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TUSC7 suppression of Notch activation through sponging MiR-146 recapitulated the asymmetric cell division in lung adenocarcinoma stem cells

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ABSTRACT

Aims: Lung adenocarcinoma consists of multiple therapeutic targets, however, patients will inevitably progress to later stage diagnosis with Tyrosine Kinase Inhibitor treatment resistance. We aim to investigate the roles of non-coding TUSC7 in ordering the cell division tendency, helping to sensitize the resistance in a miRNA incorporating way.

Materials and methods: Online study of bioinformatics analysis, molecular experiments of luciferase test, immunofluorescence staining and qRT-PCR were applied to dig out the mechanistic regulations.

Key findings: TUSC-7 inhibited the renewal ability of adenocarcinoma stem cells, yielding to asymmetric cell splitting. Informatics analysis and the luciferase testing confirmed the 3′UTR binding site, and revealed the post-transcriptional regulation of NUMB referring to miR-146. TUSC-7 sponged miR-146 and abolished its degradation toward to NUMB, and this integrated cascade made several genes become tangled to full functionality.

Significance: TUSC-7 was proved to be one strong suppressive lnc-RNA in lung adenocarcinoma stem cells, functioning through inactivating NOTCH signaling, and the turbulence on division modes precisely pointed to the key mechanisms of stem cells' renewal. The decreasing of tumor suppressive miR-146 was necessary in TUSC-7 conducted renewal repression, despite it alone could also reduce the renewal efficiency, indicating that more complicated non-coding genes may be involved in its regulation.

1. Introduction

Lung cancer is the most dangerous cancer type worldwide, as it ranks the highest among cancer-related mortalities [1,2]. Exploring more sensitive screening strategies, improving the radical operation methods, and developing more effective adjuvant therapeutic agents, are all urgent and valuable [3,4]. Lung cancer is mainly composed of non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), and lung adenocarcinoma of NSCLC is quite different from other types of lung cancer, consisting of multiple therapeutic targets for implementing the precision medicine, making therapy easier, less painful, and more beneficial [5–8]. However, patients inevitably progress to later stage diagnosis with Tyrosine Kinase Inhibitor (TKI) treatment resistance. Identifying and applying new therapeutic agents, targeting at novel and crucial molecular nodes, improving the existing therapy strategy, may all provide new ways of effective therapy.

MiRNAs have been given full attention for decades, since the post-translational regulation was identified in cellular biology. LncRNAs (long non-coding RNAs) and circRNAs (circular RNAs) were later revealed for supplementing the RNA family of non-coding members

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[9–11]. Individually, they may act as a crucial modulator to certain downstream genes [12,13]. Alternatively, they could also coordinate with each other to exert stronger inhibition, acting the opposite manner [14,15]. Cancer stem-like cells, also known as cancer initiating cells, were treated as the root for malignancy initiation, development, progression, and recurrence. Targeting at this subtype of cells group provided the potential and prospective ways of perishing the entire cancer family [16–18]. The functions of miRNAs and lncRNAs in cancer dysregulations were hotspots when looking into the micro-connections between coding and non-coding genes. In this study, we explored the role of miR-146 when TUSC7 exerted its suppressive function on stem cells expansion, tried to find one possible signaling that would affect the TKI treatment sensitivity. We successfully established the accessory mechanism of miRNA in lncRNA dominated stem cells' regulation, indicating one new possible theory of stem cells' renewal regulation.

2. Methods and materials

2.1. Cell culturing, mimics transfection and Lentiviral transduction

Non-small cell lung adenocarcinoma cancer cells lines of A549 and H1299, and HEK-293T were purchased from American Type Culture Collection and were maintained in DMEM (Gibco) or in RPMI 1640 Medium (Hyclone). 10% FBS (Gibco), 1% penicillin and 1% streptomycin (Hyclone) were be supplemented into these medium. For constructing cells with enforced TUSC7, a genomic region encoding TUSC7 was PCR-amplified using PrimeSTAR® HS DNA Polymerase (TaKaRa) and subcloned into the pcDNA3.1 vector (Invitrogen). To obtain cell lines stably expressing TUSC7, cells were transfected with the plasmid pcDNA3.1-TUSC7 or pcDNA3.1 vector by using Lipofectamine 3000 according to the manufacturer instruction. Cells were selected with neomycin (800 μg/ml) for two to four weeks.

2.2. Stem cells isolation and culturing

For studying of the stem cells' potency and features, the pretreated cells were suspended in DMEM/F12Medium, supplemented with 20 ng/ml EGF (BD Biosciences), bFGF and 4 mg/ml insulin (Sigma), and then group cells were plated in 6 cm ultra-low attachment dishes (500–1000cells/ml, Corning). After 10 days of culture, the number of tumor spheroids was counted under a Nikon Eclipse TE2000-S microscope (Nikon Imaging, Japan). The sphere formation efficiency was calculated as the percentage ratio of obtained spheres and plated cells. Single cell could be acquired by using Accutase (StemPro, GIBCO), and the daughter stem cell was kept in DMEM/F12Medium, supplemented with 20 ng/ml EGF (BD Biosciences), bFGF and 4 mg/ml insulin (Sigma).

2.3. Quantitative real-time PCR and western blot

Total mRNA was reverse-transcribed into cDNA by using a RT-PCR kit (AT301 TransGen Biotech), and real-time quantitative PCR (RT-qPCR) was performed with CFX96 Real-Time PCR Detection System (Bio-Rid). For western blot, the protein from cell extracts were separated by 10% SDS-PAGE electrophoresis, and was later transferred onto PVDF membrane. Membranes were incubated with Notch1 (1:5000; Val1744, D3B8, Rabbit mAb #4147, Cell Signaling Technology), Numb (1:2000, ab4147, Abcam), Vinculin (1:8000, #18799, Cell Signaling Technology), Jagged1 (1:1500, ab7771, Abcam), Snai1(1:1500, pSer246, SAB4504319, Sigma-Aldrich), OCT4(1:2000, 3H8L6, ABfinity™ Rabbit Monoclonal, Invitrogen, ThermoFisher), SOX2(1:3000, ab97959, Abcam), and then detected using ECL Blotting Detection Reagents (Merck Millipore). Cells of different groups were suspended in DMEM/F12 Medium supplementing with 20 ng/ml EGF (BD Biosciences), bFGF and 4 μg/ml insulin (Sigma), and then plating at 1000 cells/ml in 6-well ultra-low attachment dishes (Corning Incorporated) for about one week. To analyze the self-renewal ability, spheres number was counted by using phase contrast microscope (Nikon).

