

Article

DNA Methylation Analysis in Plasma Cell-Free DNA and Paired CTCs of NSCLC Patients before and after Osimertinib Treatment

Aliki Ntzifa ¹, Dora Londra ¹, Theodoros Rampias ², Athanasios Kotsakis ³, Vassilis Georgoulis ⁴ and Evi Lianidou ^{1,*}

¹ Analysis of Circulating Tumor Cells Lab, Lab of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece; alntzi@chem.uoa.gr (A.N.); doralo@chem.uoa.gr (D.L.)

² Basic Research Center, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece; trampias@bioacademy.gr

³ Faculty of Medicine, School of Health Sciences, University of Thessaly, 41110 Larissa, Thessaly, Greece; thankotsakis@uth.gr

⁴ Department of Medical Oncology, Hellenic Oncology Research Group (HORG), 11471 Athens, Greece; georgulv@otenet.gr

* Correspondence: lianidou@chem.uoa.gr; Tel.: +30-210-727-4311

Supplementary

Real-time MSP Protocols

APC

FORWARD: 5'-TTATTGCGGAGTGCGGGT-3'

REVERSE: 5'-CGAACTCCCGACGAAAATAAA-3'

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 2.0 mM MgCl₂ (Promega, USA), 0.15 μM of each dNTP (Invitrogen, USA), 0.15 μg/μL BSA (Sigma, Germany), 0.20 μM of each primer (Integrated DNA Technologies, USA), 1X LC Green[®] (Idaho Technology, USA) and 0.05 U/μL GoTaq[®] DNA polymerase (Promega, USA). dH₂O was added to a final volume of 10 μL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 66°C for 15s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.

RASSF10

FORWARD: 5'-CGTCGTTTTAGTAGATTTTCGGTC-3'

REVERSE: 5'-CGTCGAAACAAATAATACGACG-3'

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 2mM MgCl₂ (Promega, USA), 0.2 μM of each dNTP (Invitrogen, USA), 0.25 μg/μL BSA (Sigma, Germany), 0.25 μM of each primer (Integrated DNA Technologies, USA), 1X LC Green[®] (Idaho Technology, USA) and 0.05 U/μL GoTaq[®] DNA polymerase (Promega, USA). dH₂O was added to a final volume of 10 μL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 65°C for 20s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.

SHISA3

FORWARD: 5'-TAGTTTTAGAGGCGCGATTC-3'

REVERSE: 5'-CAAATACAAACGACCGCG-3'

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 2.5 mM MgCl₂ (Promega, USA), 0.2 μM of each dNTP (Invitrogen, USA), 0.25 μg/μL BSA (Sigma, Germany), 0.25 μM of each primer (Integrated DNA Technologies, USA), 1X LC Green[®] (Idaho Technology, USA) and 0.05 U/μL GoTaq[®] DNA polymerase (Promega,

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USA). dH₂O was added to a final volume of 10 µL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 63°C for 20s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.

FOXA1

FORWARD: 5'- GGCGGCGACGTTAAGAC -3'

REVERSE: 5'- CGCTCAACGTAAACATCTTACT -3'

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 2.0 mM MgCl₂ (Promega, USA), 0.15 µM of each dNTP (Invitrogen, USA), 0.15 µg/µL BSA (Sigma, Germany), 0.20 µM of each primer (Integrated DNA Technologies, USA), 1X LC Green® (Idaho Technology, USA) and 0.05 U/µL GoTaq® DNA polymerase (Promega, USA). dH₂O was added to a final volume of 10 µL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 66°C for 20s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.

RARβ

FORWARD: 5'- CGAGAACGCGAGCGATTC -3'

REVERSE: 5'- TCGACCAATCCAACCGAAAC -3'

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 2.0 mM MgCl₂ (Promega, USA), 0.15 µM of each dNTP (Invitrogen, USA), 0.15 µg/µL BSA (Sigma, Germany), 0.20 µM of each primer (Integrated DNA Technologies, USA), 1X LC Green® (Idaho Technology, USA) and 0.05 U/µL GoTaq® DNA polymerase (Promega, USA). dH₂O was added to a final volume of 10 µL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 65°C for 15s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.

WIF-1

FORWARD: 5'- TTATACGTTTATTTTCGCGGGC -3'

REVERSE: 5'- GACCGCCACTTAAAAACGCT -3'

PROBE: 56-FAM/ ATTGT+GAATGTAGTT +TCGGGGGTT/3BHQ_1

The PCR reaction mix consisted of 1X PCR buffer (Promega, USA), 3.0 mM MgCl₂ (Promega, USA), 0.15 µM of each dNTP (Invitrogen, USA), 0.15 µg/µL BSA (Sigma, Germany), 0.20 µM of each primer (Integrated DNA Technologies, USA), 0.30 µM of LNA probe (Integrated DNA Technologies, USA) and 0.05 U/µL GoTaq® DNA polymerase (Promega, USA). dH₂O was added to a final volume of 10 µL. Protocol conditions were: 1 cycle at 95°C for 2min, followed by 45 cycles of: 95°C for 10s, 58°C for 15s and 72°C for 20s, and a final cooling cycle at 40°C for 30s.