Electronic Supplementary Information for Analyst

Fluorometric detection of EGFR exon 19 deletion mutation in lung cancer cells using graphene oxide

Dong-Min Kim,[a] Dong Ho Kim,[b] Woong Jeong,[c] Kye Young Lee,[d] and Dong-Eun Kim*[a]

[a] Department of Bioscience and Biotechnology, Konkuk University, Gwangjin-gu, Seoul 05029, Republic of Korea

[b] Department of Pediatrics, Korea Cancer Center Hospital, Nowon-gu, Seoul 01812, Republic of Korea

[c] Department of Emergency Medicine, Kyung Hee University Hospital, Kangdong-gu, Seoul 05278, Republic of Korea

[d] Department of Internal Medicine, Konkuk University Medical Center, Gwangjin-gu, Seoul 05029, Republic of Korea

**Corresponding Author*

Supplementary experimental methods

1. Preparation of complementary DNA and PCR amplification

Total cellular RNA was extracted from PC9 cells using TRIzol® solution (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and dissolved in a final volume of 30 μL. A total of 1 μg RNA was used for reverse transcription with a PrimerScript 1st strand cDNA Synthesis Kit (Takara Bio, Inc., Shiga, Japan). Next, 2 μL of complementary DNA (cDNA) was subjected to PCR with FAM-labeled EGFR exon 19 deletion DNA probe under the above-mentioned conditions except that reverse primer 4 was used rather than reverse primer 3 (Experimental).

2. Optimization of graphene oxide and Mg^{2+} concentration

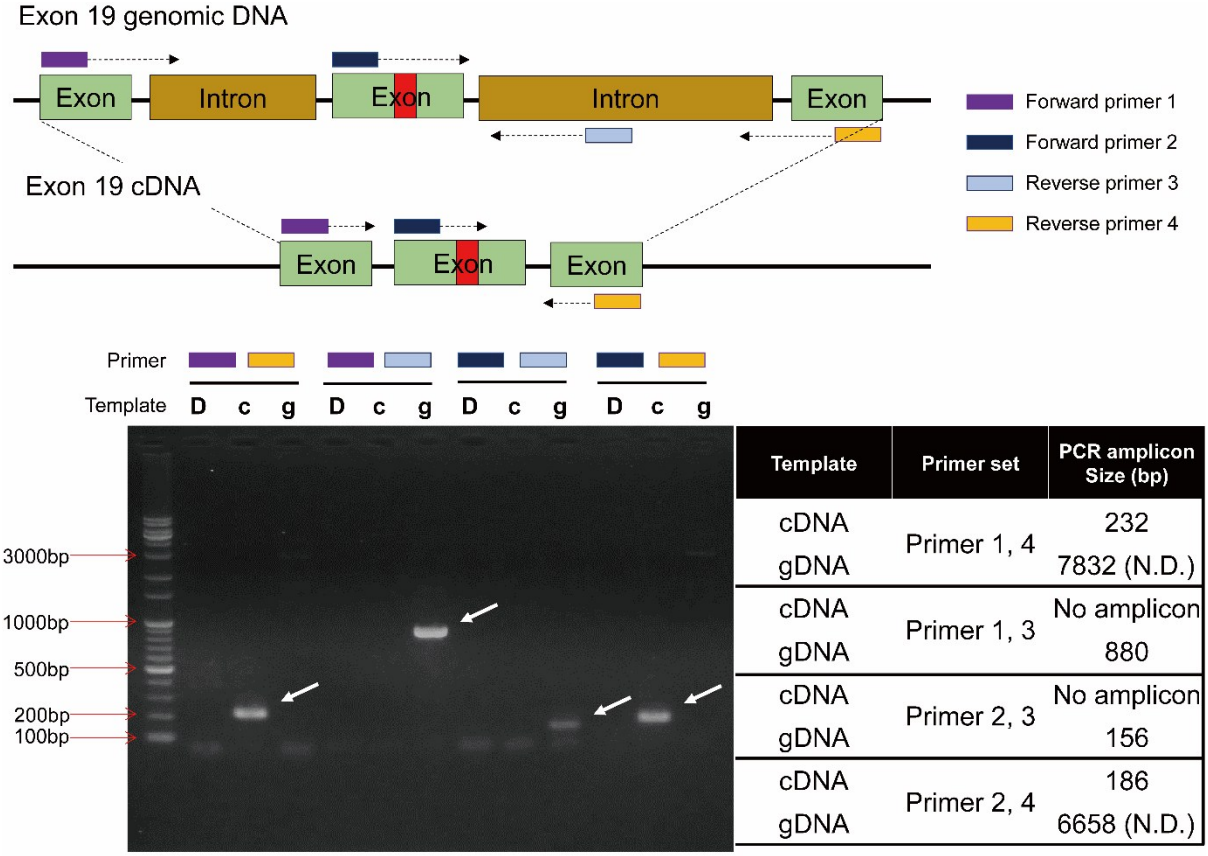
To establish the conditions with the highest difference in fluorescence between EGFR exon 19 wild-type and mutant type, the amount of GO and Mg^{2+}, which is related to the affinity for DNA binding to GO,[1] were optimized. A 5-μL aliquot of PCR product was incubated with various GO concentrations (0, 3, 5, 7, 10, 15, 20, 25 μg mL^{-1}) and the fluorescence intensity ratio (F/F_0) was measured as described above; F and F_0 represent the fluorescence intensity of the FAM-labeled probe derived from the PCR products amplified with PC9 gDNA (EGFR exon 19 deletion) and A549 gDNA (EGFR wild-type), respectively. To determine proper Mg^{2+} concentration, different concentrations of $MgCl_2$ (0, 0.1, 1, 3, 6, 10 mM) were incubated with 20 μg mL^{-1} of GO and the fluorescence intensity ratio (F/F_0) was measured in a 96-well micro plate.

