

**THE EXPRESSION PATTERN OF EPIDERMAL DIFFERENTIATION MARKER KERATIN 10
IN THE NORMAL HUMAN BREAST AND BREAST CANCER**

Jiyoung Kim and René Villadsen

SUPPLEMENTAL MATERIAL

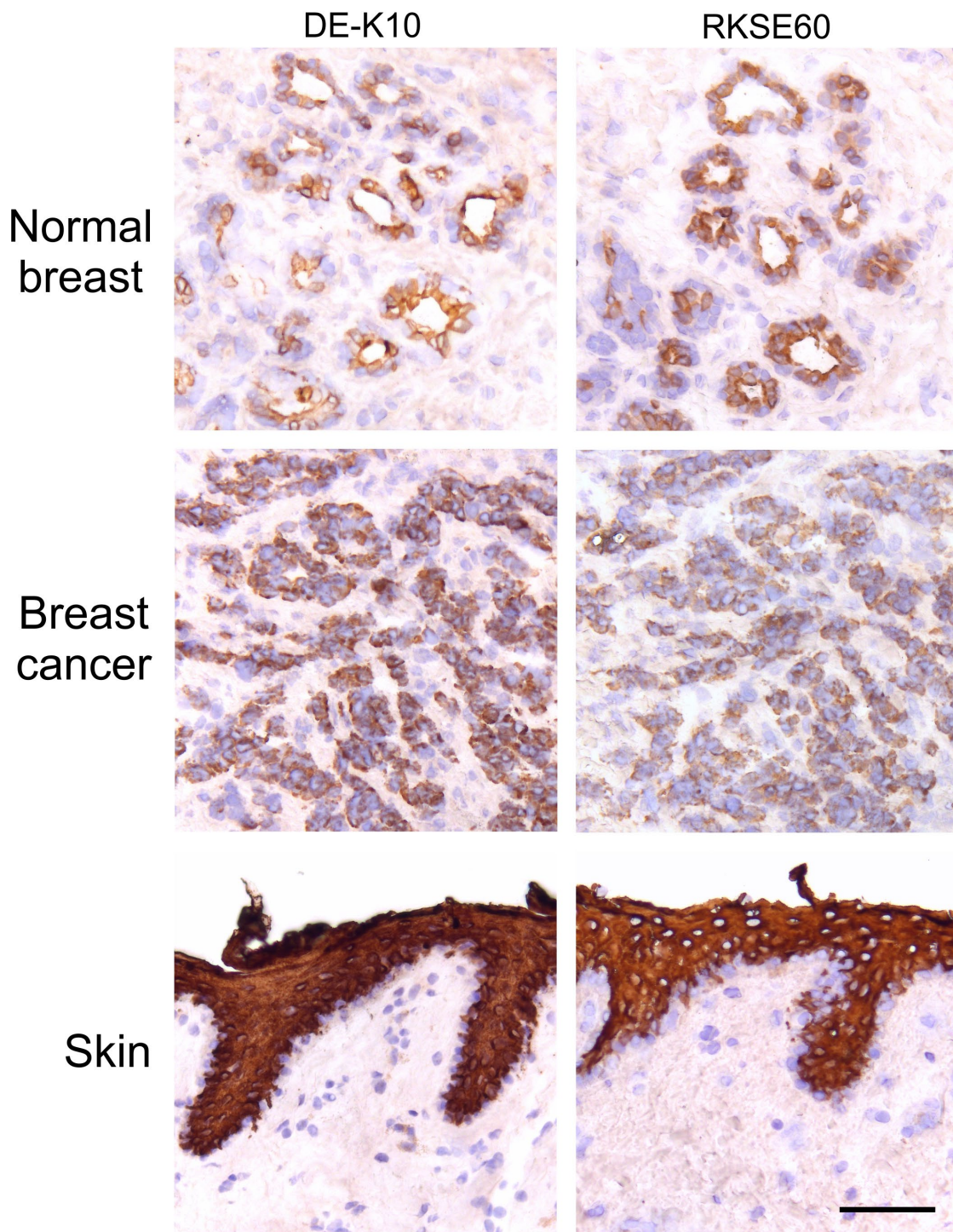


Figure S1. K10 antibodies DE-K10 and RKSE60 demonstrate similar staining patterns by immunohistochemistry. Serial sections of normal breast, breast cancer and skin stained by immunoperoxidase using either clone DE-K10 or RKSE60 against K10. Bar, 50 μ m.