2.4. Immunofluorescence and Edu staining

The cells were fixed in 4% formaldehyde, washed with PBS for 15 min, and permeabilized with 0.2% Triton X-100 for 20 min. After permeabilization, the cells were blocked with bovine serum albumin (BSA) at 37 °C for 30 min. Fixed cells were incubated with the antibodies against CCND1(1:100, #PA5-88063, ThermoFisher), DICER1(1:100, #MA5-31353, ThermoFisher), H3K9ME2(1:50, #1220, Abcam), at 4 °C overnight, followed by Alexa Fluor 594 goat anti-rabbit IgG (H + L) Secondary Antibody (1:1000, A-11012, Life Technologies, Gaithersburg) for 1 h at room temperature. The nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI, 1:15000; 4084; Cell Signaling). For EdU labeling assay, cells of different groups were seeded into chamber slides with RS glass (Nunc, Thermo Scientific) after treatments. EdU intensity was detected after cells being incubated with 10uM EdU for 24 h. Detection was performed with using Click-iT Plus EdU Alexa imaging Kit (MP 10637, Life Technologies). The fluorescence images were obtained using an Olympus microscope.

2.5. Dual luciferase report assay

Luciferase reporter assay was used to detect the proposed binding site between TUSC7 and miRNAs. PmiR-RB-Report vector (Promega) containing renilla luciferase gene (hRluc) and firefly luciferase gene (hLuc+) were applied in this experiment. The 3′UTR sequence of TUSC7 was cloned downstream of hRluc cassette. Mutations were performed in the binding sites. The miR146 mimics were obtained from GenePharma (Shanghai, China). hLuc+ cassette was used as internal control. Each miRNA or negative control oligonucleotide was co-transfected with pmiR-RB-Report vector with or without the 3′UTR sequence of TUSC7. Finally, the relative light units (RLU) of hRluc and hLuc+ were determined by Tecan M1000, and the hRluc values were normalized to the corresponding hLuc+ values.

GP-miRGLO reporter assay was used to verify NUMB as a direct target of miR-146. MiR-146 mimics and negative control oligonucleotides were co-transfected with pcDNA3 reporter vector respectively, containing the wild-type 3′UTR of NUMB or mutant 3′UTR NUMB. EGFP expression levels were normalized by RFP values.

2.6. Statistical analysis

Numerical data were presented as mean ± standard deviation (SD), of three to six independent experiments. Statistical analysis was carried out by using Graph Pad Prism 6 and Microsoft Excel software. Differences between groups were assessed by using Student's *t*-test or ANOVA individually. $P < 0.05$ was statistically significant.

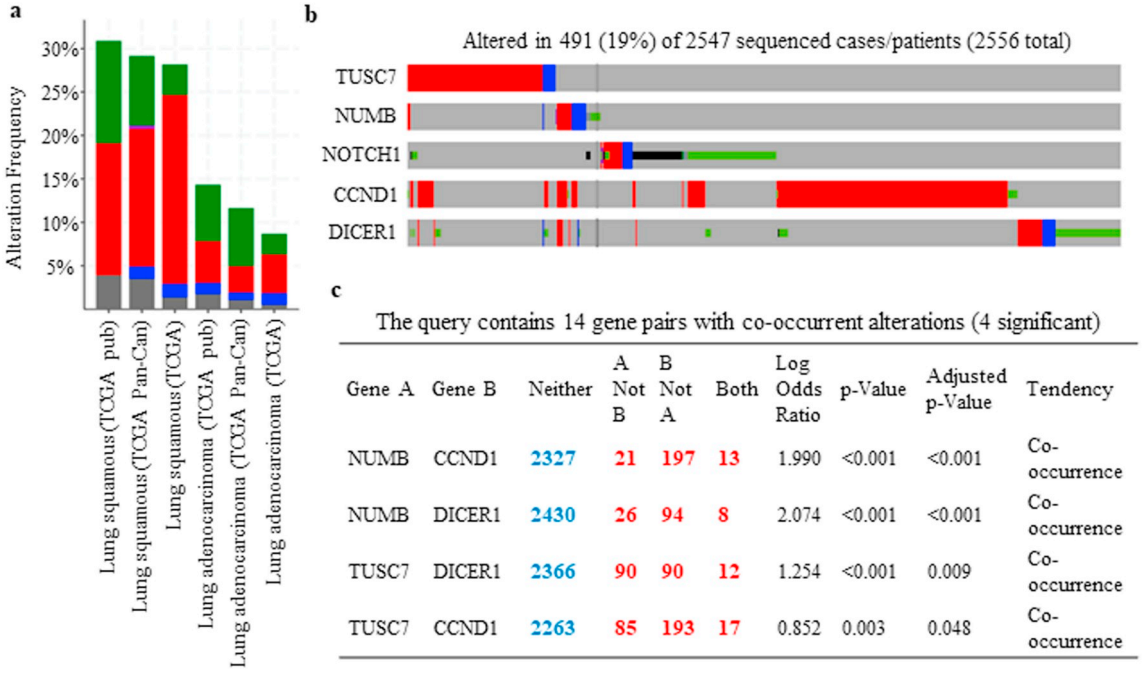
3. Results

3.1. Clinical identification of lower expressed TUSC7 in NSCLC

Suppressed TUSC7 expression in non-small cell lung cancer (NSCLC) was confirmed with an established public data base (http://www.cbioportal.org/) [19,20], and in specimens of NSCLCs. Higher TUSC7 levels, which are usually abnormal and rare in malignancies, imply better survival expectations and lower progression risk (Fig. S1a–e). Specifically, a longer survival prognosis is much clearer when analyzing TUSC7 in lung adenocarcinoma tissues (Fig. S1b, e).

3.2. Connections between TUSC7 and its related cascade factors

Based on further analysis using CANCER GENOMICS online data



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The query contains 14 gene pairs with co-occurrent alterations (4 significant)

Gene A	Gene B	Neither	A Not B	B Not A	Both	Log Odds Ratio	p-Value	Adjusted p-Value	Tendency
NUMB	CCND1	2327	21	197	13	1.990	<0.001	<0.001	Co-occurrence
NUMB	DICER1	2430	26	94	8	2.074	<0.001	<0.001	Co-occurrence
TUSC7	DICER1	2366	90	90	12	1.254	<0.001	0.009	Co-occurrence
TUSC7	CCND1	2263	85	193	17	0.852	0.003	0.048	Co-occurrence

Fig. 1. Clinical identification of TUSC7 and Notch signalings.
a. Six data bases from CANCER GENOMICS were enrolled for expression pattern analysis. b. Heat map was set up to reveal the potential connections among Notch factors and TUSC7. c. Table was drafted to show the related alternations and the negative connections between NUMB and CCND1, NUMB and DICER1, TUSC7 and DICER1, TUSC7 and CCND1 were significant.

