Clinical Significance of Gli-1 And Caveolin-1 Expression in the Human Small Cell Lung Cancer

Jie Wu1, Dingxin Di1, Chen Zhao2, Qi Pan3, Yingyi Liu1, Xue Zhang2, Xianda Zhao2, Honglei Chen1,2*

Abstract

Background: Lung cancer is the leading causes of cancer-related deaths around the world. Abnormal activation of the hedgehog (Hh) signaling pathway has been found to be involved in the occurrence, invasion, and metastasis of cancers. Autophagy also plays a significant role in the growth and metastasis of cancers. However, the correlation between the Hh signaling pathway and autophagy in small cell lung cancer (SCLC) is still poorly understood. This study aimed to investigate the significance of Hh signaling pathway and autophagy in SCLC. Materials and Methods: The expression of the Hh-induced transcriptional factor, glioma associated oncogene-1 (Gli-1) and the autophagy-related molecule caveolin-1 (Cav-1) and their clinical significance was performed to detect and assay by immunohistochemistry in tissue microarray including 70 patients with SCLC. Results: In our study, 47 (67.1%) patients had positive Gli-1 expression, 49 (70.0%) patients had positive Cav-1 expression, and 44 (62.9%) patients had negative fibroblastic Cav-1 expression. In SCLC, Gli-1 expression increased markedly, and was closely associated with decreased fibroblastic Cav-1 expression. Furthermore, we also found that Gli-1 expression was closely associated with increased Cav-1 expression. Conclusions: Our findings suggested that abnormal activation of the Hh signaling pathway is closely related to autophagy in SCLC. We envision that novel targets may come with the further investigation of Gli-1 and Cav-1 in carcinogenesis of SCLC.

Keywords: Small cell lung cancer- Gli−1- caveolin−1- hedgehog pathway- autophagy

Asian Pac J Cancer Prev, 19 (2), 401-406

Introduction

Lung cancer is one of the most frequently diagnosed cancers and the leading causes of cancer-related deaths around the world, especially in less developed countries, accounting for about 13% of newly diagnosed cancers and 18% of the total number of deaths worldwide (Torre et al., 2015). Small cell lung cancer (SCLC), accounting for 10% of clinical lung cancer cases, is an aggressive malignancy, strongly correlated with smoking (Koinis et al., 2016). Patients with SCLC can be cured by treated with chemotherapy in combination with radiotherapy currently. Nevertheless, drug resistance inevitably occurs. They relapse sometimes incredibly quick, succumbing to the disease ultimately. Because of this dismal prognosis, it is urgent to conduct more basic research about the molecular mechanism of SCLC (Santarpia et al., 2016).

The hedgehog (Hh) signaling pathway is recognized as the most significant homologue during embryonic development of vertebrates, particularly for the regulation of pattern formation and cell proliferation in numerous tissues (Ingham and McMahon, 2001). However, abnormal activation of Hh signaling pathway may cause excessive cell proliferation resulting in the development of cancer (Cochrane et al., 2015). It also allows for the modulation of the microenvironment to prepare a tumor-suitable niche, thus creating an enabling environment for cancer progression and metastasis (Hanna and Shevde, 2016). Present research reports that glioma associated oncogene-1 (Gli-1) exhibits a strong positive activating effect of downstream target genes of the Hh pathway (Lei et al., 2015). Aberrant expression of Gli-1 is involved in various types of tumors, such as gastric cancer, breast cancer, non-small cell lung cancers and so on. Recently, more and more researches regard Gli-1 as a therapeutic target for intervention of cancer metastasis (Hong et al., 2014; Wang et al., 2014; Lei et al., 2015).

