medical sciences forum

MDPI

Proceeding Paper

Experimental Identification of Aberrantly Expressed Long Non-Coding RNAs in Breast Cancer†

Irina Pronina [1,*], Svetlana Lukina [1], Vitaly Loginov [1], Aleksey Burdennyy [1], Tatiana Kazubskaya [2], Eleonora Braga [1] and Elena Filippova [1]

[1] Institute of General Pathology and Pathophysiology, Moscow, Russia; sveta_sergeevna349@mail.ru (S.L.); werwolf2000@mail.ru (V.L.); koldun.pro@mail.ru (A.B.); eleonora10_45@mail.ru (E.B.); p.lenuxa@yandex.ru (E.F.)

[2] FSBI National Medical Research Center of Oncology Named after N.N. Blokhin of the Ministry of Health of Russia, Moscow, Russia; kazubskaya@yahoo.com

* Correspondence: zolly_sten@mail.ru; Tel.: +7-985-766-83-81

† Presented at the 2nd International Electronic Conference on Biomedicines, 1–31 March 2023; Available online: https://ecb2023.sciforum.net.

Abstract: Breast cancer (BC) remains one of the leading causes of cancer deaths among women worldwide. Recently, studies of long non-coding RNAs (lncRNAs) involved in the regulation of various signaling pathways in cells have become increasingly important, since they demonstrate great prognostic potential in cancer. The aim of our work was to identify new aberrantly expressed lncRNAs in BC. We identified 30 aberrantly expressed lncRNAs in BC. For most lncRNAs, a decrease in the expression level by 2.34–13.2 times ($p < 0.05$) was noted, and only for lncRNA TERC, an increase in the expression level by 2.24 times ($p = 0.034$) was noted. Of greatest interest are the data obtained for lncRNAs ADAMTS9-AS2, EMX2OS, HOTAIRM1 and MEG3, as they are consistent with the data of bioinformatics analysis.

Keywords: long non-coding RNAs, breast cancer, biomarkers, cancer prognosis, epigenetical regulation

Citation: Pronina, I.; Lukina, S.; Loginov, V.; Burdennyy, A.; Kazubskaya, T.; Braga, E.; Filippova, E. Experimental Identification of Aberrantly Expressed Long Non-Coding RNAs in Breast Cancer. *Med. Sci. Forum* **2023**, *3*, x. https://doi.org/10.3390/xxxxx

Published: 1 March 2023

1. Introduction

According to the data of the International Agency for Research on Cancer (IARC), breast cancer (BC) is recognized as the most frequently diagnosed type of cancer (more than 2.26 million new cases) and was the cause of death of almost 685,000 people in 2020. Thus, in 2020, breast cancer overtook lung cancer in terms of the number of diagnosed cases and became the most common cause of cancer death among women and the fifth most common cause of cancer death overall. IARC estimates the incidence of breast cancer will increase to 3 million new cases per year, and mortality from breast cancer will increase to 1 million deaths per year by 2040 [1].

The BC is characterized by significant variability in cellular composition, as well as histological, expressional, genotypic and epigenetic heterogeneity. Based on immunohistochemistry data, namely the expression of estrogen and progesterone receptors in cancer cells, BC is divided into four molecular subtypes: luminal A, luminal B, Her-2 positive and triple negative breast cancer. The molecular subtype of breast cancer determines the response of patients to various therapies, including targeted anticancer therapies [2–4].

Epigenetic deregulation in cells has a decisive influence on the development and progression of cancer [5,6]. To date, such ways of epigenetic regulation as DNA methylation, histone modifications [7], abnormal expression of miRNAs, long noncoding RNAs (lncRNAs), and small nucleolar RNAs are known. lncRNAs, which genes account for up to 80% of the mammalian genome, play a key role in the regulation of gene expression

and in cancer biology through various mechanisms, including chromosome remodeling, transcriptional and posttranscriptional modifications [4,8–10].