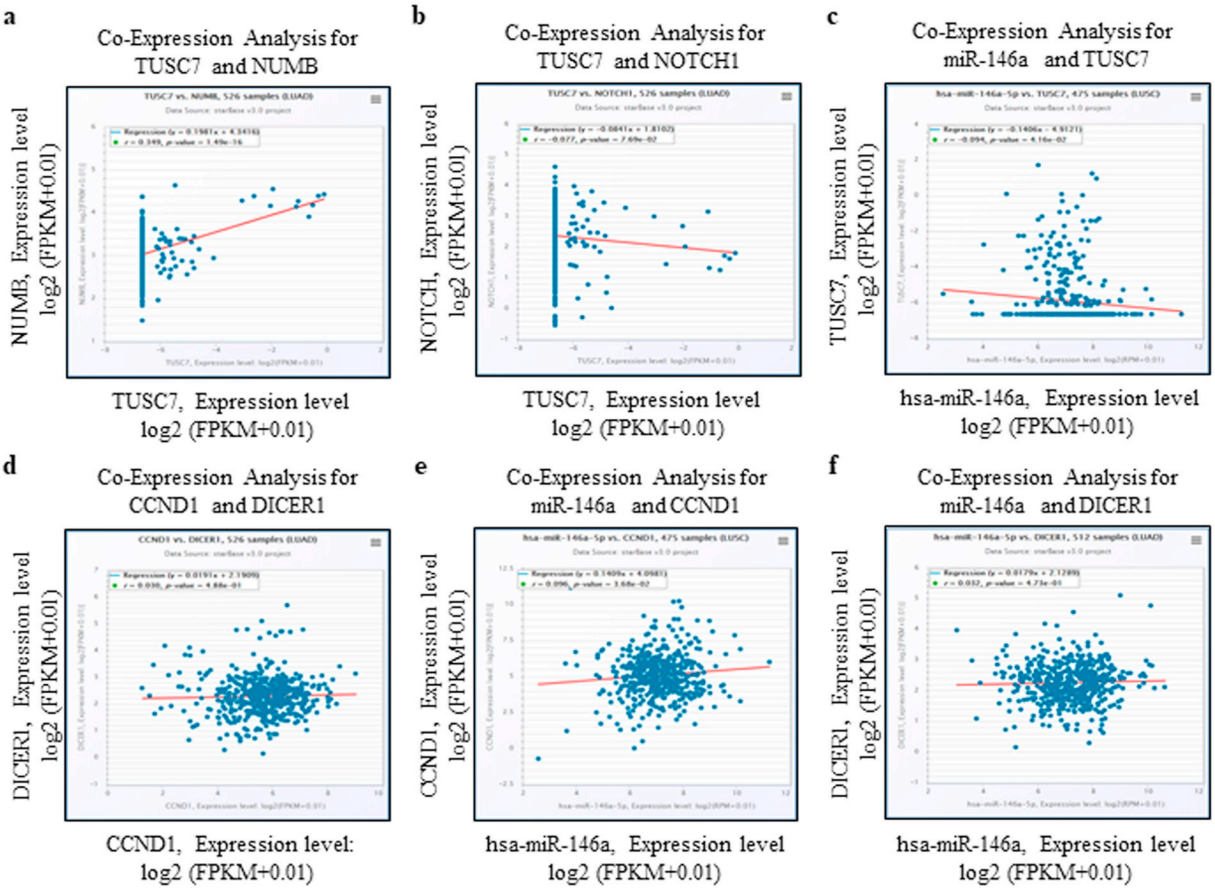

Fig. 2. Associations among cascade factors.
TUSC7 was positively related to NUMB, and was inversely correlated with NOTCH (b). Relationships among miR-146, DICER1, CCND1 were analyzed (c-d). CCND1 (e) and DICER1 (f) was both positively related to miR-146a expression.

