Supplementary tables

Multi-miRNAs panel of tumor-derived extracellular vesicles as promising diagnostic biomarkers of early-stage breast cancer

Min Woo Kim [1,†], Sunyoung Park [2,†], Hyojung Lee [1], Hogyeong Gwak [2], Kyung-A Hyun [2], Jee Ye Kim [1], Hyo-Il Jung [2, *], and Seung Il Kim [1,*]

[1] Yonsei University College of Medicine, Seoul, Republic of Korea

[2] School of Mechanical Engineering, Yonsei University, Seoul, Republic of Korea

† These authors contributed equally to this work.

* Please send Correspondence to:

Seung Il Kim – Professor, M.D./Ph.D.

Department of Surgery, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

Tel. +82-2-2228-0900 / Fax. +82-2-313-8289 / E-mail. SKIM@yuhs.ac

Hyo-Il Jung – Professor, Ph.D.

School of Mechanical Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea

Tel. +82-2-2123-7767 / Fax. +82-2-312-2821 / E-mail. URIDLE7@yonsei.ac.kr

Keywords: biomarker; extracellular vesicles; microRNA; early-stage breast cancer; liquid biopsy

Table S1. Differentially expressed miRNAs in the paired adjacent normal tissues and cancer tissues

	Adjacent normal tissues		Cancer tissues		p-value (paired t-test)
	Median (min-max)	Mean (S.D.)	Median (min-max)	Mean (S.D.)	
hsa-miR-16	308 (92.8-1709)	435 (331.9)	511 (145.9 - 3952)	628.4 (472.3)	0.006
hsa-miR-21	50117 (10121-196399)	54433 (32501)	248171 (34749-467924)	240079 (87849)	<0.0001
hsa-miR-9	250 (49.1-2628)	414.5 (473)	406.5 (44.7-51986)	3083 (8477)	0.0047
hsa-miR-429	15.1 (0-149.5)	20.9 (21.9)	94.2 (101-436.3)	120.8 (87.4)	<0.0001
hsa-miR-96	3.2 (0-36.2)	4.5 (4.5)	26.8 (2.4-153.9)	41.7 (34.8)	<0.0001
hsa-miR-155	137 (39.9-889.4)	176 (135.4)	294 (91.5-4114)	484 (589.4)	<0.0001
hsa-miR-128	41.3 (24.3-376.9)	49.9 (40.2)	63.0 (23.7-164.3)	71.7 (30.7)	<0.0001

Table S2. Evaluation of reference genes for gene expression

Methods	**Ranking order (Better-Good-Average)**		
	1	**2**	**3**
Delta C$_T$	miR-484	miR-16	let7a-
BestKeeper	miR-484	miR-16	let7a
Normfinder	miR-484	-	let7a
Genorm	miR-484/miR-16	miR-16	let7a

Table S3. Function of the selected miRNAs and the expression levels of miRNAs in breast cancer tissues and plasma in TCGA data and review of literature

miRNA	**Function** [a]	**Tissues (clinical samples)**		**Plasma**	**EVs from plasma**
		TCGA data [b]	**qPCR validated data** [c]	**(clinical samples)**	**(clinical samples)**
miR-16	Apoptosis	↑**	↑[1]	↑[14] ↓[24]	↑[22]
miR-21	Tumor growth, drug resistance	↑***	↑[2,3,4]	↑[15,16,17] ↓[18]	↑[23]
miR-9	Metastasis, angiogenesis	↑**	↑[5,6,7]	n.s.[25]↓[26]↑[27]	-
miR-429	Proliferation, migration	↑***	↑[8]	↑[19]	-
miR-96	Proliferation, migration	↑***	↑[9,10]	↑[17]	-
miR-155	Proliferation, metastasis	↑***	↑[3,11]	↑[28]	-
miR-128	Apoptosis	↑***	↑[12,13]	↑[30]↓[29]	-
miR-let7a	Immune response	↓*	-	↑[20,21]↓ [15]	-

a. Functions of miRNAs based on the breast cancer cell lines in the literature review [1-4].