Despite the increasing effectiveness of diagnostic and therapeutic strategies, the recovery for BC remains limited [11]. The lack of validated diagnostic and prognostic biomarkers is one of the reasons for failures in the early detection and treatment of breast cancer worldwide [12,13]. We conducted an experimental search for long non-coding RNAs that change expression in breast cancer, which can be used in the future as markers of this disease or targets/agents of targeted therapy, and compared our data with available online databases.

2. Materials and Methods

Samples collection. 24 paired tissue samples from the tumor and adjacent histologically normal tissue were collected and characterized at the National Medical Research Center of Oncology named after N.N. Blokhin. When performing a planned surgical operation, samples were collected, and tumor areas with a tumor cell content of at least 70% and adjacent tissue areas in which no tumor cells were found during histological analysis were taken for the study.

RNA Isolation. Total RNA was isolated by a modified guanidine thiocyanate–phenol–chloroform extraction method in an acidic acetate buffer (previously described in [16]). The concentration of total RNA was determined spectrophotometrically using NanoDrop 1000 (Thermo Scientific, USA). RIN was determined from the results of total RNA denaturing electrophoresis in 1% agarose gel [14].

Reverse transcription and PCR. 1 μg of total RNA was taken into the reverse transcription reaction, the reaction was carried out using the RT2 First Strand Kit (QIAGEN Sciences, USA) according to the manufacturer's protocol. PCR was performed using RT2 SYBR® Green qPCR Mastermix and RT2 lncRNA PCR Array #LAHS-002Z (QIAGEN Sciences, USA) according to the manufacturer's protocol. Controls for genomic DNA contamination, reverse transcription reaction and PCR, and reference genes were included in the RT2 lncRNA PCR Array panel.

Statistical processing of the results was carried out using QIAGEN's GeneGlobe Data Analysis Center cloud software (https://geneglobe.qiagen.com/us/analyze) [15], Cq was 39, because some lncRNAs are relatively weakly expressed in breast tissue. RNA isolated from histologically normal tissue was used in the Control Group, RNA from tumor tissue was used in the Experimental Group (Test Group 1).

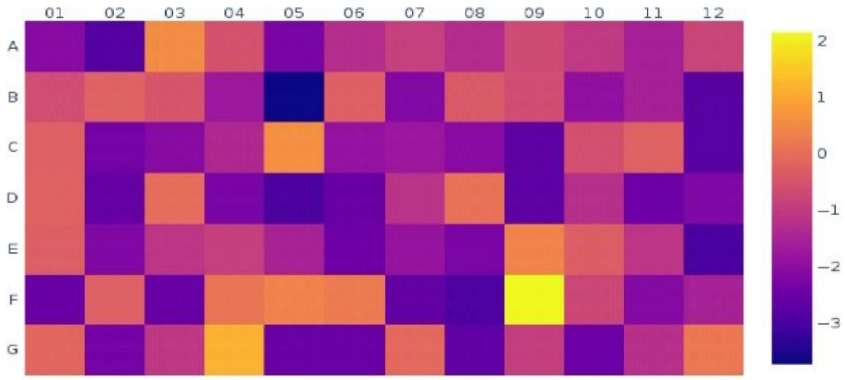
Bioinformatics. In parallel with the analysis of the expression of lncRNAs in clinical samples, a search for differentially expressed lncRNAs in BC was carried out using the GEPIA database (http://gepia.cancer-pku.cn/, accessed 6 November 2022) [16].

3. Results and Discussion

We analyzed the expression of 84 lncRNAs validated by QIAGEN Sciences for BC (Figure 1). Expression of 30 of them significantly changed in tumor tissues compared to the histological norm ($p < 0.05$), a change in expression by 2 or more times was considered significant. Moreover, for 29 lncRNAs, a decrease of 2.34–13.2 times was revealed, and only for TERC, an increase in expression by 2.24 times was found (Table 1).

In parallel with the experimental search for lncRNA aberrantly expressed in BC, we analyzed data from the Gene Expression Profiling Interactive Analysis database (GEPIA) [16] (Figure 2).