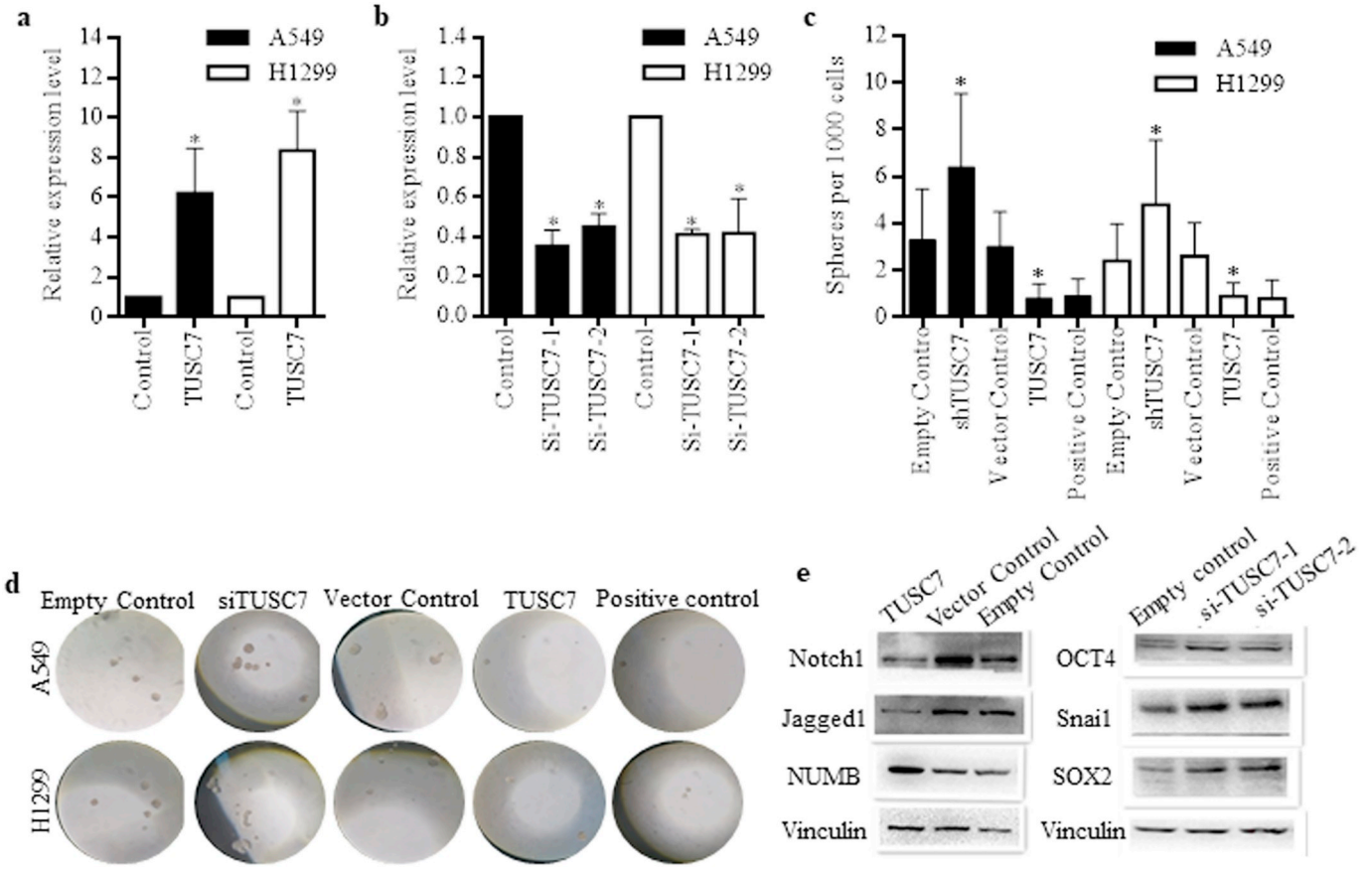

Fig. 3. TUSC7 suppressed stem cells' renewal.
Introducing TUSC7 (a) or inhibiting TUSC7 (b) into stem cells of lung adenocarcinoma changed the spheres forming efficiency greatly (c–d). e. TUSC7 inhibition promoted the NOTCH signaling and EMT factors expression.

(http://www.cbioportal.org/), a total of six databases consisted of more expansive sources of data on squamous lung cancer and adenocarcinoma tissues (Fig. 1a). Notch signaling was frequently activated in lung adenocarcinoma (Fig. S2), and heat mapping revealed its board expression signatures and relationships to associated factors (Fig. 1b). Potential relationships between TUSC7 and possible downstream affecters were drafted and shown in Fig. 1c, where the red labeling indicates a mutually exclusive existence with the exception of missing data (shown in bold blue).

3.3. Undefined miR-146a was revealed with special function referring to TUSC7

Expression pattern analysis supplemented the results of Fig. 1 and the relationship between TUSC7 and NUMB/NOTCH was consolidated with clinical data, helping to establish its suppressive roles (Fig. 2a–b). Relationships among miR-146, DICER1, CCND1 were analyzed (Fig. 2c–d). Premier clinical testing sequentially pointed to the possible cascade of TUSC7/NUMB/NOTCH, in which CCND1, DICER1 and miR-146a may be involved in generating a complicated circle loop or reciprocal affection (Fig. 2e–f).

MiR-146 was characterized with strong suppressive roles in breast cancer [21]; however, the role of miR-146a in lung cancers was uncertain. miR-146 appeared to have a positive link to CD133 stem cells markers (Fig. S3a, b), ALDH1A1 (Fig. S3c, d), KLF4 (Fig. S3e, f), SOX2 (data not shown), SOX4 (data not shown), and CMYC (data not shown). This strongly suggested its dual role in regard to renewing groups.

3.4. TUSC7 suppressed the stem cells expansion through Notch signaling inhibition

Introducing either TUSC7 (Fig. 3a) or siRNA (Fig. 3b) into the stem cells of NSCLSs resulted in expression level alternation. TUSC7 inhibition (Fig. 3c) increased the spheres number sequentially (Fig. 3c–d). Notch activation inhibitor (γ secretase inhibitor) was set as positive control to assess the Notch signaling suppression by TUSC7. TUSC7 inhibition greatly activated the renewal process through EMT factors overexpressed (Fig. 3e).

3.5. TUSC7 induced the stem cells' asymmetric division

Stem cells of ordinary nature styles tended to split asymmetrically, to maintain the mother group, and to give birth to the offspring generation (Fig. 4a). Cancer stem cells divided chaotically and often generated two identical daughter cells, as were identified and stained in Fig. 4b. Division manners were reorganized and assessed when introducing TUSC7 into stem cells. In A549 and H1299 stem cells, when a single cell was dispersed from the spheres group and adhered to low-attached slide the mother stem cells' splitting manner was affected by TUSC7 alternation, forcing TUSC7 asymmetric division, when comparing the control and negative control groups (Fig. 4c–d). Different division modes were associated with stem cells expansion of two tendencies (renewal triggering or arresting), as exhibited in Fig. 3e.

