

Table SI. Primers for HRM analysis and sequencing.

A, Primers for HRM analysis			
Fragment	Forward (5'-3')	Reverse (5'-3')	Length (bp)
Exon 18	CCAACCAAGCTCTCTTGAGG	GTGCCAGGGACCTTACCTTA	105
Exon 19	TGGATCCCAGAAGGTGAGAA	AGCAGAAACTCACATCGAGGA	106
Exon 20	GCATCTGCCTCACCTCCAC	GTCTTTGTGTTCCCGGACAT	86
Exon 21	CGCAGCATGTCAAGATCA	CCTCCTTACTTTGCCTCC	92
B, Primers for sequencing			
Fragment	Forward (5'-3')	Reverse (5'-3')	Length (bp)
Exon 18	GCTGAGGTGACCCTTGTCTC	GTGCCAGGGACCTTACCTTA	179
Exon 19	GTGCATCGCTGGTAACATCC	TGTGGAGATGAGCAGGGTCT	297
Exon 20	CTCTCCCCTGCATCTGTCA	TTATCTCCCCTCCCCGTATC	422
Exon 21	GCTCAGAGCCTGGCATGAA	CATCCTCCCCTGCATGTGT	348

Table SII. PC-9 cell countings ($\times 10^4$) (mean $\pm$ SD) of PC-9 cells at cultured alone and co-cultured with macrophages (M) at different ratios for six consecutive days

Days	PC-9	M:PC-9=1:2	M:PC-9=1:4	M:PC-9=1:8
Day 1	0.90 $\pm$ 0.29	2.07 $\pm$ 0.22 ^a	2.60 $\pm$ 0.09 ^a	2.19 $\pm$ 0.35 ^a
Day 2	1.80 $\pm$ 0.78	1.66 $\pm$ 0.13	3.51 $\pm$ 2.30	4.16 $\pm$ 0.88
Day 3	6.32 $\pm$ 0.88	5.05 $\pm$ 0.03	7.60 $\pm$ 0.53	8.52 $\pm$ 0.16 ^a
Day 4	21.10 $\pm$ 3.80	7.47 $\pm$ 0.98 ^a	12.43 $\pm$ 0.64	17.62 $\pm$ 1.74
Day 5	29.58 $\pm$ 0.59	13.57 $\pm$ 1.86 ^a	22.07 $\pm$ 3.23	29.60 $\pm$ 0.57
Day 6	28.05 $\pm$ 1.27	12.21 $\pm$ 0.33 ^a	14.18 $\pm$ 1.04 ^a	27.49 $\pm$ 0.59

PC-9 cells were all seeded with 1.5×10^4 at the beginning. ^aP<0.05 vs. PC-9.

Table SIII. The viability (%) of PC-9 and resistant sublines in the gefitinib environment (mean±SD).

Gefitinib (μM)	PC-9	PC-9/RG	PC-9/Mm/RG	PC-9/Mr/RG
20.000	--	15.96±1.24	37.22±5.24 ^{ab}	1.70±2.94 ^a
10.000	--	36.94±3.87	65.85±7.32 ^{ab}	39.50±4.69
5.000	--	56.65±2.63	80.88±9.57	91.39±16.54 ^a
2.500	--	60.04±3.25	84.29±8.16 ^a	86.90±11.87 ^a
1.250	--	69.07±1.23	80.81±4.97	92.86±11.08 ^a
0.039	29.60±2.40	--	--	--
0.020	51.98±5.35	--	--	--
0.010	77.33±8.91	--	--	--
0.005	98.04±1.86	--	--	--
0.002	100.67±4.64	--	--	--

^aP<0.05 vs. PC-9/GR; ^bP<0.05 vs. PC-9/Mr/GR

Table SIV. Cell counting ($\times 10^4$) of PC-9 and its sublines in the 5, 10 and 20 μM gefitinib environment for 5 consecutive days.