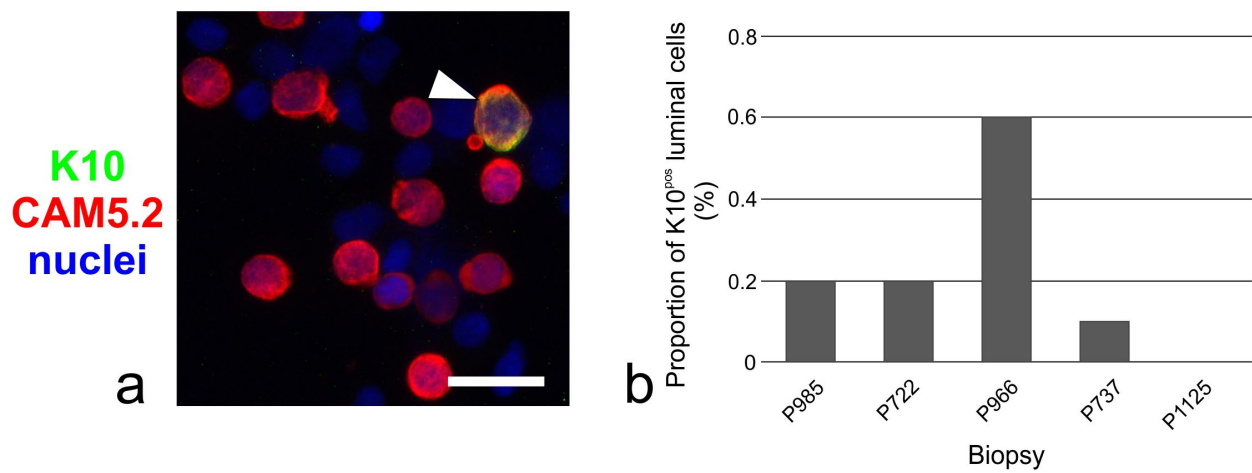
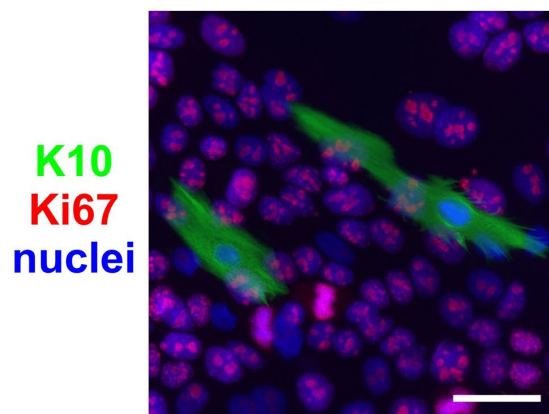
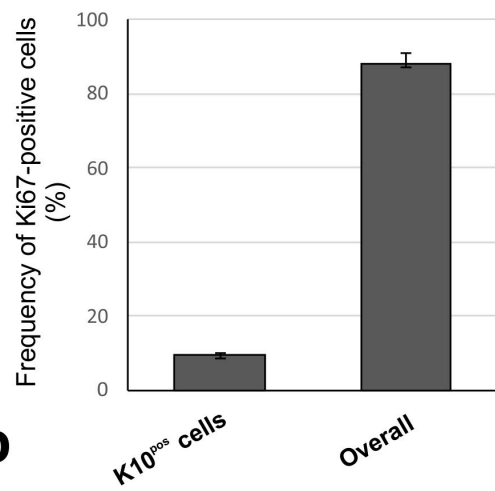


Figure S2. K10^{pos} cells constitute a rare population in the luminal compartment of the normal breast gland. (a) Immunos smear stained for K10 (green) and general luminal marker CAM5.2 (red). Arrowhead marks a double-positive cell. Bar, 25 μ m. (b) Quantification of the proportion of K10^{pos} cells within the CAM5.2-staining luminal cells in smears from 5 donors. 1000 luminal cells were counted for each biopsy.



a



b

Figure S3. K10^{pos} cells are rarely in cell cycle in normal-derived cell line MCF10A. (a) Immunofluorescent staining of cells by K10 (green) and Ki67 (red). Bar, 50 μ m. (b) Frequency of K10^{pos} cells expressing Ki67 compared to the overall frequency of Ki67^{pos} cells in MCF10A cells.