3.6. TUSC7 sponging of miR146 was critical for TUSC7/miR-146a cascade function

Bioinformatics established a possible connection between TUSC-7

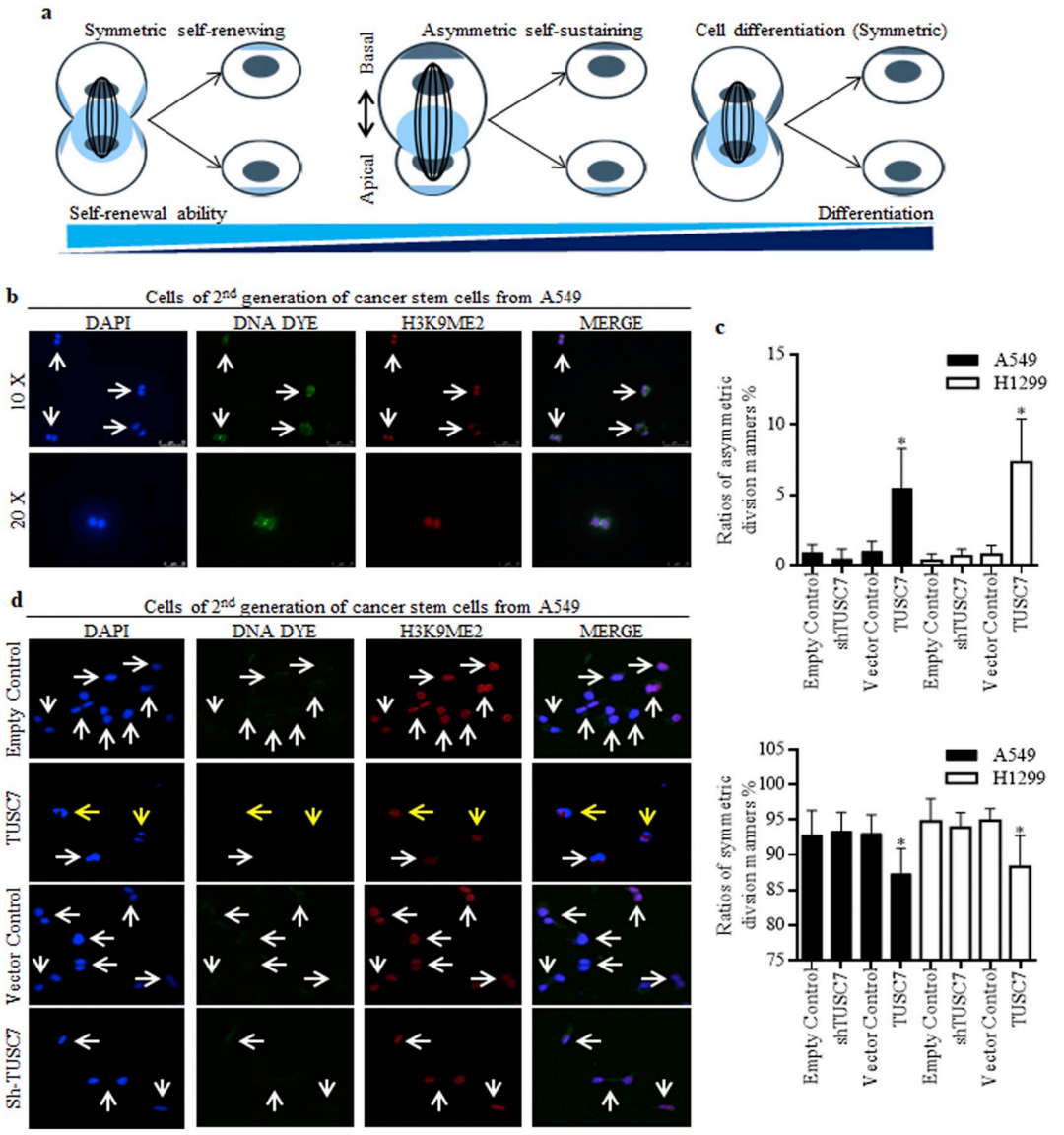

Fig. 4. Identification of division manners in lung adenocarcinoma stem cells.
a. The schematic illustration for imaging the division manners in stem cells. b. DNA dye and H3K9ME2 staining assays were applied to reveal the splitting status. d. TUSC7 enforcement stimulated the asymmetric division, and its inhibition released the symmetric nature of cancer stem cells.

and miR-146. TUSC7 was predicated to correlate with miR-146 at the possible binding site (Figs. S4, 5a). By completing sponging conjunction studies (referred to in Fig. 5b), miR-146 was mutated for its miRNA response element (Fig. 5c), and the co-transfection of luciferase reporters containing 3′UTR sequence and miR-146a mimics reduced nearly 60% of the luciferase intensity.

3.7. MiR-146 decreased NUMB expression post-transcriptionally

The alignment of miR-146a and 3′UTR of NUMB was constructed by using enhanced green fluorescent protein (EGFP) reporter assay (Fig. 5d). The wild-type 3′UTR sequence (Fig. 5e) and the mutant 3′UTR sequence (Fig. 5f) of NUMB were cloned downstream from the EGFP-coding sequence to construct a reporter plasmid and a mutant vector, and the co-transfection of miR-146a mimics and the wide-type reporter plasmid strongly reduced the EGFP expression. In TUSC7 domination of cancer suppression, miR-146 was combined to and blocked for its targeted degradation of NUMB (Fig. 5g), the strong and specific Notch signaling inhibitor. Reintroducing miR-146 into TUSC7 overexpressed cells alleviated the suppression (Fig. 5h).

3.8. CCND1-DICER1 activity helps to sustain TUSC7 expression

DICER1 activity was critical for sustaining the microRNAs and lncRNA expressions [22], and could be maintained by cyclin d1 existence as we proved in previous studies [23]. No direct binding site between 3′UTR of miR-146 and DICER1/CCND1 was ever found (data not shown), and could not be speculated by using software analysis (data not shown). The sponging of TUSC7 to miR-146 released controlling of NUMB expression (Fig. 5g–h), which manipulated the CCND1/DICER cascade inner cytoplasm (Fig. 6a–b) and decreased promoter activity (Fig. 6c–d). The absence of either CCND1 or DICER1 decreased TUSC7 (Fig. 6e), forming a closed circle to maintain balance (Fig. 6f).

4. Discussion

Interactions among varying types of non-coding genes attracted lots of attention because of the specific regulation modes (post-translational or sponge adsorption). Due to the various modulation manners, the connections could be identified in more complicated ways. We examined the unexplored non-coding world years ago, searching for