A, PC-9 and its sublines in 5 μM gefitinib environment (mean $\pm$ SD)				
Days	PC-9	PC-9/GR	PC-9/Mm/GR	PC-9/Mr/GR
Day 1	17.15 $\pm$ 1.40	11.87 $\pm$ 2.10 ^a	20.15 $\pm$ 0.57 ^b	15.88 $\pm$ 2.86
Day 2	8.49 $\pm$ 1.02	18.52 $\pm$ 0.69 ^a	20.87 $\pm$ 2.06 ^{ac}	14.29 $\pm$ 2.03 ^{ab}
Day 3	3.13 $\pm$ 0.13	6.28 $\pm$ 4.47	13.03 $\pm$ 0.41 ^{abc}	3.62 $\pm$ 0.27
Day 4	1.21 $\pm$ 0.048	6.23 $\pm$ 0.55 ^a	8.69 $\pm$ 0.44 ^{abc}	2.29 $\pm$ 0.25 ^{ab}
Day 5	0.60 $\pm$ 0.14	2.49 $\pm$ 0.19 ^a	5.58 $\pm$ 0.69 ^{abc}	0.86 $\pm$ 0.20 ^b
B, PC-9 and its sublines in the 10 μM gefitinib environment (mean $\pm$ SD)				
Days	PC-9	PC-9/GR	PC-9/Mm/GR	PC-9/Mr/GR
Day 1	17.46 $\pm$ 0.80	17.96 $\pm$ 0.59	21.03 $\pm$ 1.14 ^{abc}	14.71 $\pm$ 0.94 ^{ab}
Day 2	5.60 $\pm$ 0.07	14.27 $\pm$ 1.85	23.18 $\pm$ 6.89 ^{ac}	9.88 $\pm$ 1.20
Day 3	1.05 $\pm$ 0.15	5.84 $\pm$ 0.56	10.12 $\pm$ 0.47 ^a	4.38 $\pm$ 5.48
Day 4	0.74 $\pm$ 0.13	3.77 $\pm$ 0.44 ^a	6.94 $\pm$ 0.26 ^{abc}	1.16 $\pm$ 0.22 ^b
Day 5	0.42 $\pm$ 0.08	1.10 $\pm$ 0.18	4.50 $\pm$ 0.37 ^{abc}	0.42 $\pm$ 0.40
C, PC-9 and its sublines in the 20 μM gefitinib environment (mean $\pm$ SD)				
Days	PC-9	PC-9/GR	PC-9/Mm/GR	PC-9/Mr/GR
Day 1	12.95 $\pm$ 1.13	15.26 $\pm$ 1.03	20.13 $\pm$ 2.01 ^{abc}	11.99 $\pm$ 0.94
Day 2	2.68 $\pm$ 0.81	7.87 $\pm$ 0.39 ^a	13.07 $\pm$ 1.21 ^{abc}	6.25 $\pm$ 0.13 ^a
Day 3	0.17 $\pm$ 0.07	1.22 $\pm$ 0.00 ^a	5.39 $\pm$ 0.72 ^{abc}	0.52 $\pm$ 0.17
Day 4	0.13 $\pm$ 0.00	0.09 $\pm$ 0.16	1.32 $\pm$ 0.17 ^{abc}	0.21 $\pm$ 0.18
Day 5	0	0.02 $\pm$ 0.02	0.06 $\pm$ 0.02	0.15 $\pm$ 0.23

PC-9 cells were all seeded with 1.5×10^4 at the beginning. ^aP<0.05 vs. PC-9; ^bP<0.05 vs. PC-9/GR; ^cP<0.05 vs. PC-9/Mr/GR.

Table SV. Cell cycle distribution (%) in PC-9 and resistant sublines before and after gefitinib treatment.

