional hazards model. $P < 0.05$ was regarded as statistically significant.

Results

Cavin 2 is lowly expressed in lung adenocarcinoma

As shown in Figure 1, the mRNA levels of Cavin 2 were significantly lower in lung adenocarcinoma tissues compared with those in matched adjacent normal lung tissues ($p < 0.0001$). Moreover, Western blot results showed that Cavin 2 protein levels were also lower in lung adenocarcinoma tissues compared with those in matched adjacent normal lung tissues (Figure 1 B). Subsequently, Cavin 2 expression was further detected by IHC (Figures 1 C–F). The results showed that positive expression of Cavin 2 was mainly located in cytoplasm as brown (Figures 1 D and F). Based on ROC analysis of the IHC score, score = 4 (sensitivity = 0.815, specificity = 0.608) was considered as the cut-off to differentiate lung adenoma and normal lung tissues (Figure 2). The low expression rates of Cavin 2 in lung adenocarcinoma and adjacent normal lung tissues were 62.0% and 20.0%, respectively, and the difference was statistically significant ($p < 0.0001$, Table I). These data indicated that Cavin 2 was lowly expressed in lung adenocarcinoma.
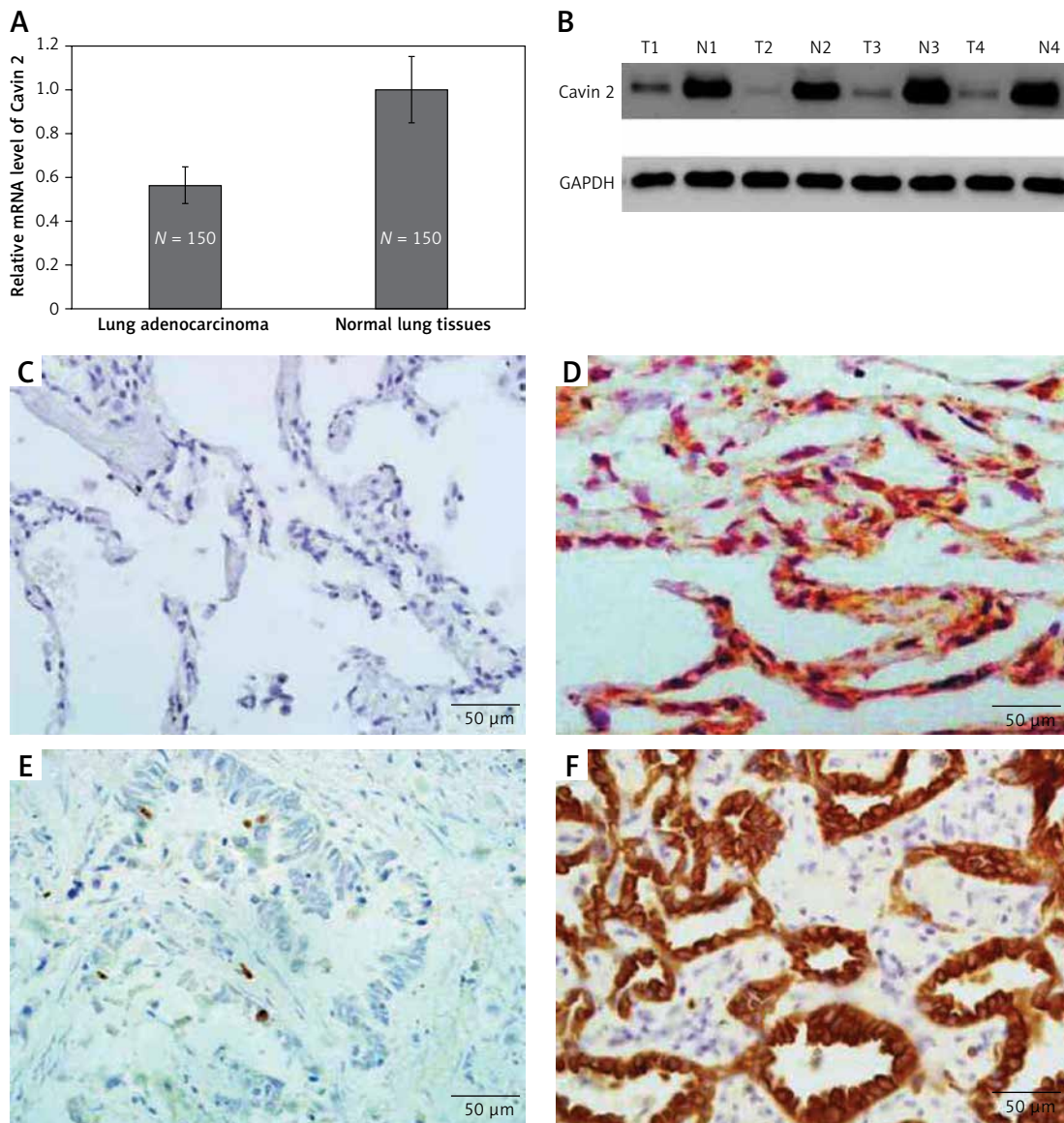