b. Sources from The cancer genome atlas were obtained (portal.gdc.cancer.gov) and the paired t-tests between primary tumor tissues and paired adjacent normal tissues were performed.

c. The expression levels of miRNAs were obtained from the literature review on breast tumor tissues, plasma, and EVs from plasmas. * $p < 0.05$, ** $p<0.01$ ***$p<0.001$, *n.s.* no significant expression'-' not validated using clinical samples.

References (Supplementary information)

1. Chan, M.; Liaw, C.S.; Ji, S.M.; Tan, H.H.; Wong, C.Y.; Thike, A.A.; Tan, P.H.; Ho, G.H.; Lee, A.S. Identification of circulating microRNA signatures for breast cancer detection. *Clin Cancer Res* **2013**, *19*, 4477-4487, doi:10.1158/1078-0432.CCR-12-3401.
2. Berber, U.; Yilmaz, I.; Narli, G.; Haholu, A.; Kucukodaci, Z.; Demirel, D. miR-205 and miR-200c: Predictive Micro RNAs for Lymph Node Metastasis in Triple Negative Breast Cancer. *J Breast Cancer* **2014**, *17*, 143-148, doi:10.4048/jbc.2014.17.2.143.
3. Iyevleva, A.G.; Kuligina, E.; Mitiushkina, N.V.; Togo, A.V.; Miki, Y.; Imyanitov, E.N. High level of miR-21, miR-10b, and miR-31 expression in bilateral vs. unilateral breast carcinomas. *Breast Cancer Res Treat* **2012**, *131*, 1049-1059, doi:10.1007/s10549-011-1845-z.
4. Min, W.; Wang, B.; Li, J.; Han, J.; Zhao, Y.; Su, W.; Dai, Z.; Wang, X.; Ma, Q. The expression and significance of five types of miRNAs in breast cancer. *Med Sci Monit Basic Res* **2014**, *20*, 97-104, doi:10.12659/MSMBR.891246.
5. Gwak, J.M.; Kim, H.J.; Kim, E.J.; Chung, Y.R.; Yun, S.; Seo, A.N.; Lee, H.J.; Park, S.Y. MicroRNA-9 is associated with epithelial-mesenchymal transition, breast cancer stem cell phenotype, and tumor progression in breast cancer. *Breast Cancer Res Tr* **2014**, *147*, 39-49, doi:10.1007/s10549-014-3069-5.
6. Zhou, X.; Marian, C.; Makambi, K.H.; Kosti, O.; Kallakury, B.V.S.; Loffredo, C.A.; Zheng, Y.L. MicroRNA-9 as Potential Biomarker for Breast Cancer Local Recurrence and Tumor Estrogen Receptor Status. *Plos One* **2012**, *7*, doi:ARTN e3901110.1371/journal.pone.0039011.
7. Jang, M.H.; Kim, H.J.; Gwak, J.M.; Chung, Y.R.; Park, S.Y. Prognostic value of microRNA-9 and microRNA-155 expression in triple-negative breast cancer. *Hum Pathol* **2017**, *68*, 69-78, doi:10.1016/j.humpath.2017.08.026.
8. Cava, C.; Novello, C.; Martelli, C.; Lodico, A.; Ottobrini, L.; Piccotti, F.; Truffi, M.; Corsi, F.; Bertoli, G.; Castiglioni, I. Theranostic application of miR-429 in HER2+breast cancer. *Theranostics* **2020**, *10*, 50-61, doi:10.7150/thno.36274.
9. Eissa, S.; Matboli, M.; Shehata, H.H. Breast tissue-based microRNA panel highlights microRNA-23a and selected target genes as putative biomarkers for breast cancer. *Transl Res* **2015**, *165*, 417-427, doi:10.1016/j.trsl.2014.10.001.
10. Zhang, Q.H.; Sun, H.M.; Zheng, R.Z.; Li, Y.C.; Zhang, Q.; Cheng, P.; Tang, Z.H.; Huang, F. Meta-analysis of microRNA-183 family expression in human cancer studies comparing cancer tissues with noncancerous tissues. *Gene* **2013**, *527*, 26-32, doi:10.1016/j.gene.2013.06.006.
11. Chen, J.; Wang, B.C.; Tang, J.H. Clinical significance of MicoRNA-155 expression in human breast cancer. *Journal of Surgical Oncology* **2012**, *106*, 260-266, doi:10.1002/jso.22153.
12. Block, I.; Burton, M.; Sorensen, K.P.; Andersen, L.; Larsen, M.J.; Bak, M.; Cold, S.; Thomassen, M.; Tan, Q.; Kruse, T.A. Association of miR-548c-5p, miR-7-5p, miR-210-3p, miR-128-3p with recurrence in systemically untreated breast cancer. *Oncotarget* **2018**, *9*, 9030-9042, doi:10.18632/oncotarget.24088.