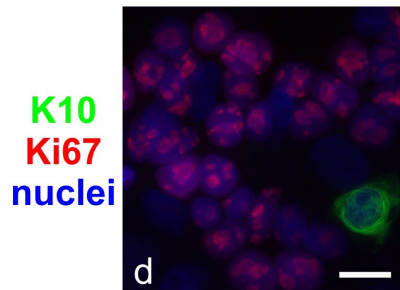
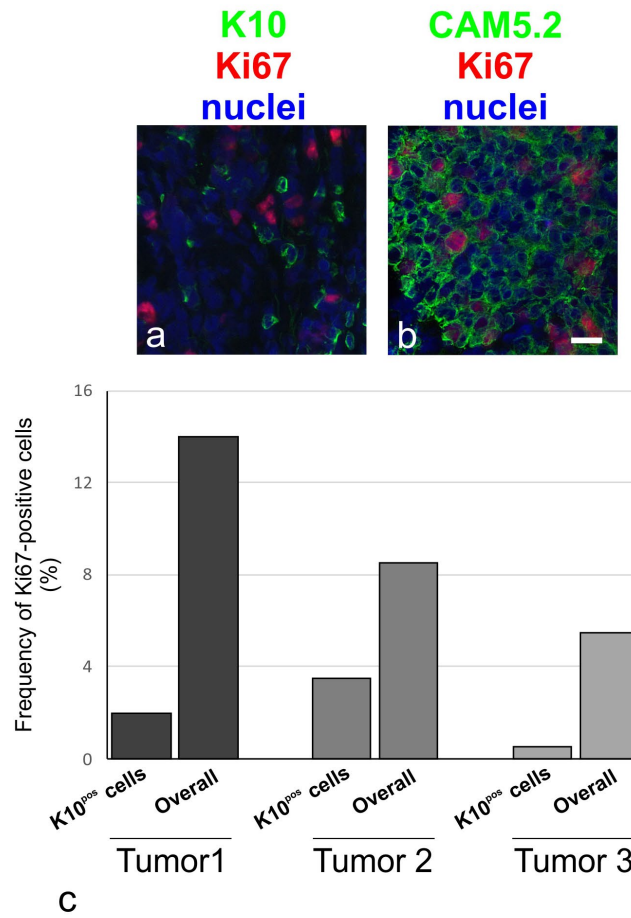


Figure S4. K10^{pos} breast cancer cells are less frequently in cell cycle. Immunofluorescence on a breast tumor stained for (a) K10 (green) and Ki67 (red) or stained for (b) luminal marker CAM5.2 (green) and Ki67 (red). Bar, 25µm. (c) Frequency of K10^{pos} cells expressing Ki67 compared to the frequency of Ki67^{pos}/CAM5.2^{pos} cells in 3 tumors. (d) MCF7 breast cancer cells stained by immunofluorescence for K10 (green) and Ki67 (red). Bar, 25µm.