A



B

Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	ACTA2-AS1 / -4.25	ADAMTS9-AS 2 / -7.21	AFAP1-AS1 / 1.45 / B	AIRN / -1.45 / B	BANCR / -4.88	BCAR4 / -2.46 / B	BLACAT1 / -1.87 / B	CAHM / -2.5 / B	CBR3-AS1 / -1.63 / B	CCAT1 / -2.04 / B	CCAT2 / -2.96 / B	CDKN2B-AS 1 / -1.72 / B
B	CRNDE / -1.54 / B	DGCR5 / -1.16 / B	DLEU2 / -1.38 / B	DLX6-AS1 / -3.34 / B	EMX2OS / -13.2	FTX / -1.21 / A	GACAT1 / -4.48 / B	GAS5 / -1.31 / A	GAS6-AS1 / -1.55 / B	GNAS-AS1 / -3.83 / B	H19 / -2.94 / A	HAND2-AS1 / -6.91
C	HEIH / -1.19 / B	HIF1A-AS1 / -5.14	HIF1A-AS2 / -4.26 / B	HNF1A-AS1 / -2.62 / B	HOTAIR / 1.53 / B	HOTAIRM1 / -3.67	HOTTIP / -3.39 / B	HOXA11-AS / -4.22 / B	HOXA-AS2 / -6.46	HULC / -1.5 / B	IPW / -1.17 / B	JADRR / -6.92
D	KCNQ1OT1 / -1.17 / B	KRASP1 / -6.06	LINC00152 / -1.01 / B	LINC00261 / -4.95 / B	LINC00312 / -7.83	LINC00538 / -5.85	LINC00887 / -2.26 / B	LINC00963 / 1.06 / B	LINC01233 / -6.62	LINC01234 / -2.36 / B	LSINCT5 / -5.66	LUCAT1 / -4.57
E	MALAT1 / -1.22 / B	MEG3 / -4.56	MIR155HG / -2.15 / B	MIR17HG / -1.9 / B	MIR31HG / -2.87	MIR7-3HG / -5.56	MRPL23-AS1 / -3.61 / B	NAMA / -4.85	NBR2 / 1.31 / B	NEAT1 / -1.24	NRON / -2.15 / B	PANDAR / -7.95 / B
F	PCA3 / -5.84	PCAT1 / -1.2 / B	PCGEM1 / -5.94	POU5F1P5 / 1.09 / B	PRNCR1 / 1.3 / B	PTCSC1 / 1.2 / B	PTCSC3 / -6.12 / B	PTENP1 / -7.68	PVT1 / 4.47 / B	RMRP / -1.68	RMST / -4.36 / B	RPS6KA2-AS 1 / -2.9 / B
G	SNHG16 / -1.1 / A	SPRY4-IT1 / -5.14	SUMO1P3 / -2.09 / B	TERC / 2.24 / A	TRERNA1 / -5.8	TSIX / -5.79	TUG1 / -1.06 / A	TUSC7 / -6.37	UCA1 / -1.92 / B	WT1-AS / -5.7	XIST / -2.34	ZFAS1 / 1.14

Figure 1. Heat map of changes in the lncRNAs expression levels in tumor tissues compared to histologically normal tissues. (**A**)—heat map, color scale of changes (log2) is given on the right. (**B**)—the table indicating the symbol of the lncRNA, the expression chages (fold regulation) and statistical significance ((**A**)—expression varies greatly within the group, which makes statistical analysis difficult; (**B**)—the value may be unsignificant, $p \geq 0.05$).