13. Zhang, Z.Q.; Xu, L.; He, L.J.; Wang, J.; Shi, X.N.; Li, Z.; Shi, S.; Hou, K.Z.; Teng, Y.E.; Qu, X.J. MiR-891a-5p as a prognostic marker and therapeutic target for hormone receptor-positive breast cancer. *J Cancer* **2020**, *11*, 3771-3782, doi:10.7150/jca.40750.

14. Stukrath, I.; Rack, B.; Janni, W.; Jager, B.; Pantel, K.; Schwarzenbach, H. Aberrant plasma levels of circulating miR-16, miR-107, miR-130a and miR-146a are associated with lymph node metastasis and receptor status of breast cancer patients. *Oncotarget* **2015**, *6*, 13387-13401.

15. Khalighfard, S.; Alizadeh, A.M.; Irani, S.; Omranipour, R. Plasma miR-21, miR-155, miR-10b, and Let-7a as the potential biomarkers for the monitoring of breast cancer patients. *Sci Rep-Uk* **2018**, *8*, doi:ARTN 1798110.1038/s41598-018-36321-3.

16. Motamedi, M.; Chaleshtori, M.H.; Ghasemi, S.; Mokarian, F. Plasma Level Of miR-21 And miR-451 In Primary And Recurrent Breast Cancer Patients. *Breast Cancer-Target* **2019**, *11*, 293-301, doi:10.2147/Bctt.S224333.

17. Matamala, N.; Vargas, M.T.; Gonzalez-Campora, R.; Minambres, R.; Arias, J.I.; Menendez, P.; Andres-Leon, E.; Gomez-Lopez, G.; Yanowsky, K.; Calvete-Candenas, J., et al. Tumor MicroRNA Expression Profiling Identifies Circulating MicroRNAs for Early Breast Cancer Detection. *Clin Chem* **2015**, *61*, 1098-1106, doi:10.1373/clinchem.2015.238691.

18. Jurkovicova, D.; Smolkova, B.; Magyerkova, M.; Sestakova, Z.; Kajabova, V.H.; Kulcsar, L.; Zmetakova, I.; Kalinkova, L.; Krivulcik, T.; Karaba, M., et al. Down-regulation of traditional oncomiRs in plasma of breast cancer patients. *Oncotarget* **2017**, *8*, 77369-77384, doi:10.18632/oncotarget.20484.

19. Madhavan, D.; Peng, C.; Wallwiener, M.; Zucknick, M.; Nees, J.; Schott, S.; Rudolph, A.; Riethdorf, S.; Trumpp, A.; Pantel, K., et al. Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis. *Carcinogenesis* **2016**, *37*, 461-470, doi:10.1093/carcin/bgw008.

