

Supplemental Material

tRNA-Derived Fragments as Novel Predictive Biomarkers for Trastuzumab- Resistant Breast Cancer

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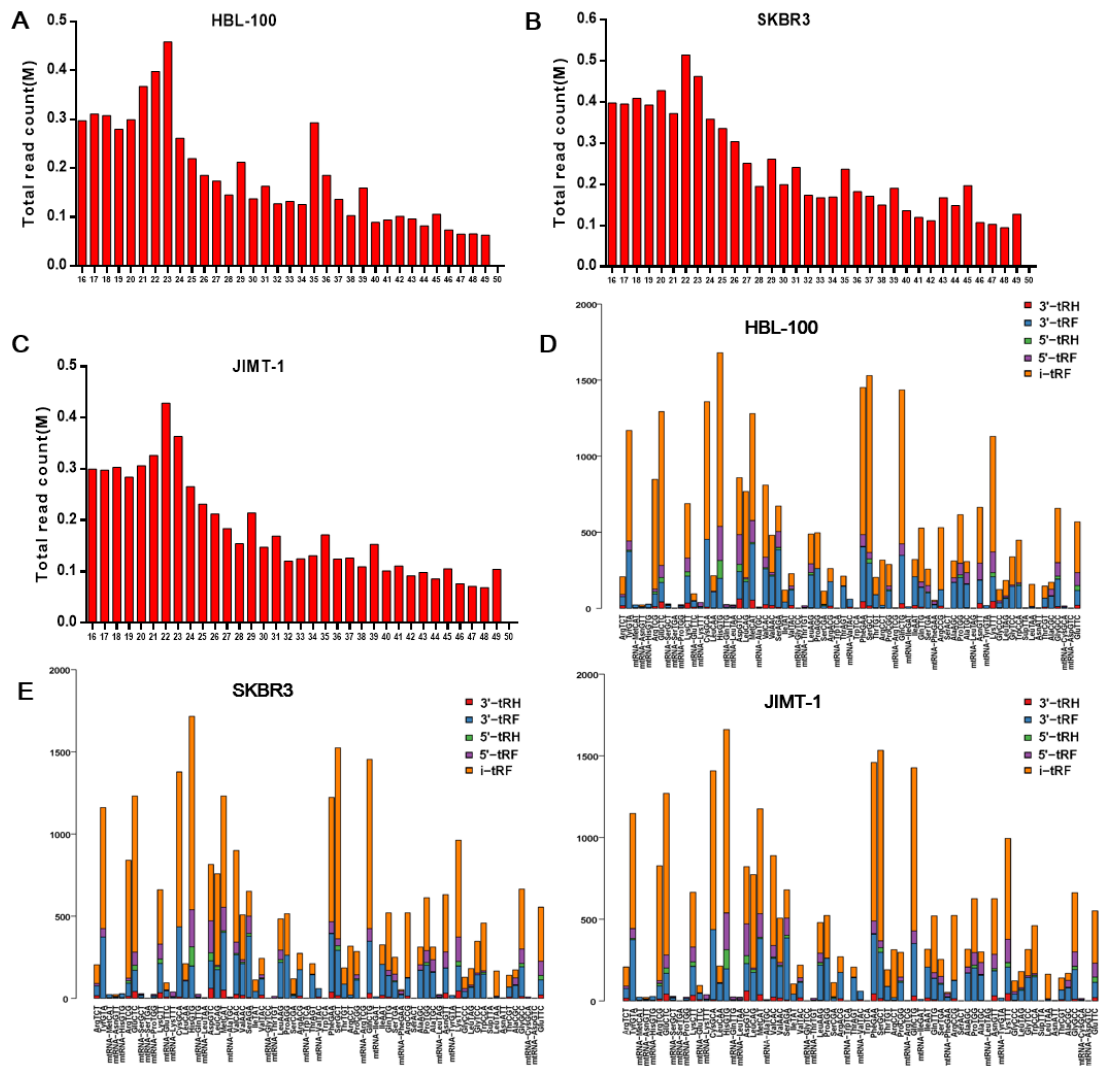
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Supplementary Figure 1. (A-C) The copy number and sequences of each unique read in the distribution of sequence read lengths. (D-F) tRFs and tRNA halves derived from the same anticodon tRNA.