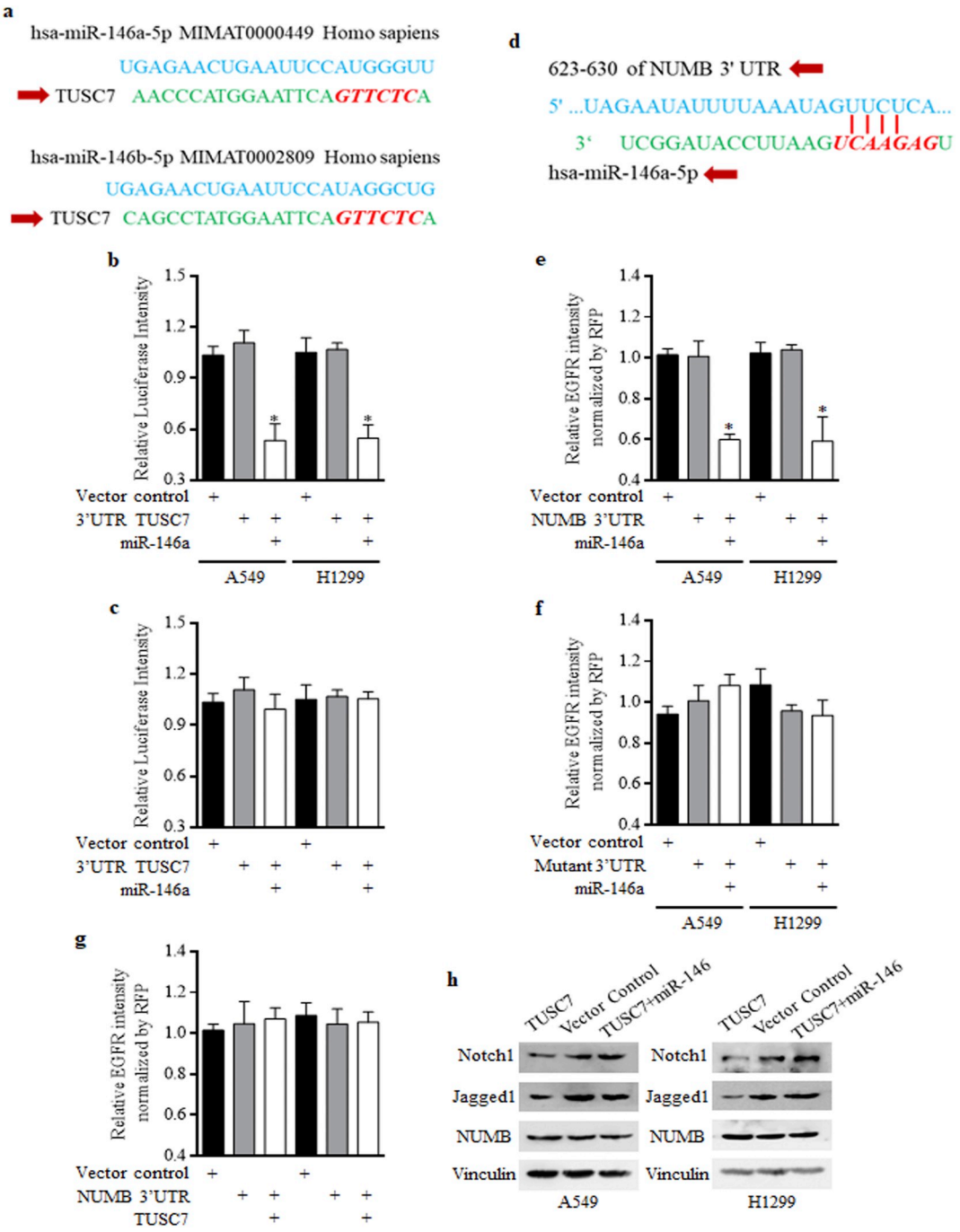

Fig. 5. TUSC7 suppressed the Notch signaling factors through sponging miR146.
a. Predication toward to the binding sited between TUSC7 and miR-146a was specially labeled for illustration. b. MiR-146 decreased the luciferase activity of wild type at TUSC7 3′UTR. c. The significant change was not detected in mutant type at TUSC7 3′UTR. d. The potential binding site at the 3′UTR of NUMB referring to miR-146 was showed. e. MiR-146 decreased the luciferase activity of wild type at NUMB 3′UTR. f. The significant change was not detected in mutant type at NUMB 3′UTR. g. Introducing TUSC7 saved the NUMB luciferase activity, defined by RFP normalized EGFR intensity. h. Overexpressing miR-146 greatly abolished the TUSC7 resulting of Notch signaling suppression.

various connection manners. In NSCLCs, stem cells expansions were not known well, and anticancer treatments seeking more efficient killing ratios encountered the bottle neck effect [24,25].

To our knowledge, the cancer group consists of heterogeneous subpopulations/ Stem cells [26,27], also named cancer stem-like cells, were considered the progenitor cells responsible for generating numerous daughter cells [28,29]. Immortalized cancer stem cells could divide symmetrically, giving birth to indefinite descendants [30]. Turbulence during the splicing stage retrieved asymmetric division, which was believed to shrink the stem cells' pool [31,32]. Traditional anticancer agents could only perish those pools at rapid proliferation, unwittingly enriching sleeping ones, awakening them to regenerate and repopulated.

The disturbance exerted by one specific or cluster of non-coding genes work to cut off the link between the splitting motor and splitting daughter cells, instead of massive perishing or toxic killing (which is against the strategy of “Precision Medicine”). In the current study, our group explored promising members that could support the “Precision