Figure 1. Cavin 2 was lowly expressed in lung adenocarcinoma. **A** – Cavin 2 mRNA level was measured in lung adenocarcinoma and normal lung tissues by RT-qPCR. **B** – Cavin 2 protein expression level was detected in lung adenocarcinoma and normal lung tissues by Western blotting. **C** – Low Cavin 2 expression was observed in normal lung tissues by IHC. **D** – High Cavin 2 expression was observed in normal lung tissues by IHC. **E** – Low Cavin 2 expression was observed in lung adenocarcinoma tissues by IHC. **F** – High Cavin 2 expression was observed in lung adenocarcinoma tissues by IHC

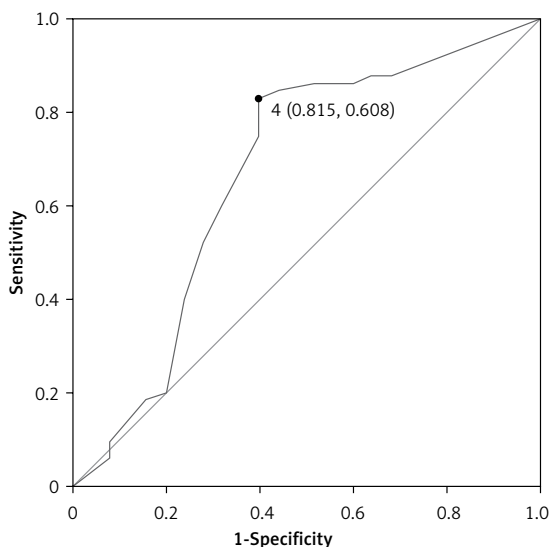*$P < 0.0001$, N – normal lung tissue, T – tumor tissue (lung adenocarcinoma).

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Figure 2. Score = 4 was selected as the cut-off by ROC analysis and the sensitivity and specificity were 0.815 and 0.608, respectively

Cavin 2 expression correlates with clinicopathological characters and predicts prognosis in lung adenocarcinoma

Based on IHC analysis, the correlation between Cavin 2 expression and clinicopathological characters was analyzed in lung adenocarcinoma. The results showed that lower expression of Cavin 2 was significantly associated with tumor size, lymph node metastasis and TNM stage (Table II, $p < 0.05$), while it was not associated with age, sex, smoking history or tumor differentiation (Table II, $p > 0.05$). Cavin 2 low expression was more easily observed in patients with lymph node metastasis. Patients with stage III presented lower levels of Cavin 2 compared with those with stage I–II. In addition, the correlation between Cavin 2 mRNA expression level and TNM stage was analyzed. As shown in Figure 3, the mRNA level of Cavin 2 in patients with stage III was significantly lower compared with those with stage I–II ($p < 0.0001$).

Then, the association between Cavin 2 expression and prognosis was analyzed by Kaplan-Meier method and Cox's proportional hazards model. Kaplan-Meier survival analysis showed that the median survival time of patients with low Cavin 2 expression was 45 months, which was significant-ly shorter than that of patients with high Cavin 2 expression (66 months) (Table III, Figure 4, $p < 0.0001$). In addition, patients' prognosis was associated with lymph node metastasis and TNM stage (Table III, $p < 0.05$), while it was not connected with age, sex, smoking history, tumor size or tumor differentiation (Table III, $p > 0.05$). Cox regression survival analysis showed that Cavin 2, lymph node metastasis and TNM stage were independent factors for predicting patients' prognosis in lung adenocarcinoma (Table IV, $p < 0.05$).

