



1995 1998  
keratin-19  
RT-PCR 59  
acid guanidine  
phenol chloroform total RNA  
keratin-19 RT primer  
PCR (Table 1). RT-PCR  
DNA 2% agarose gell  
Total RNA control (-actin)

1) RNA

10 ml lymphoprep  
(Nycomed) 2,700 rpm 20  
-70°C . Trizol (Gibco  
BRL) 가 total RNA  
260 280 nm

2) Oligonucleotide primer

가 oligonucleotide primer human keratin 19 DNA  
DNA external primer  
set K 19ES (external sense) 5'-aggtggattccgctccgggca-3',  
K 19EA (external antisense) 5'-atcttctgtccctcgagc-3' RT-  
PCR 461  
inner primer set K 19IS (internal sense) 5'-agtgtgtctccaagg-  
cagc-3', K 19IA (internal antisense) 5'-gacatgccaagccaat-  
atgagg-3' nested PCR 221  
PCR (Table 1).

3) RT-PCR nested PCR

PCR (50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1  
% Triton X-100, 200 uM dNTP, 2.5 mM MgCl2, 0.1 mM DTT,

Table 1. External and internal primer for keratin-19

External	5'-AGGTGGATTCCGCTCCGGGCA-3'' (739 759)
	5'-ATCTTCCTGTCCCTCGAGCA-3'' (1180 1199)
Internal	5'-GACATGCGAAGCCAATAIGAGG-3' (783 804)
	5'-AGTGTGTICTTCCAAGGCAGC-3'' (984 1003)
-actin	5'-CGCTGTGTTGGCGTACAGGT-3''
	5'-TCATCACCAATTGGCAATGAG-3''

\*Underlying areas are different base from pseudogene.

60 U M-MLV reverse transcriptase, 0.5 U taq polymerase, 12  
U RNase inhibitor, 10 mM sense primer, 10 mM antisense  
primer) total RNA 1 µg DEPC DW  
30 µl . DNA thermal cyclcer (Perkin  
Elmer Cetus) 42°C 1 cDNA  
denaturation (94°C, 1 ), annealing (57°C, 1.5 ),  
extension (72°C, 2 ) PCR cycle 35  
extension 10 RT-PCR  
2 µl nested PCR  
2% agarose gel  
ethidium bromide UV illuminator  
. mRNA  
-actin RNA (Fig. 1).  
MCF7 10 ml 10<sup>7</sup>  
10<sup>5</sup> 1  
nested RT-PCR

4)

3  
, 6  
Mann-Whitney  
Kaplan-  
log-rank  
Meier  
SPSS 10.0 . P <  
0.05

59 가  
10<sup>7</sup> 가 10 가  
가 10<sup>6</sup>  
가 (Fig. 2).  
1) RT-PCR  
DCIS 4 1 T1

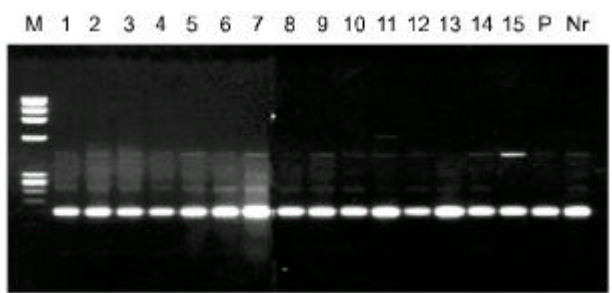
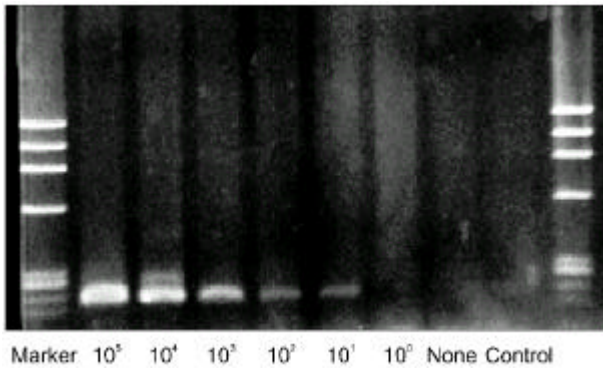
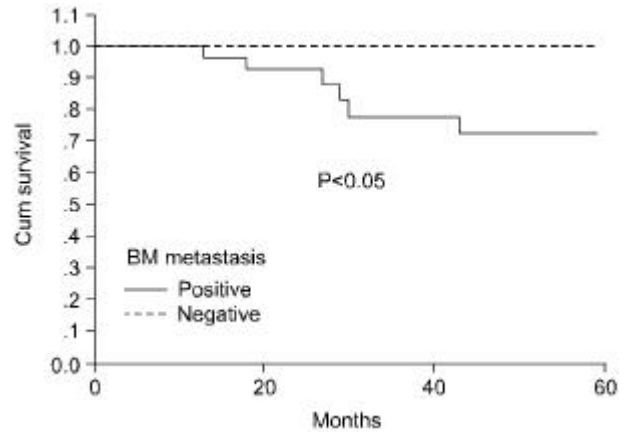