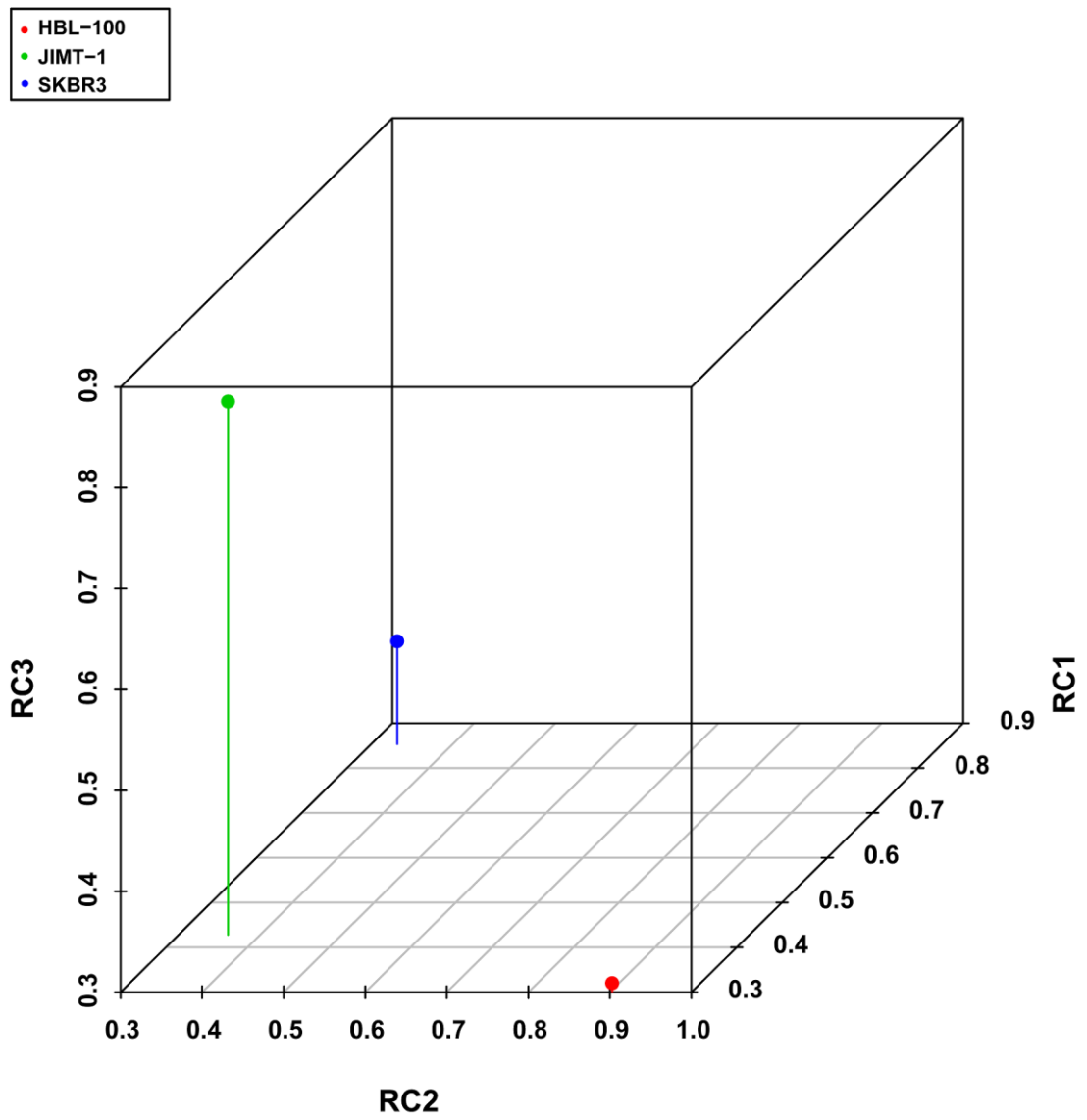
Supplementary Figure 2. The principal component analysis showed the differences among the three cell lines.