Discussion

Cavin 2, a serum deprivation protein response gene, is enriched in lung tissue [12]. In this study, our data showed that low Cavin 2 expression was more easily observed in lung adenocarcinoma compared with normal lung tissues, indicating that Cavin 2 might be a tumor suppressor gene and Cavin 2 depletion might be implicated in the formation of lung adenocarcinoma. Moreover, Cavin 2 was also lowly expressed in oral cancer, prostate cancer, kidney cancer, breast cancers and hepatocellular carcinoma, which was consistent with our findings [21–25]. However, Codenotti *et al.* reported that Cavin 2, along with Cavin 1, was mostly expressed in the well-differentiated liposarcoma, while being almost undetectable in the more aggressive tumors [17]. These data suggested that Cavin 2 expression might be connected with tumor heterogeneity.

In addition, the correlation between Cavin 2 expression and clinicopathological characters was evaluated in lung adenocarcinoma. The results showed that Cavin 2 expression was significantly associated with tumor size, suggesting that Cavin 2 depletion might contribute to cell growth and proliferation. Furthermore, Cavin 2 expression was confirmed to be correlated with lymph node metastasis and TNM stage, indicating that low Cavin 2 expression correlated with the progression of lung adenocarcinoma. Survival analysis indicated that Cavin 2 as well as lymph node metastasis and TNM stage correlated with patients' prognosis and could be independent prognostic factors in lung adenocarcinoma. Thus, these data indicated that Cavin 2 was a prognostic biomarker in lung adenocarcinoma. Cavin 2 depletion might promote tumor progression and result in an unfavorable

Table I. Cavin 2 expression was detected in lung adenocarcinoma by IHC

Tissue types	N	Cavin 2		P-value
		High expression, n (%)	Low expression, n (%)	
Lung adenocarcinoma	150	57 (38.0)	93 (62.0)	< 0.0001
Normal lung	150	120 (80.0)	30 (20.0)	

Table II. Cavin 2 expression correlated with clinicopathological characters in lung adenocarcinoma

Clinicopathological characters	N	Cavin 2		P-value
		High expression	Low expression	
Age [years]:				
≤ 50	85	29	56	0.309
> 50	65	28	37	
Sex:				
Male	84	28	56	0.236
Female	66	29	37	
Smoking history:				
Negative	87	32	55	0.736
Positive	63	25	38	
Tumor size [cm]:				
≤ 3	42	22	20	0.026
> 3	108	35	73	
Tumor differentiation:				
High-middle grade	107	39	68	0.579
Low grade	43	18	25	
Lymph node metastasis:				
Negative	81	42	39	< 0.0001
Positive	69	15	54	
TNM stage:				
I–II	87	44	43	< 0.0001
III	63	13	50	

prognosis. Unozawa *et al.* reported that Cavin 2 was a potential predictor for tumor progression in oral cancer [18]. In hepatocellular carcinoma, Cavin 2 was regarded as a potential diagnostic and prognostic biomarker [24].

Recently, Cavin 2 was reported to be involved in cell differentiation, proliferation, migration and invasion. Codenotti *et al.* reported that Cavin 2 was connected with adipogenic differentiation [17]. Unozawa *et al.* reported that Cavin 2 up-regulation slowed cellular proliferation by regulating cell-cycle arrest in oral cancer [18]. Boopathy *et al.* reported that Cavin 2 was required for endothelial cell proliferation, invasion and migration [12]. However, the effect of Cavin 2 expression on cell differentiation, proliferation, migration and invasion is still unclear in lung adenocarcinoma. Cavin 2 depletion may promote the progression of lung adenocarcinoma by regulating cell differentiation, proliferation, migration or invasion. However, this hypothesis needs to be validated by more evidence in future.
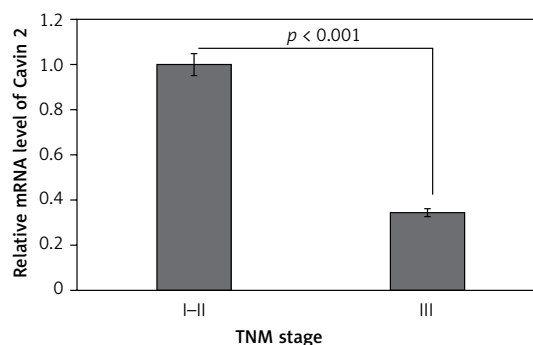
Figure 3. Lower mRNA level of Cavin 2 correlated with TNM stage

In conclusion, these data indicate that Cavin 2 is lowly expressed in lung adenocarcinoma and correlates with tumor formation and progression, which may be regarded as a potential prognostic and diagnostic biomarker in lung adenocarcinoma. Cavin 2 depletion forebodes poorer prognosis, which may be helpful for prognosis assessment in lung adenocarcinoma. However, there are some