20. Heneghan, H.M.; Miller, N.; Lowery, A.J.; Sweeney, K.J.; Newell, J.; Kerin, M.J. Circulating microRNAs as Novel Minimally Invasive Biomarkers for Breast Cancer. *Ann Surg* **2010**, *251*, 499-505, doi:10.1097/SLA.0b013e3181cc939f.

21. Lin, Z.Y.; Li, J.W.; Wang, Y.H.; Chen, T.; Ren, N.N.; Yang, L.W.; Xu, W.Y.; He, H.J.; Jiang, Y.; Chen, X.D., et al. Abnormal miRNA-30e Expression is Associated with Breast Cancer Progression. *Clin Lab* **2016**, *62*, 121-128, doi:10.7754/Clin.Lab.2015.150607.

22. Ni, Q.T.; Stevic, I.; Pan, C.; Muller, V.; Oliviera-Ferrer, L.; Pantel, K.; Schwarzenbach, H. Different signatures of miR-16, miR-30b and miR-93 in exosomes from breast cancer and DCIS patients. *Sci Rep-Uk* **2018**, *8*, doi:ARTN 1297410.1038/s41598-018-31108-y.

23. Hannafon, B.N.; Trigoso, Y.D.; Calloway, C.L.; Zhao, Y.D.; Lum, D.H.; Welm, A.L.; Zhao, Z.Z.J.; Blick, K.E.; Dooley, W.C.; Ding, W.Q. Plasma exosome microRNAs are indicative of breast cancer. *Breast Cancer Research* **2016**, *18*, doi:ARTN 9010.1186/s13058-016-0753-x.

24. Ye, T.; Liang, Y.; Zhang, D.; Zhang, X. MicroRNA-16-1-3p represses breast tumor growth and metastasis by inhibiting PGK1-Mediated Warbug Effect. *Front. Cell Dev. Biol* **2020**, 8, 615154, doi: 10.3389/fcell.2020.615154.

25. Orangi, E.; Motovali-Bashi, M. Evaluation of miRNA-9 and miRNA-34a as potential biomarkers for diagnosis of breast cancer in Iranian women. *Gene* **2019**, 687, 272-279, doi.org/10.1016/j.gene.2018.11.036.

26. Tavakolian, S.; Goudarzi, H.; Torfi, F.; Faghihloo, E. Evaluation of microRNA-9 and -192 expression levels as biomarkers in patients suffering from breast cancer. *Biomedical report* **2019**, 12, 30-34, doi: 10.3892/br.2019.1257.

27. Li, X.; Zeng, Z.; Wang, J.; Wu, Y.; Chen, W.; Zheng, L.; Xi, T.; Wang, A.; Lu, Y. MicroRNA-9 and breast cancer. *Biomedicine & pharmacotherapy* **2020**, 122, 109687, doi:10.1016/j.biopha.2019.109687.

28. Han, J.; Jiang, Y.; Zhang, C.; Yang, Y.; Pang, D.; Song, Y.; Zhang, G. A novel panel of serum miR-21/miR-155/miR-365 as a potential diagnostic biomarker for breast cancer. *Ann Surg Treat Res* **2017**, 92, 55-66, doi: 10.4174/astr.2017.92.2.55.

29. Eterno, V. et al. AurkA controls self-renewal of breast cancer-initiating cells promoting wnt3a stabilization through suppression of miR-128. *Scientific reports* **2016**, 6, 28436, doi: 10.1038/srep28436.

30. Bao, S.; Hu, T.; Liu, J.; Su, J.; Sun, J.; Ming, Y.; Li, J.; Wu, N.; Chen, H.; Zhou, M. Genomic instability-derived plasma extracellular vesicle-microRNA signature as a minimally invasive predictor of risk and unfavorable prognosis in breast cancer. *J nanobiotechnology* **2021**, 19, 22, doi: 10.1186/s12951-020-00767-3