Supplementary Table 1. Clinical characteristic of the serum samples of trastuzumab sensitive and resistant patients.

Supplementary Table 2. Sequence of primers of tDRs used for qRT-PCR.



Supplementary Figure 1. (A-C)The copy number and sequences of each unique read in the distribution of sequence read lengths. **(D-F)** tRFs and tRNA halves derived from the same anticodon tRNA.



Supplementary Figure 2. The principal component analysis showed the differences among the three cell lines.

Supplementary Table 1. Clinical characteristic of the serum samples of trastuzumab sensitive and resistant patients

patient no.	age at diagnosis	initial diagnosis/subtype	initial stage	metastasis in	ER	PR	HER2	Ki67	treatment	outcome
1	62	06/2008/HER-2	IV	10/2014	(+/-)	(+/-)	(3+)	10%	Trastuzumab/liposome paclitaxel	Improved
2	66	10/2013/HER-2	IIA	04/2015	(-)	(-)	(-)	70%	Trastuzumab/Vinorelbine/Lobaplatin	Improved
3	34	02/2013/HER-2	II	05/2015	(-)	(-)	(3+)	50–60%	Trastuzumab/liposome paclitaxel/capecitabine	Improved
4	47	05/2015/HER-2	II	none					EC-TH	Improved and maintained on trastuzumab monotherapy
5	57	06/2012/LB	III	06/2015	(-)	(-)	(2+)	15%	Trastuzumab/liposome paclitaxel/ Cisplatin	Improved and maintained on trastuzumab monotherapy
6	40	03/2010/LB	I	02/2015	(-)	(-)	(3+)		Trastuzumab/liposome paclitaxel	Improved
7	54	02/2015/HER-2	II	none					EC-TH	Improved and maintained on trastuzumab monotherapy
8	50	06/2015/LB	II	none					EC-TH	Improved and maintained on trastuzumab monotherapy
9	54	01/2010/HER-2	II	09/2015	(-)	(-)	(2+)	10%	Trastuzumab/liposome paclitaxel/ capecitabine	Improved
10	51	03/2012/HER-2	II	05/2015	(-)	(-)	(3+)	15%	Trastuzumab/docetaxel/carboplatin	Improved and maintained on trastuzumab monotherapy
11	57	11/2008/LB	II	05/2015	(+)	(-)	3(%3+)	20%	Trastuzumab/Vinorelbine/Everolimus	Improved
12	46	04/2014/LB	I	02/2016	(+)	(+)	(3+)	25%	Trastuzumab/liposome paclitaxel/ capecitabine	Improved and maintained on trastuzumab monotherapy
13	50	02/2016/HER-2	IV		(-)	(-)	(2+)	30%	Trastuzumab/gemcitabine/cisplatin	Improved and maintained on trastuzumab monotherapy
14	54	01/2016/HER-2	II	none					EC-TH	Improved and maintained on trastuzumab monotherapy
15	55	04/2016/HER-2	I	none					Trastuzumab/Cyclophosphamide/ docetaxel	Improved
16	46	02/2009/LB	I	04/2013	(2+)	(-)	(2+)	30%	Trastuzumab/liposome paclitaxel/ capecitabine	Improved
17	57	03/2006/HER-2	I	02/2013	(-)	(-)	(3+)	20%	Trastuzumab/Pertuzumab/ albumin paclitaxel	Improved
18	71	01/2013/HER-2	III	02/2015	(-)	(-)	(3+)	40%	Trastuzumab/liposome paclitaxel	Improved
19	37	05/2010/HER-2	I	03/2014	(-)	(-)	(3+)	60%	Trastuzumab/liposome paclitaxel	Improved
20	38	03/2012/LB	III	09/2016	(2+)	(-)	(2+)	25%	Trastuzumab/capecitabine	Improved and maintained on trastuzumab monotherapy
21	58	10/2014/HER-2	III	04/2016	(-)	(-)	(3+)	20%	Trastuzumab/liposome paclitaxel	Improved and maintained on trastuzumab monotherapy
22	43	02/2010/LB	II	03/2016	(2+)	(2+)	(3+)	30%	Trastuzumab/liposome paclitaxel	Improved
23	57	04/2016/HER-2	IV		(-)	(-)	(3+)	25%	Trastuzumab/liposome paclitaxel	Improved
24	52	04/2016/HER-2	III	09/2016	(-)	(-)	(3+)	60%	Trastuzumab/liposome paclitaxel	Improved and maintained on trastuzumab monotherapy
25	72	06/2013/HER-2	III	07/2015	(-)	(-)	(3+)	25%	Trastuzumab/liposome paclitaxel	Improved
26	48	02/2012/HER-2	II	03/2015	(-)	(-)	(3+)	5%	Trastuzumab/docetaxel/carboplatin	Improved and maintained on trastuzumab monotherapy
27	25	09/2011/LB	I	11/2015	(+)	(+)	(3+)		Trastuzumab/albumin paclitaxel	Improved
28	50	09/2015/HER-2	IV		(-)	(-)	(3+)	20%	Trastuzumab/ Pertuzumab/ Paclitaxel- albumin	Improved
29	43	11/2014/HER-2	III	05/2015	(-)	(-)	(2+)	10%	Trastuzumab/gemcitabine/ capecitabine	Disease progression after 4th cycles
30	56	03/2010/HER-2	II	06/2015	(-)	(-)	(2+)	75%	Trastuzumab/albumin paclitaxel	Disease progression after 4th cycles
31	41	03/2011/HER-2	II	04/2015	(-)	(-)	(3+)	75%	Trastuzumab/docetaxel/capecitabine	Failed on trastuzumab treatment and local radiotherapy
32	56	03/2015/HER-2	IV		(-)	(-)	(2+)	35%	Trastuzumab/liposome paclitaxel/ capecitabine	Disease progression after 4th cycles
33	40	02/2014/HER-2	III	02/2015	(-)	(-)	(3+)	35%	Trastuzumab/paclitaxel/ capecitabine	Disease progression after 4th cycles
34	35	02/2012/HER-2	III	09/2014	didn't undergo biopsy of metastasis lesion				Trastuzumab/docetaxel	Failed on trastuzumab multi-line treatment
35	48	02/2011/LB	II	02/2014	(+)	(-)	(3+)	35%	Trastuzumab/docetaxel/capecitabine	Failed on trastuzumab multi-line treatment
36	30	04/2013/HER-2	II	03/2015	(-)	(-)	(3+)	70%	Trastuzumab/Vinorelbine/Everolimus	Failed on every chemotherapeutic agents
37	65	12/2014/LB	IV		(+)	(-)	(3+)	40%	Trastuzumab/Letrozole	Disease progression after 10months and then failed on chemotherapeutic agents
38	44	9/2011/LA	I	04/2016	(2+)	(-)	(2+)	60%	Trastuzumab/liposome paclitaxel	Disease progression after 2th cycles