Supplementary data (tables and figures)

Table S1. Oligonucleotide sequences (5′ to 3′).

Name	Sequence* (5′ → 3′)	Size (nts)
Forward primer 1	GATCAAAGTGCTGGGCTCCGGTG	23
Forward primer 2 for plasma	CTCTGGATCCCAGAAGGTGAGAAAGTT	27
Reverse primer 3	AGAAAAGGTGGGCCTGAGGTTCAGA	25
Exon19 deletion probe	**FAM**-CCCGTCGCTATCAAGACATCTCCGAAAGCCAACAAGG AAATCCTCGATGTGAGTTTCTGCTTTGCTGTGTGGGGGTCCAT-**P**	80
Taqman probe	**FAM**-CCCGTCGCTATCAAGACATCTCCGAAAGCC-**TAMRA**	30
Reverse primer 4 for cDNA	CTGCGTGATGAGCTGCACGGT	21
Reverse primer 5 for sequencing	CTGGGTAGATGCCAGTAATTGCCTG	25

*FAM, 6-fluorescein amidite modification; TAMRA, 5-Carboxy-tetramethylrhodamine modification; P, phosphorylation; nts, nucleotides.



Template	Primer set	PCR amplicon Size (bp)
cDNA	Primer 1, 4	232
gDNA		7832 (N.D.)
cDNA	Primer 1, 3	No amplicon
gDNA		880
cDNA	Primer 2, 3	No amplicon
gDNA		156
cDNA	Primer 2, 4	186
gDNA		6658 (N.D.)

Fig. S1. Agarose gel electrophoresis analysis of PCR products amplified (indicated with arrows) with different templates and primers. PCR products of big size (longer than 1,000 bp) was not detected (denoted as N.D.), because template with big span was not amplified due to a short extension time (30 s) during PCR. Abbreviations: D, Distilled water; c, complementary DNA (cDNA); g, genomic DNA (gDNA).