Fig. 1. Nested RT-PCR for beta actin. -actin shows positive result in all smpls.



**Fig. 2.** Sensitivity test of RT-PCR. Nested RT-PCR for keratin-19 shows positive result in 10 cells in 10 million peripheral nucleated blood cells.



**Fig. 3.** Bone marrow micrometastasis and disease Free Survival. Disease free survival curves according to bone marrow micrometastasis were statistically significant difference between groups.

**Table 2.** Tumor staging and bone marrow micrometastasis

	BM mets (-)	BM mets (+)	Total	P-value
<b>Tumor size</b>				
DCIS	3	1	4	
T1	17	13	30	
T2	9	11	20	
T3	0	4	4	
T4	1	0	1	0.091
<b>Node staging</b>				
N0	21	20	41	
N1	5	6	11	
N2	4	3	7	1.00
<b>Staging</b>				
DCIS	3	1	4	
Stage I	15	11	26	
Stage II	9	13	22	
Stage III	3	4	7	0.148
<b>Total</b>	<b>30</b>	<b>29</b>	<b>54</b>	

\*BM = bone marrow; mets = metastasis.

30	13	가		T2	20	11
				T3	4	
				T4	1	
				. N staging		
				N0	41	20 가
				N1		
11	6			N2	7	3
				DCIS	4	1
				, 1	26	11 , 2
						22
	13 , 3			7	4	(Table
2).						

**Table 3.** Prognostic factors and tumor recurrence

	Recur (-)	Recur (+)	Total	P-value
<b>Tumor size</b>				
DCIS	4	0	4	
T1	29	1	30	
T2	16	4	20	
T3	2	2	4	
T4	1	0	1	0.004
<b>Node staging</b>				
N0	38	3	41	
N1	9	2	11	
N2	5	2	7	0.088
<b>Stage</b>				
DCIS	4	0	4	
Stage I	26	0	26	
Stage II	18	4	22	
Stage III	4	3	7	0.002
<b>Bone marrow micrometastasis</b>				
BM mets (-)	30	0	30	
BM mets (+)	22	7	29	0.004
<b>Total</b>	<b>52</b>	<b>7</b>	<b>59</b>	

**2) RT-PCR**

59	7		DCIS	4
		T1	30	1 가
		T2		
20	4		T3	4 2
		T4	1	
		N0	41	3 , N1



RT-PCR  
 Keratin 19 pseudogene 가 가  
 pseudogene Keratin 19 가  
 가 (19-23)  
 Primer가 external primer sense primer 2  
 nucleotide (749, 754 )가 antisense 3  
 nucleotide pseudogene internal keratin 19  
 primer sense primer 4 (783,793,794,800  
 ), antisense primer 2 nucleotide  
 pseudogen 가  
 pseudogene primer  
 pseudogene  
 (Table 1). Keratin 19 keratin 8,18

nested RT-PCR RT-PCR  
 primer primer  
 PCR  
 Nested RT-PCR  
 42% 49%  
 RT-PCR  
 (24,25)  
 RT-PCR  
 30% 51%가  
 sensitivity test  
 DCIS 4 1  
 RT-PCR  
 RT-PCR  
 가  
 2 3 가  
 가  
 (26)  
 가  
 가 가 가  
 가 mammo-  
 globlin

mammoglobin MCF  
 가  
 가  
 가  
 가  
 가  
 가  
 DCIS  
 가  
 RT-PCR  
 가  
 가

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