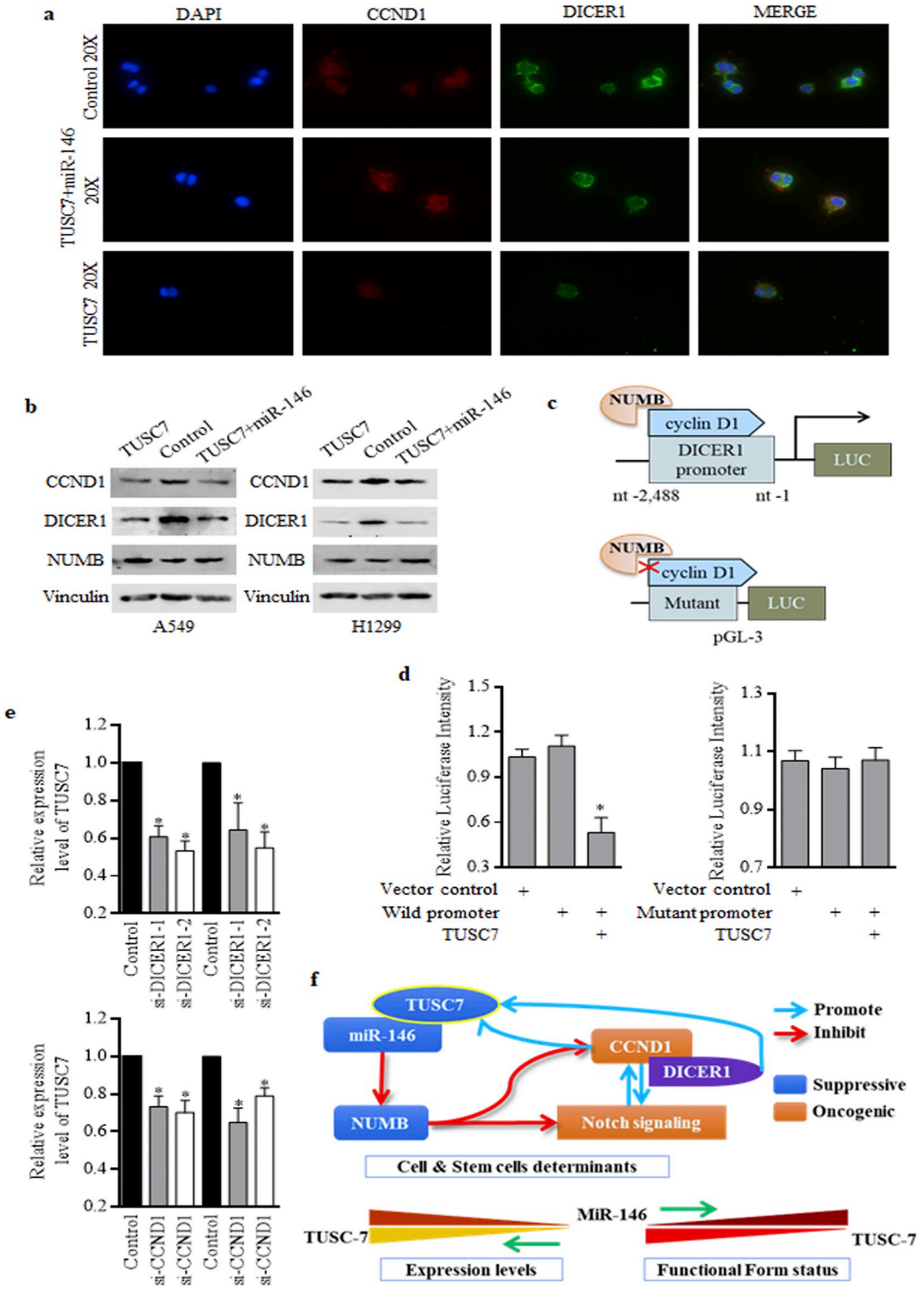

Fig. 6. Homeostasis of TUSC7 level was determined by its downstream cascade.
Immunofluorescence staining (a) and western blot (b) indicated thatTUSC7 decreased the cytoplasmic expression of CCND1 and DICER1, and reintroducing miR-146 saved their expression level. c. Schematic image of NUMB dependent DICER1 promoter activity. d. TUSC7 decreased the promoter activity of wild Dicer1. e. TUSC7 expression decreased significantly in A549 cells (above) and H1299 cells (below) when reducing the Dicer1 level. f. The feedback loop was drafted to illustrate the network, where TUSC7 and miR-146 functioned through.

Medicine" strategy. MiR-146 was disclosed with dual characters in various malignancy types and in lung cancer [33–36]. The good site took the upper hand. The role of TUSC-7 was rarely studied in lung cancer progression. Long non-coding RNA of TUSC-7 in lung cancer was tested with cancer suppressive affections in our study. Stem cells originated from different lung adenocarcinoma cell lines, and cell expansion was blocked from symmetric division cessation.

Bioinformatics testing and the Luciferase study confirmed the 3′UTR binding site, and revealed the post-transcriptional of NUMB referring to miR-146. MiR-146 was identified with oncogenic actions in colon cancer stem cells. Here we also confirmed its suppression on NUMB, one crucial member in restraining the NOTCH signaling. In lung cancer,

suppressive roles of miR-146 on malignancy were established with solid data from a public database and bench work; however, in cyto-network, it could only be treated as one downstream executor. Specifically, TUSC-7 sponged miR-146 and abolished its degradation toward to NUMB, and this integrated cascade made several genes become tangled to full functionality.

In conclusion, TUSC-7 proved to be one strong suppressive lnc-RNA in lung adenocarcinoma stem cells, functioning through inactivated NOTCH signaling. Data from clinical analysis and molecular analysis revealed its roles in stem cells expansion. Most importantly, the turbulence on division modes precisely pointed to the key mechanisms of stem cells' renewal. The decreasing of tumor suppressive miR-146 was necessary in TUSC-7 conducted renewal repression, unless it alone could also reduce the renewal efficiency. This indicates that more complicated non-coding genes may be involved in its regulation.

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CRediT authorship contribution statement

Guanglin Huang: Investigation, Methodology.**Meng Wang:**Software, Formal analysis.**Xiang Li:**Methodology, Data curation, Formal analysis.**Jie Wu:**Data curation, Formal analysis.**Sisi Chen:**Data curation.**Ning Du:**Resources, Investigation.**Kai Li:**Methodology, Data curation, Formal analysis.**Jichang Wang:**Methodology, Project administration, Writing - original draft.**Chongwen Xu:**Methodology, Project administration, Writing - original draft.**Hong Ren:**Supervision, Project administration.**Shou-Ching Tang:**Conceptualization, Project administration, Supervision, Validation, Writing - original draft, Writing - review & editing.**Xin Sun:**Conceptualization, Project administration, Funding acquisition, Resources, Software, Supervision, Validation.

Declaration of Competing Interest

Authors declare each has approved this article to be published and that this research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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References