Mediated by the lysosomal degradation pathway, autophagy degrades superfluous or damaged organelles, misfolded proteins, and invading micro-organisms, in order to maintain quality control, sustain cell homeostasis as well as provide energy and nutrients. This self-digestion to some extent is a process potently triggered by fasting (Levine and Kroemer, 2008; Martinez-Outschoorn et al., 2010). Autophagy can also promote tumor in established cancer through autophagy-mediated intracellular recycling.
Jie Wu et al
Asian Pacific Journal of Cancer Prevention, Vol 19

graded alcohol washes. Antigen retrieval was performed on TMAs were deparaffinized in xylene and rehydrated in a stepwise dilution with alcohol and water. Heat-induced epitope retrieval (HIER) was performed using citric acid buffer (pH 6.0) in a pressure cooker. The slides were allowed to cool for 10 min before the addition of primary antibody. Rabbit polyclonal antibodies to human Caveolin-1 (Cav-1) (sc-8948, H-300, Santa Cruz, USA) and Gli-1 (sc-8008, Santa Cruz, USA) were used to visualize antibody binding. Briefly, TMA sections were incubated for 1 h with secondary antibody, followed by DAB visualization. TMAs were then counterstained with hematoxylin.

Materials and Methods

Selection of patients and tissue microarray construction

A total of seventy patients receiving surgical intervention due to SCLC were recruited from the Department of Pathology, Zhongnan Hospital of Wuhan University. The clinical and pathological information of each patient were recorded, including age, gender, depth of invasion, depth of tumor invasion (T), lymph node metastasis (N), distant metastasis (M), and pTNM stage (UICC/AJC TNM staging system, 2016) (Goldstraw et al., 2016). Age at diagnosis ranged from 19 to 73 years (average: 54 years). This study was approved by the Ethical Committee of Zhongnan Hospital of Wuhan University. Written informed consent was obtained from all the patients preoperatively.

Seventy SCLC and ten matched adjacent noncancerous lung tissues were collected from each patient, fixed in 10% buffered formalin, embedded in paraffin. Hematoxylin and eosin stained slides were screened for the most representative tumor tissues and matched adjacent noncancerous tissues. Two tissue microarray (TMA) slides with a diameter of 1.5mm were constructed with a tissue manual arraying instrument. Each TMA slide consisted of 160 specimens of 70 SCLC tissues and 10 matched adjacent noncancerous lung tissues (each case has two specimens).

Immunohistochemistry

Immunohistochemistry was performed to detect the expression of Gli-1 (rabbit anti-human polyclonal antibody, H-300, 1:50 dilution; GenWay Biotech, CA, USA), and Cav-1 (rabbit anti-human polyclonal antibody, sc-8948, 1:150 dilution; Santa Cruz, USA) proteins, according to manufacturer’s instructions. HRP-conjugated secondary antibody and DAB kit (Dako, Agilent Technologies, CA, USA) were used to visualize antibody binding. Briefly, TMAs were deparaffinized in xylene and rehydrated in graded alcohol washes. Antigen retrieval was performed in citric acid (10 mM, pH 6.0) at 95 °C for 15 min by microwave, followed by cooling for 30 min. TMAs were washed in PBS and treated with 0.3% hydrogen peroxide for 30 min, in order to block endogenous peroxide activity, and then washed again in PBS. TMAs were then incubated in 2% BSA buffer at 37 °C for 30 min, and then at 4 °C overnight in rabbit anti-Cav-1 polyclonal antibody and rabbit anti-Gli-1 polyclonal antibody respectively, to permit antibody binding. TMAs were then washed three times with PBS for 5 min each time and incubated in HRP-conjugated second antibody at 37 °C for 30 min. The sites of peroxidase activity were visualized by using DAB. TMAs were then counterstained with haematoxylin. Immunostaining reactivity was observed by using light microscopy (Olympus BX-53 with CCD DP73). The standard positive control provided by the manufacturer served as a positive control, and the primary antibody was replaced with PBS in negative controls.

Determination of the proportion of cells positive for each marker

We counted the Cav-1- or Gli-1-stained tumor tissues of each specimen at high magnification (200×) and estimated the positive area (PA) that was determined independently by two pathologists (Zhao XD and Chen HL) who were blinded to the clinical features independently. PA was graded as follows: 0 (PA ≤ 20%), 1 (PA 21%-40%), 2 (PA 41%-60%), 3 (PA 61%-80%) and 4 (PA > 81%). Then the intensity of staining (IS) was evaluated in hot spots at high-power magnification and was scored as: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The Cav-1 and Gli-1 intensity distribution (ID) scores for each case were calculated by the following equation: ID = PA × IS, where ID ≤ 4 represented negative (−) or low expression and ID > 4 represented positive (+) or high expression. This standard was applied for Cav-1 and Gli-1, which could well present the relationship between the markers and the clinical characteristics.