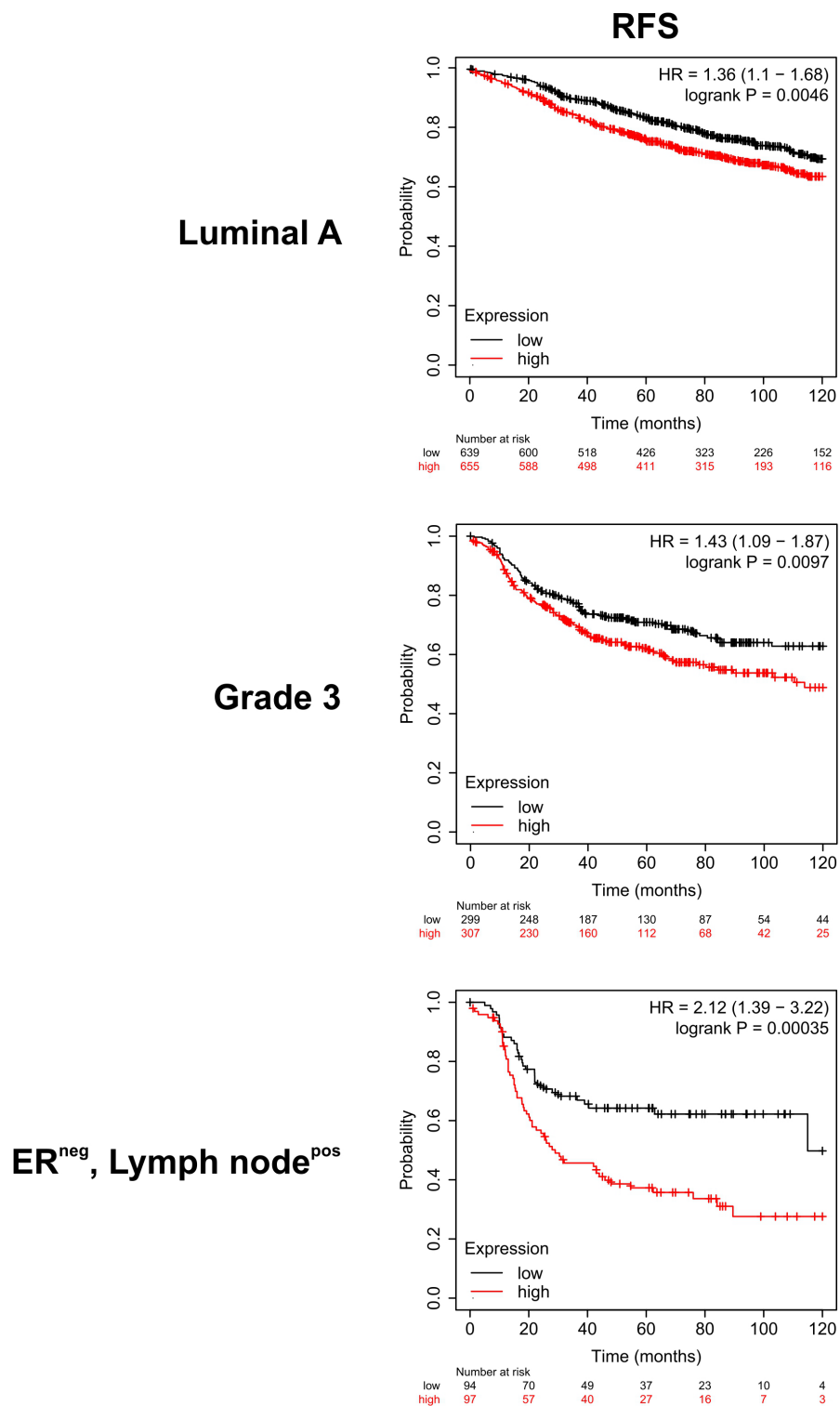


Figure S5. Kaplan-Meier plots of relapse free survival (RFS) demonstrate that higher levels of *KRT10* correlate to a worse outcome in different subsegments of breast cancer.

Table S1. Distribution of K10^{pos} structures in lobules (LOB) and ducts (DUCT) in normal-derived samples.

Data from this table were used to generate the graph shown in Figure 1b.

No	Biopsy ID	Age (years)	LOB ^{pos}	LOB- Total	DUCT ^{pos}	DUCT- Total	Positive
1	P648	13	0	25	0	14	
2	W428/1	17	0	10	0	3	
3	W430/1	17	0	6	0	7	
4	W432/1	18	0	7	0	4	
5	P944	18	0	10	0	1	
6	W637	18	0	0	0	10	
7	P790	18	4	5	5	6	√
8	P957	19	0	6	0	5	
9	P985	19	3	13	0	6	√
10	F526	19	0	11	0	7	
11	P722	20	0	7	0	7	
12	W445/1	20	0	13	0	6	
13	P816	20	0	25	0	9	
14	W562/1	21	0	12	0	10	
15	P960	22	0	8	0	8	

16	W458/1	22	0	3	0	8	
17	W1908/IV	23	0	31	0	11	
18	F518	24	0	21	0	5	
19	P644	25	0	16	0	4	
20	W1916/6	27	0	12	1	11	√
21	P941	27	0	28	0	9	
22	P820	29	0	5	0	6	
23	P945	29	0	10	0	6	
24	P832	32	0	6	0	3	
25	P636	33	0	26	0	14	
26	P671	36	0	24	0	18	
27	P809	39	0	7	0	3	
28	P880	43	8	21	3	12	√
29	W426/1	45	3	19	0	7	√
30	P819	45	0	22	0	7	
31	P959	45	2	9	0	7	√
32	P1075	45	4	34	2	7	√
33	W438	46	1	18	0	2	√

34	F498	46	0	13	0	5	
35	F519	46	3	6	1	7	√
36	P964	47	3	7	3	6	√
37	P637	49	3	11	0	4	√
38	W431/2	50	0	9	0	15	
39	W371/3	52	3	22	1	9	√
40	P647	56	0	5	0	7	
41	P634	56	0	9	0	3	
42	W430/2	59	0	16	0	7	
43	P940	59	0	25	0	22	
44	P662	60	1	5	0	7	√
45	P798	63	0	3	0	7	
46	P828	74	0	3	0	3	
TOTAL			38	604	16	352	13

Table S2. Expression of K10 in breast carcinomas by IHC.