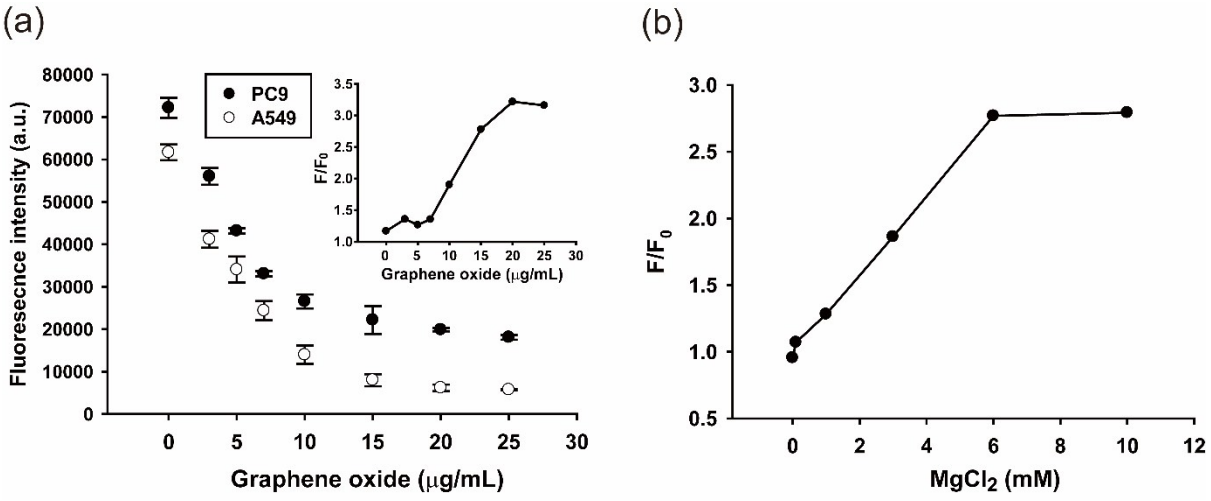


Fig. S2. Optimization of GO and Mg^{2+} concentration for GO-based fluorometric assay. (a) The fluorescence intensity ratio (F/F_o) change, which was derived from fluorescence intensity ratio between PC9 (mutant gene, F) and A549 (wild-type gene, F_o), with the amount of GO. The maximal fluorescence intensity ratio was observed with 20 μg mL^{-1} of GO. *Inset:* Fluorescence intensity ratio (F/F_0) with different concentrations of GO. (b) Optimization of Mg^{2+} concentration. The highest fluorescence intensity ratio (F/F_0) was obtained in $MgCl_2$ over 6 mM.

Table S2. Comparison of experimental detection limit and selectivity between GO-based PCR and published methods for detecting EGFR exon 19 deletion mutation.

Method	Labeling[a]	Detection limit[b]	Selectivity[c]	Reference
GO-based PCR	1	49 pg	0.1 %	This study
qPCR	2	10 ng uL^{-1}	5 ~ 10 %	2
Cast PCR	2	-	0.1 %	3
PNA clamping PCR	1	8.6 ng uL^{-1}	-	4
Scorpion ARMS	2	2.3 ng uL^{-1}	1 %	5
Droplet digital PCR	2	-	0.04 %	6
NGS	4	-	~ 1 %	7

[a] Labels such as fluorophore or quencher required for assay

[b] The lowest quantity of a mutant gene required to distinguish the sample from the blank.

[c] Minimum percentage of mutant gene in an excess wild-type gene required for reliable mutation detection.

Abbreviation: qPCR, quantitative PCR; cast PCR, Competitive allele-specific Taqman PCR; PNA, peptide nucleic acid; scorpion SARMS, scorpion amplified refractory mutation system; NGS, next-generation sequencing

References

1. M. Wu, R. Kempaiah, P. J. J. Huang, V. Maheshwari and J. W. Liu, *Langmuir*, 2011, **27**, 2731-2738.
2. K. Endo, A. Konishi, H. Sasaki, M. Takada, H. Tanaka, M. Okumura, M. Kawahara, H. Sugiura, Y. Kuwabara, I. Fukai, A. Matsumura, M. Yano, Y. Kobayashi, K. Mizuno, H. Haneda, E. Suzuki, K. Iuchi and Y. Fujii, *Lung Cancer-J Iaslc*, 2005, **50**, 375-384.
3. C. Roma, C. Esposito, A. M. Rachiglio, R. Pasquale, A. Iannaccone, N. Chicchinelli, R. Franco, R. Mancini, S. Pisconti, A. De Luca, G. Botti, A. Morabito and N. Normanno, *Biomed Res Int*, 2013, **2013**, 485087
4. H. R. Kim, S. Y. Lee, D. S. Hyun, M. K. Lee, H. K. Lee, C. M. Choi, S. H. Yang, Y. C. Kim, Y. C. Lee, S. Y. Kim, S. H. Jang, J. C. Lee and K. Y. Lee, *J Exp Clin Canc Res*, 2013, **32**, 50.
5. H. L. Duan, J. L. Lu, T. Lu, J. Gao, J. Zhang, Y. Xu, M. Z. Wang, H. W. Wu, Z. Y. Liang and T. H. Liu, *Int J Clin Exp Patho*, 2015, **8**, 13136-13145.
6. G. S. Zhu, X. Ye, Z. W. Dong, Y. C. Lu, Y. Sun, Y. Liu, R. McCormack, Y. Gu and X. Q. Liu, *J Mol Diagn*, 2015, **17**, 265-272.
7. D. de Biase, M. Visani, U. Malapelle, F. Simonato, V. Cesari, C. Bellevicine, A. Pession, G. Troncone, A. Fassina and G. Tallini, *Plos One*, 2013, **8,** e83607.