[1] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[2] L.A. Torre, R.L. Siegel, A. Jemal, Lung cancer statistics, Adv. Exp. Med. Biol. 893 (2016) 1–19.
[3] P. Bertuccio, G. Alicandro, M. Malvezzi, G. Carioli, P. Boffetta, F. Levi, et al., Cancer mortality in Europe in 2015, and an overview of trends since 1990, Ann. Oncol. (2019), https://academic.oup.com/annonc/advance-article-abstract/doi/10.1093/annonc/mdz179/5506635?redirectedFrom=fulltext.
[4] M. Malvezzi, G. Carioli, P. Bertuccio, P. Boffetta, F. Levi, C. La Vecchia, et al., European cancer mortality predictions for the year 2019 with focus on breast cancer, Ann. Oncol. 30 (5) (2019) 781–787 May 2019.
[5] M. Cabanero, R. Sangha, B.S. Sheffield, M. Sukhai, M. Pakkal, S. Kamel-Reid, et al., Management of EGFR-mutated non-small-cell lung cancer: practical implications from a clinical and pathology perspective, Curr. Oncol. 24 (2017) 111–119.
[6] A.F. Cardona, O. Arrieta, M.I. Zapata, L. Rojas, B. Wills, N. Reguart, et al., Acquired resistance to Erlotinib in EGFR mutation-positive lung adenocarcinoma among Hispanics (CLICaP), Target. Oncol. 12 (2017) 513–523.
[7] M. Forcella, M. Oldani, S. Epistolio, S. Freguia, E. Monti, P. Fusi, et al., Non-small cell lung cancer (NSCLC), EGFR downstream pathway activation and TKI targeted therapies sensitivity: effect of the plasma membrane-associated NEU3, PLoS One 12 (2017) e0187289.
[8] Y. Kobayashi, T. Mitsudomi, Not all epidermal growth factor receptor mutations in lung cancer are created equal: perspectives for individualized treatment strategy, Cancer Sci. 107 (2016) 1179–1186.
[9] C. Daniel, J. Lagergren, M. Ohman, RNA editing of non-coding RNA and its role in gene regulation, Biochimie 117 (2015) 22–27.
[10] L. Kumar, Shamsuzzama, R. Haque, T. Baghel, A. Nazir, Circular RNAs: the emerging class of non-coding RNAs and their potential role in human neurodegenerative diseases, Mol. Neurobiol. 54 (2017) 7224–7234.
[11] R. Tripathi, P. Chakraborty, P.K. Varadwaj, Unraveling long non-coding RNAs through analysis of high-throughput RNA-sequencing data, Noncoding RNA Res 2 (2017) 111–118.
[12] C.M. Roake, S.E. Artandi, Approaching TERRA firma: genomic functions of telomeric noncoding RNA, Cell 170 (2017) 8–9.
[13] X. Sun, X. Jiao, T.G. Pestell, C. Fan, S. Qin, E. Mirabelli, et al., MicroRNAs and cancer stem cells: the sword and the shield, Oncogene 33 (2014) 4967–4977.
[14] E. Anastasiadou, L.S. Jacob, F.J. Slack, Non-coding RNA networks in cancer, Nat. Rev. Cancer 18 (2017) 5.
[15] T.R. Cech, J.A. Steitz, The noncoding RNA revolution-trashing old rules to forge new ones, Cell 157 (2014) 77–94.
[16] L. Laplane, Cancer stem cells modulate patterns and processes of evolution in cancers, Biology & Philosophy 33 (2018) 18.
[17] K.J. Lenos, D.M. Miedema, S.C. Lodestijn, L.E. Nijman, T. van den Bosch, X. Romero Ros, et al., Stem cell functionality is microenvironmentally defined during tumour expansion and therapy response in colon cancer, Nat. Cell Biol. 20 (2018) 1193–1202.
[18] S. Skvortsov, I.-I. Skvortsova, D.G. Tang, A. Dubrovska, Concise review: prostate cancer stem cells: current understanding, Stem Cells 36 (2018) 1457–1474.
[19] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discovery 2 (2012) 401.
[20] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) pl1.
[21] R. Liang, Y. Li, M. Wang, S.-C. Tang, G. Xiao, X. Sun, et al., MiR-146a promotes the asymmetric division and inhibits the self-renewal ability of breast cancer stem-like cells via indirect upregulation of Let-7, Cell Cycle 17 (2018) 1445–1456.
[22] G.X. Zheng, B.T. Do, D.E. Webster, P.A. Khavari, H.Y. Chang, Dicer-microRNA-Myc circuit promotes transcription of hundreds of long noncoding RNAs, Nat. Struct. Mol. Biol. 21 (2014) 585–590.
[23] X. Sun, S.-C. Tang, C. Xu, C. Wang, S. Qin, N. Du, et al., DICER1 regulated let-7 expression levels in p53-induced cancer repression requires cyclin D1, J. Cell. Mol. Med. 19 (2015) 1357–1365.
[24] A. Guinot, F. Oeztuerk-Winder, J.J. Ventura, miR-17-92/p38alpha dysregulation enhances Wnt signaling and selects Lgr6+ cancer stem-like cells during lung adenocarcinoma progression, Cancer Res. 76 (2016) 4012–4022.
[25] G. Leon, L. MacDonagh, S.P. Finn, S. Cuffe, M.P. Barr, Cancer stem cells in drug resistant lung cancer: targeting cell surface markers and signaling pathways, Pharmacol. Ther. 158 (2016) 71–90.
[26] J.K. Das, Q. Felty, R. Poppiti, R.M. Jackson, D. Roy, Nuclear respiratory factor 1 acting as an oncoprotein drives estrogen-induced breast carcinogenesis, Cells 7 (2018).
[27] J.A. Hackett, T. Kobayashi, S. Dietmann, M.A. Surani, Activation of lineage regulators and transposable elements across a pluripotent spectrum, Stem Cell Reports 8 (2017) 1645–1658.
[28] S. Mukherjee, D.J. Brat, Molecular programs underlying asymmetric stem cell division and their disruption in malignancy, Results Probl. Cell Differ. 61 (2017) 401–421.
[29] R.J. Winquist, A.B. Hall, B.K. Eustace, B.F. Furey, Evaluating the immortal strand hypothesis in cancer stem cells: symmetric/self-renewal as the relevant surrogate marker of tumorigenicity, Biochem. Pharmacol. 91 (2014) 129–134.
[30] K. Zhang, Y. Guo, X. Wang, H. Zhao, Z. Ji, C. Cheng, et al., WNT/beta-catenin directs self-renewal symmetric cell division of hTERT(high) prostate cancer stem cells, Cancer Res. 77 (2017) 2534–2547.
[31] J. Bajaj, B. Zimdahl, T. Reya, Fearful symmetry: subversion of asymmetric division in cancer development and progression, Cancer Res. 75 (2015) 792–797.
[32] C.C. Nwaeburu, A. Abukiwan, Z. Zhao, I. Herr, Quercetin-induced miR-200b-3p regulates the mode of self-renewing divisions in pancreatic cancer, Mol. Cancer 16 (2017) 23.
[33] R. Liang, Y. Li, M. Wang, S.C. Tang, G. Xiao, X. Sun, et al., MiR-146a promotes the asymmetric division and inhibits the self-renewal ability of breast cancer stem-like cells via indirect upregulation of Let-7, Cell Cycle 17 (2018) 1445–1456.
[34] R. Liu, C. Liu, D. Chen, W.H. Yang, X. Liu, C.G. Liu, et al., FOXP3 controls an miR-146/NF-kappaB negative feedback loop that inhibits apoptosis in breast cancer cells, Cancer Res. 75 (2015) 1703–1713.
[35] M.R. Paterson, A.J. Kriegel, MiR-146a/b: a family with shared seeds and different roots, Physiol. Genomics 49 (2017) 243–252.
[36] X. Sun, S. Cui, X. Fu, C. Liu, Z. Wang, Y. Liu, MicroRNA-146-5p promotes proliferation, migration and invasion in lung cancer cells by targeting claudin-12, Cancer Biomark 25 (2019) 89–99.