Statistical analysis

SPSS Statistics software package, version 19.0 (Chicago, IL, USA) was used for the statistical analysis. Demographic characteristics were summarized by count and percentage for categorical variables, and comparisons were performed by Chi-square test or Fisher’s exact test. For binary categorical data, the phi coefficient, a measure of the degree of association between two binary variables, were used to determine association between two markers. All statistic assessments were evaluated at a two-sided P value of 0.05.

Results

Demographics and clinical characteristics

Demographic and clinical characteristics of SCLC patients were summarized in Table 1. Seventy SCLC patients entered the study, including 13 (18.6%) women and 57 (81.4%) men. The largest group of patients in the study was younger than 60 years of age (62.9%). Fifty-eights (82.9%) had shallow (T1/T2) invasion. Forty-two (60.0%) had no lymph node invasion. Fifty-nine (84.3%) had...
Gli-1 and Caveolin-1 Expression in SCLC

Gli-1 expression was present only at a low level. In SCLC, 67.1% (47/70) of the specimens were positive for Gli-1, which was a significantly higher than that in normal lung tissues. Cav-1 was expressed in both SCLC tissues and normal alveolar and bronchial epithelium. In tumor tissues, Cav-1 was predominantly expressed on the cell membrane or in the cytoplasm. In normal lung tissues, Cav-1 was also expressed in the fibroblasts, however, Cav-1 was absent at most of stromal fibroblasts of SCLC. There was negative Cav-1 expression in inflammatory cells, but positive expression in vascular endothelial cell (Figure 2). Our results showed that forty-nine (70.0%) patients had positive Cav-1 expression, and forty-four (62.9%) patients had negative fibroblastic Cav-1 expression.

We also evaluated the clinicopathological significance between negative and positive groups for these markers. From Table 2 showed, just found both of Gli-1 and Cav-1 positive expression group in the tumor cells had a higher percentage with pTNM stage III/IV of SCLC (both P ≤ 0.05).

Association between Gli-1 expression with Cav-1/fibroblastic Cav-1 expression in SCLC

The association between Gli-1 and Cav-1 expression was shown in Table 3. There was a positive association between Gli-1 and Cav-1 expression (P < 0.001, phi coefficient = 0.737); and a negative association between Gli-1 and fibroblastic Cav-1 expression (P < 0.001, phi coefficient = 0.737).

Table 1. Patient Characteristics (N=70)

| Characteristics       | Sub-characteristics | Value (%) |
|-----------------------|---------------------|-----------|
| Age (years)           |                     | 54 (range = 19-73) |
| Gender                | Male                | 57 (81.4) |
|                       | Female              | 13 (18.6) |
| Invasion deep (T)     | T1                  | 1 (1.4)   |
|                       | T2                  | 57 (81.5) |
|                       | T3                  | 11 (15.7) |
|                       | T4                  | 1 (1.4)   |
| Lymph node metastasis | N0                  | 42 (60.0) |
|                       | N1                  | 25 (35.7) |
|                       | N2                  | 3 (4.3)   |
| Distant metastasis (M)| M0                  | 69 (98.6) |
|                       | M1                  | 1 (1.4)   |
| pTNM stage            | I                   | 29 (41.4) |
|                       | II                  | 30 (42.9) |
|                       | III                 | 10 (14.3) |
|                       | IV                  | 1 (1.4)   |
| Total                 |                     | 70 (100)  |

pTNM stage I / II SCLC.

Expression of Gli-1, Cav-1 and fibroblastic Cav-1

Gli-1 was mainly expressed in SCLC tissues, was primarily localized in the cytoplasm and or cell nucleus. There was almost negative Gli-1 expression in the stromal fibroblasts of SCLC or inflammatory cells, but there was positive Gli-1 expression in vascular endothelial cell (Figure 1). In normal alveolar and bronchial epithelium, Gli-1 expression was present only at a low level. In SCLC, 67.1% (47/70) of the specimens were positive for Gli-1, which was a significantly higher than that in normal lung tissues.