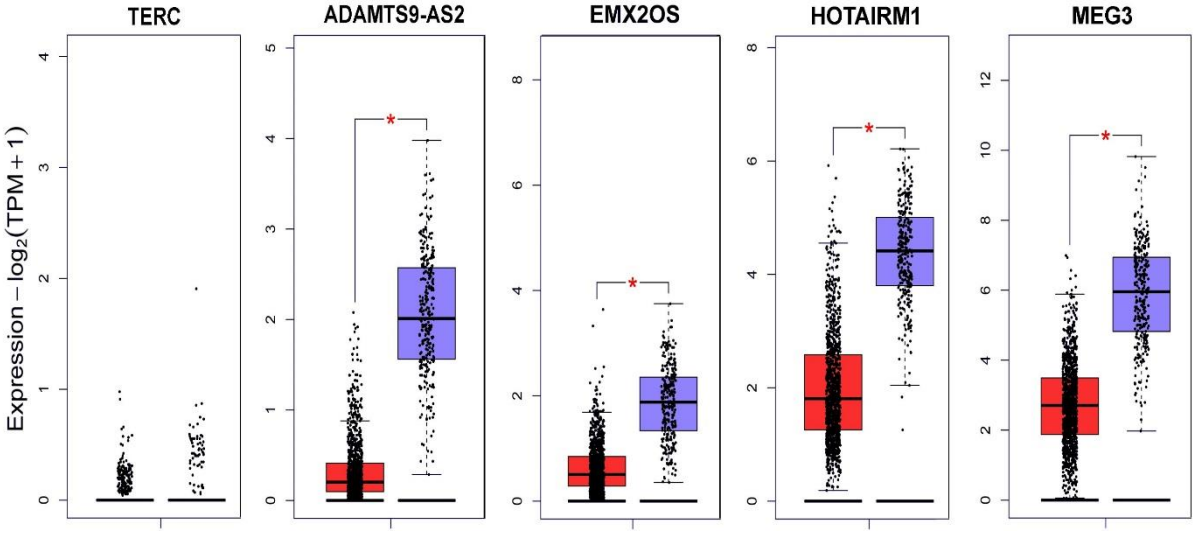


Figure 2. LncRNA expression profile of TERC, ADAMTS9-AS2, EMX2OS, HOTAIRM1 and MEG3 in BC according to the GEPIA database [18]. Red indicates the expression level in the tumor tissue (1085 samples); blue indicates the expression level in normal tissue (291 samples); * $p < 0.01$.

Table 1. The most significant lncRNA expression changes in tumor tissue compared to the histological norm (mean values are shown).

Symbol	Fold Regulation (Mean)	*p*-Value
TERC	2.24	0.034325
ACTA2-AS1	−4.25	0.02341
ADAMTS9-AS2	−7.21	0.000352
BANCR	−4.88	0.043833
EMX2OS	−13.2	0.004726
H19	−2.94	0.020427
HAND2-AS1	−6.91	0.030796
HIF1A-AS1	−5.14	0.044182
HOTAIRM1	−3.67	0.003605
HOXA-AS2	−6.46	0.031769
JADRR	−6.92	0.03366
KRASP1	−6.06	0.040312
LINC00312	−7.83	0.002035
LINC00538	−5.85	0.036459
LINC01233	−6.62	0.02316
LSINCT5	−5.66	0.033926
LUCAT1	−4.57	0.003831
MEG3	−4.56	0.044958
MIR31HG	−2.87	0.04044
MIR7-3HG	−5.56	0.043805
NAMA	−4.85	0.037223
PCA3	−5.84	0.045823
PCGEM1	−5.94	0.04299
PTENP1	−7.68	0.028923
SPRY4-IT1	−5.14	0.001182
TRERNA1	−5.8	0.043079
TSIX	−5.79	0.023387
TUSC7	−6.37	0.03224
WT1-AS	−5.7	0.039399
XIST	−2.34	0.03889

TERC (telomerase RNA component) serves as a template for telomere replication catalyzed by the telomerase enzyme, which contributes to the uncontrolled proliferation of cancer cells. Despite the fact that our experimental data were not confirmed by the data of the GEPIA database for breast cancer, an increase in TERC expression was shown for gastric cancer [17], TERC was proposed as a diagnostic marker in cervical cancer [18] and lung cancer [19].

MEG3 (maternally expressed 3) is a relatively well-studied lncRNA demonstrating oncosuppressive properties. A systemic meta-analysis showed an association between suppression of MEG3 expression and poor outcome in patients with cancer of different localizations [20]. MEG3 expression is reduced in cell lines and tissues of ovarian cancer [21]. An increase in MEG3 expression prevented cell proliferation and induced apoptosis in cell lines of ovarian, cervical, and other cancers [22]. Competitively interacting with miR-421, MEG3 enhances the action of E-cadherin, which blocks the epithelial-mesenchymal transition [23].