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Table III. Survival analysis was performed by Kaplan-Meier method

| Variables	N	Survival time, median (95% CI) [months]	P-value
Cavin 2 expression:			
High expression	57	66 (61–70)	< 0.0001
Low expression	93	45 (42–48)	
Age [years]:			
≤ 50	85	59 (52–66)	0.259
> 50	65	53 (49–56)	
Sex:			
Male	84	52 (49–57)	0.489
Female	66	57 (51–63)	
Smoking history:			
Negative	87	54 (49–59)	0.558
Positive	63	55 (50–59)	
Tumor size [cm]:			
≤ 3	42	61 (53–68)	0.186
> 3	108	53 (49–56)	
Tumor differentiation:			
High-middle grade	107	57 (53–61)	0.426
Low grade	43	48 (44–52)	
Lymph node metastasis:			
Negative	81	62 (57–67)	0.001
Positive	69	48 (44–51)	
TNM stage:			
I–II	87	63 (58–68)	< 0.0001
III	63	44 (40–47)	
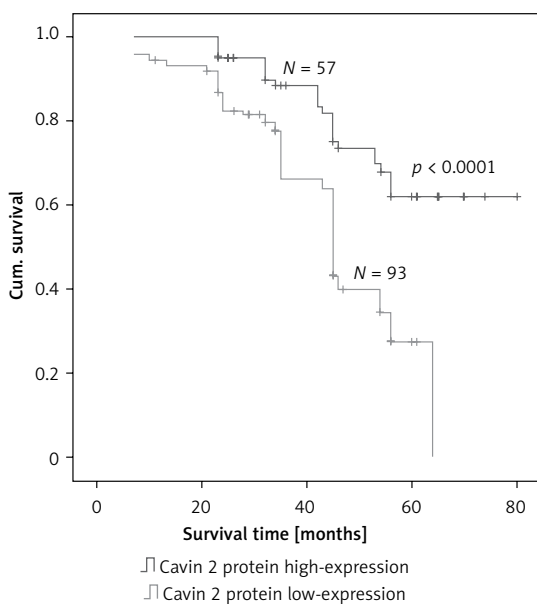
Figure 4. Kaplan-Meier analysis showed that the protein level of Cavin 2 correlated with prognosis in lung adenocarcinoma

limitations of the current study. For one thing, all patients were from a single clinical center, which means it is hard to eliminate the selection bias. For another, the biological functions and mechanism of Cavin 2 in lung adenocarcinoma remain unknown. Therefore, further investigations are needed.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

Table IV. Prognostic factors were further evaluated by multivariate Cox regression

Variables	Hazard rate	95% CI	*P*-value
Cavin 2 (low expression vs. high expression)	2.716	1.681–4.387	< 0.0001
Lymph node metastasis (positive vs. negative)	2.088	1.332–3.270	0.001
TNM stage (III vs. I–II)	2.492	1.615–3.847	< 0.0001
Age (> 50 years vs. ≤ 50)	1.354	0.642–2.857	0.426
Sex (male vs. female)	0.858	0.360–2.041	0.728
Smoking history (positive vs. negative)	0.735	0.440–1.227	0.239
Tumor size (> 3 cm vs. ≤ 3 cm)	1.485	0.700–3.15	0.302
Tumor differentiation (low grade vs. ≤ high-middle grade)	1.093	0.654–1.828	0.734