Cav-1 was expressed in both SCLC tissues and normal alveolar and bronchial epithelium. In tumor tissues, Cav-1 was predominantly expressed on the cell membrane or in the cytoplasm. In normal lung tissues, Cav-1 was also expressed in the fibroblasts, however, Cav-1 was absent at most of stromal fibroblasts of SCLC. There was negative Cav-1 expression in inflammatory cells, but positive expression in vascular endothelial cell (Figure 2). Our results showed that forty-nine (70.0%) patients had positive Cav-1 expression, and forty-four (62.9%) patients had negative fibroblastic Cav-1 expression.

We also evaluated the clinicopathological significance between negative and positive groups for these markers. From Table 2 showed, just found both of Gli-1 and Cav-1 positive expression group in the tumor cells had a higher percentage with pTNM stage III/IV of SCLC (both P ≤ 0.05).
Table 2. Demographics and Clinical Characteristics of SCLC Patients According to their Gli-1 and Cav-1 Expression

| Parameters | All patients (n=70) | Gli-1 Positive (n=47) | Cav-1 Positive (n=49) | Fibroblastic Cav-1 Positive (n=26) |
|-----------|---------------------|----------------------|----------------------|-----------------------------------|
| Age       |                     |                      |                      |                                   |
| <60 years | 44 (62.9%)          | 14 (60.9%)           | 13 (61.9%)           | 21 (81.1%)                       |
| ≥60 years | 26 (37.1%)          | 9 (39.1%)            | 8 (38.1%)            | 5 (19.2%)                        |
| Gender    |                     |                      |                      |                                   |
| Women     | 13 (18.6%)          | 7 (30.4%)            | 6 (28.6%)            | 5 (19.2%)                        |
| Men       | 57 (81.4%)          | 16 (69.7%)           | 15 (71.4%)           | 21 (81.1%)                       |
| Invasion deep |                |                      |                      |                                   |
| T1/T2     | 58 (82.9%)          | 21 (91.3%)           | 20 (95.2%)           | 13 (61.9%)                       |
| T3/T4     | 12 (17.1%)          | 2 (8.7%)             | 1 (4.8%)             | 3 (11.5%)                        |
| Lymph node metastasis |          |                      |                      |                                   |
| N0        | 42 (60.0%)          | 14 (60.9%)           | 13 (61.9%)           | 26 (91.3%)                       |
| N1/N2     | 28 (40.0%)          | 9 (39.1%)            | 8 (38.1%)            | 3 (11.5%)                        |
| pTNM stage | 0.029*              | 0.146                | 0.145                | 0.53                             |
| I/II      | 59 (84.3%)          | 23 (100.0%)          | 21 (100.0%)          | 10 (38.5%)                       |
| III/IV    | 11 (15.7%)          | 0 (0.0%)             | 0 (0.0%)             | 21 (81.1%)                       |

Data expressed as count and percentage for categorical variables, and were performed by Chi-square test or Continuity correction; *P <0.05 between negative and positive groups

Table 3. Association between Gli-1 with Cav-1 and Fibroblastic Cav-1 Expression

| Gli-1 | Cav-1 | Fibroblastic Cav-1 |
|-------|-------|--------------------|
|       | Positive | Low | High |
|       | P value | P value | P value |
| Negative | 18 (78.3%) | 3 (6.4%) | 2 (8.7%) |
| Positive | 5 (21.7%) | 44 (93.6%) | 21 (91.3%) |

P<0.05 between Gli-1 with Cav-1 and fibroblastic Cav-1 expression coefficient = -0.784.

Discussion

SCLC is an aggressive malignancy belonging to lung cancer, which remains the first cause of death in the malignant carcinomas. In this study, we assessed the expression of Gli-1 and Cav-1 in SCLC for the first time, and the results revealed that forty-seven (67.1%) patients had positive Gli-1 expression, and forty-four (62.9%) patients had negative fibroblastic Cav-1 expression. Gli-1 was related to TNM stage of SCLC (P = 0.029). What’s more, we first revealed the relationship between the Hh signaling pathway and autophagy in SCLC: the Hh pathway marker Gli-1 increased markedly and was found to be associated with the autophagy-related marker fibroblastic Cav-1 in a negative way(P < 0.001, phi coefficient = -0.784).