ADAMTS9-AS2 also exhibits suppressor properties in cancer. Various authors have studied ADAMTS9-AS2 in regulating the development of gastric cancer [24] and lung cancer [25]. EMX2OS is still relatively little studied; however, competitive interaction of

this lncRNA with some microRNAs in Wilms tumor [26] and its prognostic value in gastric cancer have been shown [27].

The lnc RNA HOTAIRM1 has already been proposed as a biomarker for oral squamous cell carcinoma [28], and its role proliferation and metastasis of breast cancer [29], development of resistance to tamoxifen therapy due to the regulation of HOXA1 protein expression is being studied [30].

4. Conclusions

LncRNAs are involved in the regulation of multiple cancer features. Their association with inhibition of apoptosis, initiation of invasion and metastasis, and activation of angiogenesis has been shown (see review [31]).

During experimental and bioinformatic analysis of changes in lncRNA expression in breast cancer, we have identified TERC, ADAMTS9-AS2, EMX2OS, HOTAIRM1, and MEG3 lncRNAs with biomarker potential. Further study of lncRNA expression and its regulation will also help in the development of targeted anticancer therapy.

Author Contributions: Conceptualization, I.P. and E.F.; methodology, I.P.; software, V.L., A.B. and E.F.; validation, I.P., S.L. and E.B.; formal analysis, E.B.; investigation, I.P and E.F.; resources, T.K.; data curation, T.K. and E.B.; writing—original draft preparation, I.P.; writing—review and editing, I.P, V.L, E.B and E.F.; visualization, I.P. and V.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Higher Education of the Russian Federation, the state task number FGFU-2022-0007 (sample collection, RNA isolation and online databases analysis), and the Russian Science Foundation grant no. 22-75-00132 (experimental RT2 lncRNA PCR Array analysis).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. International Agency for Research on Cancer. Available online: https://www.iarc.who.int/featured-news/breast-cancer-awareness-month-2021/ (accessed on 19 January 2023)
2. Joseph, C.; Papadaki, A.; Althobiti, M.; Alsaleem, M.; Aleskandarany, M.A.; Rakha, E.A. Breast cancer intratumour heterogeneity: current status and clinical implications. *Histopathology* **2018**, *73*, 717–731. https://doi.org/10.1111/his.13642.
3. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature* **2012**, *490*, 61–70. https://doi.org/10.1038/nature11412.
4. Kansara, S.; Pandey, V.; Lobie, P.E.; Sethi, G.; Garg, M.; Pandey, A.K. Mechanistic Involvement of Long Non-Coding RNAs in Oncotherapeutics Resistance in Triple-Negative Breast Cancer. *Cells* **2020**, *9*, 1511. https://doi.org/10.3390/cells9061511.
5. Kumar, R.; Paul, A.M.; Rameshwar, P.; Pillai, M.R. Epigenetic Dysregulation at the Crossroad of Women's Cancer. *Cancers* **2019**, *11*, 1193. https://doi.org/10.3390/cancers11081193.
6. Kagohara, L.T.; Stein-O'Brien, G.L.; Kelley, D.; Flam, E.; Wick, H.C.; Danilova, L.V.; Easwaran, H.; Favorov, A.V.; Qian, J.; A Gaykalova, D.; et al. Epigenetic regulation of gene expression in cancer: techniques, resources and analysis. *Briefings Funct. Genom.* **2017**, *17*, 49–63. https://doi.org/10.1093/bfgp/elx018.
7. Shanmugam, M.K.; Arfuso, F.; Arumugam, S.; Chinnathambi, A.; Jinsong, B.; Warrier, S.; Wang, L.Z.; Kumar, A.P.; Ahn, K.S.; Sethi, G.; et al. Role of novel histone modifications in cancer. *Oncotarget* **2018**, *9*, 11414–11426. https://doi.org/10.18632/oncotarget.23356.
8. Gao, P.; Wei, G.-H. Genomic Insight into the Role of lncRNAs in Cancer Susceptibility. *Int. J. Mol. Sci.* **2017**, *18*, 1239. https://doi.org/10.3390/ijms18061239.
9. Esteller, M. Non-coding RNAs in human disease. *Nat. Rev. Genet.* **2011**, *12*, 861–874. https://doi.org/10.1038/nrg3074.
10. Ghafouri-Fard, S.; Tamizkar, K.H.; Hussen, B.M.; Taheri, M. An update on the role of long non-coding RNAs in the pathogenesis of breast cancer. *Pathol.-Res. Pract.* **2021**, *219*, 153373. https://doi.org/10.1016/j.prp.2021.153373.