											and give up further treatment
39	43	04/2013/LB	II	12/2015	(+)	(-)	(3+)	35%	Trastuzumab/Zoladex/anastrozole	Disease progression after 4th cycles	
40	57	04/2010/HER-2	I	07/2014	(2+)	(-)	(3+)	10%	Trastuzumab/liposome paclitaxel	Disease progression after 4th cycles	
41	46	11/2008/LA	III	04/2014	didn't undergo biopsy of metastasis lesion				Trastuzumab/liposome paclitaxel	Disease progression after 4th cycles	
42	48	04/2010/LB	I	05/2014	(-)	(-)	(2+)	50%	Trastuzumab/docetaxel	Disease progression after 4th cycles and failed on chemotherapeutic agents	
43	56	05/2013/LA	III	10/2014	(+)	(-)	(2+)	20%	Trastuzumab/gemcitabine/cisplatin	Disease progression after 4th cycles failed on trastuzumab multi-line treatment	
44	33	12/2006/LA	II	10/2014	(+)	(-)	(3+)	80%	Trastuzumab/liposome paclitaxel	Disease progression after 5th cycles and failed on multiple chemotherapeutic agents	
45	42	09/2013/HER-2	II	11/2014	(-)	(-)	(3+)	40%	Trastuzumab/Vinorelbine	Disease progression after 4th cycles	
46	36	01/2014/HER-2	II	09/2014	(2+)	(+)	(2+)	40%	Trastuzumab/liposome paclitaxel	Disease progression after 2th cycles	
47	52	12/2008/LA	II	02/2014	(+)	(-)	(3+)	30%	Trastuzumab/Vinorelbine/ local radiotherapy	Disease progression after 4th cycles	
48	43	05/2012/HER-2	II	10/2014	(-)	(-)	(3+)	60%	Trastuzumab/liposome paclitaxel	Failed on trastuzumab multi-line treatment and brain radiotherapy	
49	50	05/2015/HER-2	III	06/2016	(-)	(-)	(3+)	50%	Trastuzumab/liposome paclitaxel	Disease progression after 4th cycles	
50	61	04/2014/HER-2	IV	08/2015	(+)	(-)	(3+)	30%	Trastuzumab/ liposome paclitaxel	Disease progression after 2th cycles and failed on multiple chemotherapeutic agents	
51	54	04/2015/HER-2	III	01/2016	(-)	(-)	(3+)	40%	Trastuzumab/gemcitabine/cisplatin	Disease progression after 3 months	
52	58	06/2011/HER-2	II	11/2013	(-)	(-)	(3+)	10%	Trastuzumab/liposome paclitaxel/capecitabine	Disease progression after 3 months	
53	36	04/2015/LB	III	02/2016	(+)	(-)	(3+)	45%	Trastuzumab/ liposome paclitaxel	Disease progression after 2cycles	
54	43	05/2013/LB	III	10/2015	(+)	(-)	(3+)	40%	Trastuzumab/ lapatinib//capecitabine	Disease progression after 2cycles	
55	51	04/2010/HER-2	III	08/2013	didn't undergo biopsy of metastasis lesion				Trastuzumab/capecitabine	Disease progression after 4cycles	
56	49	04/2008/HER-2	II	01/2016	didn't undergo biopsy of metastasis lesion				Trastuzumab/liposome paclitaxel/capecitabine	Disease progression after 2th cycles and failed on whole brain radiotherapy	
57	47	04/2014/LB	III	06/2016	(+)	(-)	(2+)	40%	Trastuzumab/Vinorelbine/ cisplatin	Disease progression after 2th cycles and failed on multiple chemotherapeutic agents	