The Hh signaling pathway plays an important role in embryonic development, formation of mature organs, and maintenance of morphology. Abnormal activation of the Hh signaling pathway is closely related to the occurrence, invasion, and metastasis of some cancers, including SCLC. Watkins et al., (2003); Abe and Tanaka, (2016) Gli proteins, belong to the zinc finger protein family and act as the downstream regulatory factors of classic Hh signaling pathway. Hui and Angers, (2011) Gli proteins are vital for embryogenesis and adult homeostasis. Matise and Joyner, (1999) On the other hand, as a member of Gli families, the aberrant expression of Gli-1 will promote carcinogenesis, according to epithelial-mesenchymal transition or angiogenesis or other channels. Cui et al., (2012) Abnormal expression of Gli-1 has been discovered in various types of tumors. In recent years, more and more researches focus on Gli-1 and take it as therapeutic targets. Inhibitor of classical Hh pathway such as cyclopamine is also further explored in cancer targeted therapy (Chen, 2016)

Autophagy plays a significant role in maintaining quality control, sustaining cell homeostasis as well as providing energy and nutrients. On the other hand, autophagy can also promote tumor in established cancer through autophagy-mediated intracellular recycling. Levine, (2007) Caveolin-1 (Cav-1), with multiple binding partners, localized in membrane subdomains called caveolae, is a multifunctional scaffolding protein. Fu et al., (2017) Increasing evidence suggests that Cav-1 regulates multiple cancer-associated processes, including cell proliferation, migration and metastasis, cell apoptosis and survival, mutations through the interactions with all these well-known factors, and multidrug resistance. Yeh et al., (2009) It has been reported recently that the loss of Cav-1 in the tumor stroma brings about an activation of tumor microenvironment, which is significantly related to early tumor recurrence, metastasis, and poor clinical outcome in cancer. Shi et al., (2016) Mean while, researches illuminated that tumor proliferation and progression involve autophagy in tumor stromal fibroblasts and Cav-1 expression deficiency. He et al., (2012) In that case, we can draw a conclusion that the loss
of stromal Cav-1 results in the metabolic reprogramming of cancer-associated fibroblasts, primarily caused by activated autophagy in fibroblasts, which has been proved in present investigations (Guan et al., 2016).

In this research, we have explored the association between the expression of Hh signaling pathway and autophagy-related gene in SCLC, and showed that the high expression level of the Hh pathway marker Gli-1 was related to the loss of the autophagy-related marker Cav-1 in the tumor stroma. Consequently, it can be ascertained that in the invasion and metastasis of SCLC, abnormal activation of the Hh signaling pathway is closely related to the induction of autophagy. Hence, we conjecture that Hh signaling pathway may bring about an activation of tumor microenvironment via autophagy, and also provides an alternative therapeutic strategy for SCLC.

Interestingly, during the analysis of the experimental results, a positive association between Gli-1 and Cav-1 expression in tumor cells was discovered. We proposed a possible mechanism to explain this association: Cav-1 mediated Gli-1 expression through PI3K/AKT/mTOR pathway. On the one hand, Cav-1 regulates PI3K/AKT signaling pathway which involved in cancer initiation and progression. PI3K/AKT pathway motivates regulation of proliferation, survival and inhibition of apoptosis, which is important in carcinogenesis. Ersahin et al., (2015) PI3K/AKT pathway is aberrantly activated in different tumor entities, providing a unique foundation for pharmacological target. Li et al., (2016b); Sharma et al., (2017) and Liang et al., (2014) found that Cav-1 activation-induced PI3K/AKT signaling pathway promoted an invasive phenotype in bladder cancer cells. Yang et al., (2016) revealed that Cav-1 could be activated by low shear stress (LSS) to trigger PI3K/AKT/mTOR pathway in breast carcinoma MDA-MB-231 cells. Yang et al., (2016) Consequently, we suppose that PI3K/AKT signaling pathway can be activated by Cav-1 in pathogenesis, invasion and metastasis of tumors. On the other hand, PI3K/AKT pathway also upregulates Gli-1 in a Smo-independent manner. It has been demonstrated that PI3K-AKT can lead to mTORC-mediated phosphorylation of 70S6K, which cause an abolishment of GSK3β-dependent Gli-1-degradation. Kebenko et al., (2015) To be consistent, Smo-independent Gli-1 activation has also been reported in renal cell carcinoma, esophageal adenocarcinoma, and refractory acute myeloid leukemia, which are mediated by the PI3K/AKT signaling pathway. Kebenko et al., (2015); Li et al., (2016a) and Zhou et al., (2016) Therefore, we assume that in the occurrence and metastasis of SCLC, Gli-1 expression is possible to be mediated by the Cav-1 regulation of PI3K/AKT pathway. However, it is better to have some in vitro results to identify this assumption, which is a limitation of this study. Our findings highlight the complicate role of Gli-1 in carcinogenesis. This explains why approaches aiming to block Hh signaling pathway have met with limited success. Thus, we envision that novel targets may come with the further investigation of Cav-1/PI3K/AKT/Gli-1 pathway in carcinogenesis.