11. Nounou, M.I.; ElAmrawy, F.; Ahmed, N.; Abdelraouf, K.; Goda, S.; Syed-Sha-Qhattal, H. Breast Cancer: Conventional Diagnosis and Treatment Modalities and Recent Patents and Technologies. *Breast Cancer Basic Clin. Res.* **2015**, *9*, 17–34. https://doi.org/10.4137/bcbcr.s29420.
12. Feldman, R.; Kim, E.S. Prognostic and predictive biomarkers post curative intent therapy. *Ann. Transl. Med.* **2017**, *5*, 374–374. https://doi.org/10.21037/atm.2017.07.34.
13. Kashyap, D.; Kaur, H. Cell-free miRNAs as non-invasive biomarkers in breast cancer: Significance in early diagnosis and metastasis prediction. *Life Sci.* **2020**, *246*, 117417. https://doi.org/10.1016/j.lfs.2020.117417.
14. Pronina, I.V.; Loginov, V.I.; Prasolov, V.S.; Klimov, E.A.; Khodyrev, D.S.; Kazubskaya, T.P.; Gar'Kavtseva, R.F.; Sulimova, G.E.; Braga, E.A. Altered expression of the SEMA3B gene in epithelial tumors. *Mol. Biol.* **2009**, *43*, 403–409. https://doi.org/10.1134/s002689330903008x.
15. QIAGEN's GeneGlobe Data Analysis Center. Available online: https://geneglobe.qiagen.com/us/analyze (accessed on 30 October 2022)
16. Tang, Z.; Li, C.; Kang, B.; Gao, G.; Li, C.; Zhang, Z. GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* **2017**, *45*, W98–W102. https://doi.org/10.1093/nar/gkx247.
17. Wang, J.; Wu, M.; Chang, L.; Jin, Z.; Yang, X.; Li, D.; Wang, J.; Qu, J.; Hou, Q.; Huang, X.; et al. The lncRNA TERC promotes gastric cancer cell proliferation, migration, and invasion by sponging miR-423-5p to regulate SOX12 expression. *Ann. Transl. Med.* **2022**, *10*, 963–963. https://doi.org/10.21037/atm-22-3545.
18. Zhao, H.; He, Y.; Fan, B.; Wang, Y.; Wu, Y.-M. Human papillomavirus E6E7 mRNA and TERC lncRNA in situ detection in cervical scraped cells and cervical disease progression assessment. *Virol. J.* **2022**, *19*, 18. https://doi.org/10.1186/s12985-021-01696-9.
19. Storti, C.B.; de Oliveira, R.A.; de Carvalho, M.; Hasimoto, E.N.; Cataneo, D.C.; Cataneo, A.J.M.; De Faveri, J.; Vasconcelos, E.J.R.; dos Reis, P.P.; Cano, M.I.N. Telomere-associated genes and telomeric lncRNAs are biomarker candidates in lung squamous cell carcinoma (LUSC). *Exp. Mol. Pathol.* **2020**, *112*, 104354. https://doi.org/10.1016/j.yexmp.2019.104354.
20. Binabaj, M.M.; Bahreyni, A.; Khazaei, M.; Avan, A.; Hassanian, S.M. The prognostic value of long noncoding RNA MEG3 expression in the survival of patients with cancer: A meta-analysis—response. *J. Cell. Biochem.* **2019**, *120*, 18599–18599. https://doi.org/10.1002/jcb.28532.
21. Tao, P.; Yang, B.; Zhang, H.; Sun, L.; Wang, Y.; Zheng, W. The overexpression of lncRNA MEG3 inhibits cell viability and invasion and promotes apoptosis in ovarian cancer by sponging miR-205-5p. *Int. J. Clin. Exp. Pathol.* **2020**, *13*, 869–879.