A, Before gefitinib treatment (mean $\pm$ SD)			
Cell line	G ₀ /G ₁	S	G ₂
PC-9	52.9 $\pm$ 4.7	36.3 $\pm$ 3.6	10.7 $\pm$ 1.5
PC-9/GR	41.1 $\pm$ 3.0 ^a	55.1 $\pm$ 3.8 ^b	3.8 $\pm$ 1.4 ^c
PC-9/Mr/GR	48.9 $\pm$ 2.8	47.9 $\pm$ 2.5 ^b	3.2 $\pm$ 1.1 ^c
PC-9/Mm/GR	47.5 $\pm$ 4.4	45.3 $\pm$ 5.0	7.3 $\pm$ 0.6
B, After gefitinib treatment (mean $\pm$ SD)			
Cell line	G ₀ /G ₁	S	G ₂
PC-9	84.0 $\pm$ 0.6	8.7 $\pm$ 0.6	7.3 $\pm$ 1.2
PC-9/GR	85.1 $\pm$ 0.8	10.7 $\pm$ 0.2	4.2 $\pm$ 0.7 ^d
PC-9/Mr/GR	84.9 $\pm$ 1.3	9.8 $\pm$ 1.7	5.3 $\pm$ 0.4
PC-9/Mm/GR	80.1 $\pm$ 0.6 ^e	13.0 $\pm$ 1.2 ^f	6.9 $\pm$ 1.0

^aP<0.05 vs. PC-9 in G₀/G₁ phase; ^bP<0.05 vs. PC-9 in S phase; ^cP<0.05 vs. PC-9 in G₀/G₁ phase; ^dP<0.05 vs. PC-9 in G₀/G₁ phase; ^eP<0.05 vs. PC-9, PC9/GR, PC-9/Mr/GR in G₀/G₁ phase; ^fP<0.05 vs. PC-9, PC-9/Mr/GR in S phase.

Table SVI. Total of S and G₂ phases (%) in PC-9 and resistant sublines after gefitinib treatment.

Sublines	S + G ₂ (mean ± SD)	S + G ₂ (mean ± SE)
PC-9	16.0±0.6	16.0±0.4
PC-9/GR	14.9±0.8	14.9±0.5
PC-9/Mr/GR	15.1±1.3	15.1±0.8
PC-9/Mm/GR	19.9±0.6 ^a	19.9±0.4

^aP<0.05 vs. PC-9.

Table VII. TGF- β levels (mean $\pm$ SD) in the supernatant of PC-9 cells and co-culture with macrophages in Mm and Mr way

Day	PC-9	PC-9/Mm	PC-9/Mr
Day 1 ^a	1,434.8 $\pm$ 59.3	1,289.6 $\pm$ 70.1	1,023.3 $\pm$ 131.0
Day 2 ^b	1,410.0 $\pm$ 25.9	1,450.8 $\pm$ 124.1	1,091.7 $\pm$ 44.4

^aP=0.0131, ^bP=0.007 among three groups (PC-9/GR, PC-9/Mm/GR and PC-9/Mr/GR). TGF- β levels (pg/ml) in the supernatant of cells were detected with ELISA. The beginning of TGF- β level was detected in the culture medium (1114.9 pg/ml).

Table SVIII. TGF- β levels (mean $\pm$ SD) in the supernatant of resistant sublines from PC-9 cells

Day	PC-9/GR	PC-9/Mm/GR	PC-9/Mr/GR
Day 1 ^a	1,041.7 $\pm$ 33.3	1,208.4 $\pm$ 165.3	1,174.1 $\pm$ 19.3
Day 2 ^b	1,213.1 $\pm$ 4.4	1,258.2 $\pm$ 4.6	1,001.8 $\pm$ 96.9

^aP=0.381 and ^bP=0.027 among three groups (PC-9/GR, PC-9/Mm/GR and PC-9/Mr/GR). TGF- β levels (pg/ml) in the supernatant of cells were detected using ELISA. The beginning of TGF- β level was detected in the culture medium (1114.9 pg/ml).