Fraction of K10 ^{pos} breast carcinomas				
<i>ERα status</i>	<i>subtype</i>	<i>No. of carcinomas</i>	<i>No. of K10^{pos} carcinomas</i>	<i>%</i>
ERα ^{pos}		84	17	20.2%
	Luminal A	74	13	17.6%
	Luminal B	10	4	40%
ERα ^{neg}		51	5	9.8%
	HER2-enriched	16	1	6.3%
	TN	35	4	11.4%
<i>Total</i>		<i>135</i>	<i>22</i>	<i>16.3%</i>

Table S3. Expression of cKIT and Ks20.8 in 22 K10^{pos} breast carcinomas. -: negative, +c: >1% positive, +d >10% positive, +e: >50% positive.

No.	Biopsy ID	K10	cKIT	Ks20.8
ERα^{pos}				
1	w204-2	+d	-	+e
2	P688	+c	+d	+d
3	P720	+c	-	+d
4	P760	+c	-	+e
5	P788	+d	-	+e
6	P807	+c	-	+e
7	P808	+c	-	+e
8	P834	+c	-	+d
9	P875	+d	+e	+d
10	P953	+e	-	+e
11	P883	+d	-	+e
12	P990	+d	-	+e
13	P996	+e	-	+e
14	P1000	+c	-	+e
15	P1040	+c	-	+e

16	P1062	+d	-	-
17	P1066	+d	-	+e
ERα^{neg}				
18	P687	+e	-	+d
19	P757	+c	+d	+c
20	P811	+c	-	+e
21	p1065	+d	-	+e
22	W3141/6	+d	-	+c

Table S4. Clinical outcome as a function of *KRT10* expression in breast cancer patients. Patients were trichotomized and the high (upper tertile, T3) and low groups (lower tertile, T1) were compared for Relapse free survival (RFS) (A) and overall survival (OS) (B) in all patients, ER α -positive and ER α -negative patients based on *KRT10* expression. (C) Additional subsegmentation of data that showed significance. The follow up threshold was set at 120 months. To measure a significance of RFS and OS, P values based on Bonferroni multiple testing correction and hazard ratio (HR) were analyzed.

A	No. of patients in test			Multivariate analysis of selected gene		Relapse Free Survival	
Group	Total	Low, T1	High, T3	P value	HR	P value	HR
All	3951	1305	1343	0.0014	1.25 (1.09-1.43)	0.0189	1.17 (1.03-1.34)
ER α^{pos}	2061	680	701	0.0253	1.27 (1.03-1.56)	0.0434	1.23 (1.01-1.52)
ER α^{neg}	801	264	272	0.0009	1.62 (1.22-2.16)	0.001	1.59 (1.2-2.11)

B	No. of patients in test			Multivariate analysis of selected gene		Overall Survival	
Group	Total	Low, T1	High, T3	P value	HR	P value	HR
All	1402	464	435	0.0035	1.52 (1.15-2.01)	0.0039	1.5 (1.14-1.97)
ER α^{pos}	548	173	172	0.0068	1.9 (1.19-3.02)	0.0077	1.85 (1.17-2.92)
ER α^{neg}	251	84	85	0.0234	2 (1.1-3.66)	0.0451	1.81 (1-3.24)

C	No. of patients in test			Multivariate analysis of selected gene		Relapse Free Survival	
Group	Total	Low, T1	High, T3	P value	HR	P value	HR
Luminal A	1933	639	655	0.0048	1.36 (1.1-1.68)	0.0046	1.36 (1.1-1.68)
Grade 3	903	299	307	0.0057	1.48 (1.12-1.96)	0.0097	1.43 (1.09-1.87)
Grade 3, ER α^{neg}	385	127	131	0.0178	1.62 (1.09-2.42)	0.01	1.67 (1.13-2.48)
ER α^{neg} , lymph node positive	284	49	50	0.0002	2.32 (1.5-3.6)	0.00035	2.12 (1.39-3.22)