References

1. Lei B, Qi W, Zhao Y, et al. PBK/TOPK expression correlates with mutant p53 and affects patients' prognosis and cell proliferation and viability in lung adenocarcinoma. Hum Pathol 2015; 46: 217-24.
2. Liu H, Qie P, Yang G, et al. miR-181b inhibits chemoresistance in cisplatin-resistant H446 small cell lung cancer cells by targeting Bcl-2. Arch Med Sci 2018; 14: 745-51.
3. Lortet-Tieulent J, Soerjomataram I, Ferlay J, et al. International trends in lung cancer incidence by histological subtype: adenocarcinoma stabilizing in men but still increasing in women. Lung Cancer 2014; 84: 13-22.
4. Liu S, Li Y, Qi W, et al. Expression of Tiam1 predicts lymph node metastasis and poor survival of lung adenocarcinoma patients. Diagn Pathol 2014; 9: 69.
5. Chen X, Fu Y, Xu H, et al. SOX5 predicts poor prognosis in lung adenocarcinoma and promotes tumor metastasis through epithelial-mesenchymal transition. Oncotarget 2018; 9: 10891-4.
6. Li C, Kuo SW, Hsu HH, et al. Lung adenocarcinoma with intraoperatively diagnosed pleural seeding: Is main tumor resection beneficial for prognosis? J Thorac Cardiovasc Surg 2018; 155: 1238-49e1231.
7. Zhao S, Shen W, Yu J, et al. TBX21 predicts prognosis of patients and drives cancer stem cell maintenance via the TBX21-IL-4 pathway in lung adenocarcinoma. Stem Cell Res Ther 2018; 9: 89.
8. Hung JJ. Histologic subtype component predicts lymph node micrometastasis and prognosis in patients with stage I lung adenocarcinoma. J Thorac Dis 2017; 9: 3623-5.
9. Bucciarelli PR, Tan KS, Chudgar NP, et al. BRMS1 expression in surgically resected lung adenocarcinoma predicts future metastases and is associated with a poor prognosis. J Thorac Oncol 2018; 13: 73-84.
10. Ettinger DS. Ten years of progress in non-small cell lung cancer. J Natl Compr Canc Netw 2012; 10: 292-5.
11. Sun Y, Chen Y, Li S, et al. NanoVelcro-captured CTC number concomitant with enhanced serum levels of MMP7 and MMP9 enables accurate prediction of metastasis and poor prognosis in patients with lung adenocarcinoma. Int J Nanomed 2017; 12: 6399-412.
12. Boopathy GTK, Kulkarni M, Ho SY, et al. Cavin 2 regulates the activity and stability of endothelial nitric-oxide synthase (eNOS) in angiogenesis. J Biol Chem 2017; 292: 17760-6.
13. Toufaily C, Charfi C, Annabi B, et al. A role for the cavin-3/matrix metalloproteinase-9 signaling axis in the regulation of PMA-activated human HT1080 fibrosarcoma cell neoplastic phenotype. Cancer Growth Metastasis 2014; 7: 43-51.
14. Yi JS, Mun DG, Lee H, et al. PTRF/cavin-1 is essential for multidrug resistance in cancer cells. J Proteome Res 2013; 12: 605-14.
15. Gupta R, Toufaily C, Annabi B. Caveolin and cavin family members: dual roles in cancer. Biochimie 2014; 107: 188-202.
16. Gustincich S, Vatta P, Goruppi S, et al. The human serum deprivation response gene (SDPR) maps to 2q32-q33 and codes for a phosphatidylserine-binding protein. Genomics 1999; 57: 120-9.
17. Codenotti S, Vezzoli M, Poliani PL, et al. Cavin-2 is a specific marker for detection of well-differentiated liposarcoma. Biochem Biophys Res Commun 2017; 493: 660-5.
18. Unozawa M, Kasamatsu A, Higo M, et al. Cavin-2 in oral cancer: a potential predictor for tumor progression. Mol Carcinog 2016; 55: 1037-47.
19. Jędroszka D, Orzechowska M, Bednarek AK. Predictive values of Notch signalling in renal carcinoma. Arch Med Sci 2017; 13: 1249-54.
20. Jen J, Tang YA, Lu YH, et al. Oct4 transcriptionally regulates the expression of long non-coding RNAs NEAT1 and MALAT1 to promote lung cancer progression. Mol Cancer 2017; 16: 104.
21. Altintas DM, Allioli N, Decaussin M, et al. Differentially expressed androgen-regulated genes in androgen-sensitive tissues reveal potential biomarkers of early prostate cancer. PLoS One 2013; 8: e66278.
22. Ozturk S, Papageorgis P, Wong CK, et al. SDPR functions as a metastasis suppressor in breast cancer by promoting apoptosis. Proc Natl Acad Sci USA 2016; 113: 638-43.
23. Li X, Jia Z, Shen Y, et al. Coordinate suppression of sdpr and fhl1 expression in tumors of the breast, kidney, and prostate. Cancer Sci 2008; 99: 1326-33.
24. Jing W, Luo P, Zhu M, et al. Prognostic and diagnostic significance of SDPR-Cavin-2 in hepatocellular carcinoma. Cell Physiol Biochem 2016; 39: 950-60.
25. Aung CS, Hill MM, Bastiani M, et al. PTRF-cavin-1 expression decreases the migration of PC3 prostate cancer cells: role of matrix metalloprotease 9. Eur J Cell Biol 2011; 90: 136-42.