In conclusion, this was the first study that demonstrated the expression of Gli-1 and Cav-1 in SCLC patients. Our data elucidated that the high expression level of Gli-1 is related to the low levels of fibroblastic Cav-1 in SCLC, which identified that Hh signaling pathway induced autophagy in the occurrence and metastasis of SCLC. Our results indicate the clinical importance of the expression of Gli-1 and fibroblastic Cav-1 and will potentially attract more attention to the exploration of the complicate tumor microenvironment.

Conflict of interests
The authors declare no conflict of interest.

Acknowledgments
This work was supported by grants from the National Undergraduate Innovation Project of China (No. 201510486090) and Innovation Project of Wuhan University Medical School (No. MS2015004), and Public Welfare Technology Application Research of Zhejiang Province (No. 2016C33236).

References
Abe Y, Tanaka N (2016). The hedgehog signaling networks in lung cancer: The mechanisms and roles in tumor progression and implications for cancer therapy. Biomed Res Int, 2016, 7969286.

Chen JK (2016). I only have eye for ewe: the discovery of cyclopamine and development of Hedgehog pathway-targeting drugs. Nat Prod Rep, 33, 595-601.

Cochran CR, Szczepny A, Watkins DN, et al (2015). Hedgehog signaling in the maintenance of cancer stem cells. Cancers (Basel), 7, 1554-85.

Cui D, Chen X, Yin J, et al (2012). Aberrant activation of Hedgehog/Gli1 pathway on angiogenesis in gliomas. Neuro India, 60, 589-96.

Ersahin T, Tuncbag N, Cetin-Atalay R (2015). The PI3K/AKT/mTOR interactive pathway. Mol Biosyst, 11, 1946-54.

Fu P, Chen F, Pan Q, et al (2017). The different functions and clinical significances of caveolin-1 in human adenocarcinoma and squamous cell carcinoma. Oncol Targets Ther, 10, 819-35.

Goldstraw P, Chansky K, Crowley J, et al (2016). The IASLC lung cancer staging project: Proposals for revision of the TNM stage groupings in the forthcoming (Eighth) edition of the TNM classification for lung cancer. J Thorac Oncol, 11, 39-51.

Guan J, Yuan Z, He J, et al (2016). Overexpression of caveolin-1 reduces Taxol resistance in human osteosarcoma cells by attenuating PI3K-Akt-JNK dependent autophagy. Exp Ther Med, 12, 2815-22.

Hanna A, Shevde LA (2016). Hedgehog signaling: modulation of cancer properties and tumor microenvironment. Mol Cancer, 15, 24.

He Y, Zhao X, Gao J, et al (2012). Quantum dots-based immunofluorescent imaging of stromal fibroblasts Caveolin-1 and light chain 3B expression and identification of their clinical significance in human gastric cancer. Int J Mol Sci, 13, 13764-80.

Hong Z, Bi A, Chen D, et al (2014). Activiation of hedgehog signaling pathway in human non-small cell lung cancers. Pathol Oncol Res, 20, 917-22.

Hui CC, Angers S (2011). Gli proteins in development and disease. Annu Rev Cell Dev Biol, 27, 513-37.

Ingham PW, McMahon AP (2001). Hedgehog signaling in animal development: paradigms and principles. Genes Dev,
Jie Wu et al.

15, 3059-87.