The first 28 patients are trastuzumab-responsive and the last 29 patients are trastuzumab non-responsive.

Supplementary Table 2. Sequence of primers of tDRs used for qRT-PCR

Primer name	Sequence
tRF-30-JZOYJE22RR33:	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCGGGGA
Forward	AGCCCGCATTTGACTGCAGATCAAGAGG
Reverse	CAGTGCAGGGTCCGAGGT
tRF-27-ZDXPHO53KSN: :	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCGGGG
Forward	AGCCCGTTGACTGCAGATCAAGAGGT
Reverse	CAGTGCAGGGTCCGAGGT
tRF-26-XIP2801MK8E:	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCGGGG
Forward	AGCCCGTGACTGCAGATCAAGAGGT
Reverse	CAGTGCAGGGTCCGAGGT
tRF-29-IYEVFMD0SR1Z:	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCGGGGA
Forward	AGCCCGATTTGACTGCAGATCAAGAGG
Reverse	CAGTGCAGGGTCCGAGGT
tRF-22-8B8871K92:	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGGAGG
Forward	AGCCCGTCAAATCCGGGTGCC
Reverse	CAGTGCAGGGTCCGAGGT
tRF-30-SERXPIN2NYDR	
RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGATTCAA
Forward	AGCCCGTAATGGTTAGCACTCTGGACT
Reverse	CAGTGCAGGGTCCGAGGT