22. He, Y.; Luo, Y.; Liang, B.; Ye, L.; Lu, G.; He, W. Potential applications of MEG3 in cancer diagnosis and prognosis. *Oncotarget* **2017**, *8*, 73282–73295. https://doi.org/10.18632/oncotarget.19931.
23. Zhang, W.; Shi, S.; Jiang, J.; Li, X.; Lu, H.; Ren, F. LncRNA MEG3 inhibits cell epithelial-mesenchymal transition by sponging miR-421 targeting E-cadherin in breast cancer. *Biomed. Pharmacother.* **2017**, *91*, 312–319. https://doi.org/10.1016/j.biopha.2017.04.085.
24. Wang, S.; Li, X.-C.; Zhu, J.-R.; Ma, Z.-J.; Ran, J.-T.; Zhou, Y.-N. Construction of a novel ceRNA network and identification of lncRNA ADAMTS9-AS2 and PVT1 as hub regulators of miRNA and coding gene expression in gastric cancer. *Transl. Cancer Res.* **2021**, *10*, 938–952. https://doi.org/10.21037/tcr-20-2168.
25. Lin, Z.; Huang, W.; Yi, Y.; Li, D.; Xie, Z.; Li, Z.; Ye, M. LncRNA ADAMTS9-AS2 is a Prognostic Biomarker and Correlated with Immune Infiltrates in Lung Adenocarcinoma. *Int. J. Gen. Med.* **2021**, *14*, 8541–8555. https://doi.org/10.2147/ijgm.s340683.
26. Chen, Z.H.; Cui, M.Y.; Zhang, H.M. EMX2OS Delays Wilms' Tumor Progression via Targeting miR-654-3p. *Ann. Clin. Lab. Sci.* **2022**, *52*, 12–20.
27. Liu, G.-X.; Tan, Y.-Z.; He, G.-C.; Zhang, Q.-L.; Liu, P. EMX2OS plays a prognosis-associated enhancer RNA role in gastric cancer. *Medicine* **2021**, *100*, e27535. https://doi.org/10.1097/md.0000000000027535.
28. Yu, Y.; Niu, J.; Zhang, X.; Wang, X.; Song, H.; Liu, Y.; Jiao, X.; Chen, F. Identification and Validation of HOTAIRM1 as a Novel Biomarker for Oral Squamous Cell Carcinoma. *Front. Bioeng. Biotechnol.* **2022**, *9*, 798584. https://doi.org/10.3389/fbioe.2021.798584.
29. Lai, G.-E.; Zhou, J.; Huang, C.-L.; Mai, C.-L.H.C.-J.; Lai, Y.-M.; Lin, Z.-Q.; Peng, T.; Luo, Y.; Liu, F.-E. A combination of transcriptome and methylation analyses reveals the role of lncRNA HOTAIRM1 in the proliferation and metastasis of breast cancer. *Gland. Surg.* **2022**, *11*, 826–836. https://doi.org/10.21037/gs-22-164.
30. Kim, C.Y.; Oh, J.H.; Lee, J.-Y.; Kim, M.H. The LncRNA HOTAIRM1 Promotes Tamoxifen Resistance by Mediating HOXA1 Expression in ER+ Breast Cancer Cells. *J. Cancer* **2020**, *11*, 3416–3423. https://doi.org/10.7150/jca.38728.
31. Kashyap, D.; Sharma, R.; Goel, N.; Buttar, H.S.; Garg, V.K.; Pal, D.; Rajab, K.; Shaikh, A. Coding roles of long non-coding RNAs in breast cancer: Emerging molecular diagnostic biomarkers and potential therapeutic targets with special reference to chemotherapy resistance. *Front. Genet.* **2023**, *13*, 993687. https://doi.org/10.3389/fgene.2022.993687.