Kebenko M, Drenckhan A, Gros SJ, et al (2015). ErbB2 signaling activates the Hedgehog pathway via PI3K-Akt in human esophageal adenocarcinoma: identification of novel targets for concerted therapy concepts. Cell Signal, 27, 373-81.

Koinis F, Kotsakis A, Georgoulas V (2016). Small cell lung cancer (SCLC): no treatment advances in recent years. Transl Lung Cancer Res, 5, 59-50.

Lei J, Fan L, Wei G, et al (2015). Gli-1 is crucial for hypoxia-induced epithelial-mesenchymal transition and invasion of breast cancer. Tumour Biol, 36, 3119-26.

Levine B (2007). Cell biology: autophagy and cancer. Nature, 446, 745-7.

Levine B, Kroemer G (2008). Autophagy in the pathogenesis of disease. Cell, 132, 27-42.

Li X, Chen F, Zhu Q, et al (2016a). Gli-1/PI3K/AKT/NF-kB pathway mediates resistance to radiation and is a target for reversion of responses in refractory acute myeloid leukemia cells. Oncotarget, 7, 33004-15.

Li X, Wu C, Chen N, et al (2016b). PI3K/Akt/mTOR signaling pathway and targeted therapy for glioblastoma. Oncotarget, 7, 33440-50.

Liang W, Hao Z, Han JL, et al (2014). CA V-1 contributes to bladder cancer progression by inducing epithelial-to-mesenchymal transition. Urol Oncol, 32, 855-63.

Martinez-Outschoorn U, Sotgia F, Lisanti MP (2014). Tumor microenvironment and metabolic synergy in breast cancers: critical importance of mitochondrial fuels and function. Semin Oncol, 41, 195-216.

Martinez-Outschoorn UE, Trimmer C, Lin Z, et al (2010). Autophagy in cancer associated fibroblasts promotes tumor cell survival: Role of hypoxia, HIF1 induction and NFkappaB activation in the tumor stromal microenvironment. Cell Cycle, 9, 3515-33.

Matise MP, Joyner AL (1999). Gli genes in development and cancer. Oncogene, 18, 7852-9.

Pabhu VV, Warfel NA, El-Deiry WS (2012). CTGF-mediated autophagy-senescence transition in tumor stroma promotes anabolic tumor growth and metastasis. Cell Cycle, 11, 2592-3.

Santarpia M, Daffina MG, Karachaliou N, et al (2016). Targeted drugs in small-cell lung cancer. Transl Lung Cancer Res, 5, 51-70.

Sharma VR, Gupta GK, Sharma AK, et al (2017). PI3K/Akt/mTOR intracellular pathway and breast cancer: Factors, mechanism and regulation. Curr Pharm Des, 23, 1633-8.

Shi D, Liu Y, Xi R, et al (2016). Caveolin-1 contributes to realgar nanoparticle therapy in human chronic myelogenous leukemia K562 cells. Int J Nanomedicine, 11, 5823-35.

Torre LA, Bray F, Siegel RL, et al (2015). Global cancer statistics, 2012. CA Cancer J Clin, 65, 87-108.

Wang ZS, Shen Y, Li X, et al (2014). Significance and prognostic value of Gli-1 and Snail/E-cadherin expression in progressive gastric cancer. Tumour Biol, 35, 1357-63.

Watkins DN, Berman DM, Burkholder SG, et al (2003). Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. Nature, 422, 313-7.

White E (2012). Deconvoluting the context-dependent role for autophagy in cancer. Nat Rev Cancer, 12, 401-10.

Yang H, Guan L, Li S, et al (2016). Mechanosensitive caveolin-1 activation-induced PI3K/Akt/mTOR signaling pathway promotes breast cancer motility, invadopodia formation and metastasis in vivo. Oncotarget, 7, 16227-47.

Yeh D, Chen C, Sun MZ, et al (2009). Caveolin-1 is an important factor for the metastasis and proliferation of human small cell lung cancer NCI-H446 cell. Anat Rec (Hoboken), 292, 1584-92.

Zhou J, Zhu G, Huang J, et al (2016). Non-canonical GLI1/2 activation by PI3K/AKT signaling in renal cell carcinoma: A novel potential therapeutic target. Cancer Lett, 370, 313-23.

This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.