Table S1 PCR condition and primer sequences used in this study

|  |
| --- |
| List of primers for PCR, sequencing and their cycling conditions |
| Primer Name | Sequence | Direction | Size | Tm | Ta | Ext. | Description |
| EGFR\_18\_NF | TCCAAATGAGCTGGCAAGTG | Forward | 400 bps | 60.76 oC | 60-54oC | 30s | Nested PCR for EGFR exon 18 in genomic DNA |
| EGFR\_18\_NR | TCCCAAACACTCAGTGAAACAAA | Reverse | 60.36 oC |
| EGFR\_18\_F1\* | CTTACACCCAGTGGAGAGGC | Forward | 192 bps | 58.18 oC | 60-54oC | 30s |
| EGFR\_18\_R1 | TACAGCTTGCAAGGACTCTG | Reverse | 55.22 oC |
| EGFR\_19\_NF | CCTTAGGTGCGGCTCCACAGC | Forward | 249 bps | 67.05 oC | 60-54oC | 45s | Nested PCR for EGFR exon 19 in genomic DNA |
| EGFR\_19\_NR | CATTTAGGATGTGGAGATGAGC | Reverse | 57.21 oC |
| EGFR\_19\_F1\* | ATTGCCAGTTAACGTCTTCC | Forward | 160 bps | 55.33 oC | 60-54oC | 30s |
| EGFR\_19\_R1 | CACACAGCAAAGCAGAAACT | Reverse | 55.12 oC |
| EGFR\_20\_NF | GAAACTCAAGATCGCATTCATGC | Forward | 378 bps | 61.75 oC | 60-54oC | 30s | Nested PCR for EGFR exon 20 in genomic DNA |
| EGFR\_20\_NR | GCAAACTCTTGCTATCCCAGGAG | Reverse | 62.17 oC |
| EGFR\_20\_F1\* | CATGCGAAGCCACACTGAC | Forward | 198 bps | 59.83 oC | 60-54oC | 30s |
| EGFR\_20\_R1 | GCAGGTACTGGGAGCCAAT | Reverse | 58.54 oC |
| EGFR\_21\_NF | GCTCAGAGCCTGGCATGAA | Forward | 347 bps | 61.05 oC | 60-54oC | 30s | Nested PCR for EGFR exon 21 in genomic DNA |
| EGFR\_21\_NR | CATCCTCCCCTGCATGTGT | Reverse | 60.33 oC |
| EGFR\_21\_F1\* | CAGCAGGGTCTTCTCTGTTT | Forward | 200 bps | 55.55 oC | 60-54oC | 30s |
| EGFR\_21\_R1 | GACCTAAAGCCACCTCCTTA | Reverse | 54.59 oC |
| Kras\_2F | GTATTAACCTTATGTGTGA | Forward | 222 bps | 41.00 oC | 60-54oC | 30s | Semi-nested PCR for Kras exon 2 in genomic DNA |
| Kras\_2R | GTCCTGCACCAGTAATATG | Reverse | 49.63 oC |
| Kras\_2R\_in\* | TTGGATCATATTCGTCCAC | Reverse | 177 bps | 52.48 oC | 60-54oC | 30s |

con't Table S1 - List of primers for PCR and PCR conditions for direct PCR and sequencing experiments

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Primer Name | Sequence | Direction | Size | Tm | Ta | Ext. | Description |
| Kras\_c61\_F | GATGGAGAAACCTGTCTCTTGG | Forward | 112 bps | 58.64 oC | 60-54oC | 30s | Semi-nested PCR for Kras codon 61 mutations |
| Kras\_c61\_Fin | TGGAGAAACCTGTCTCTTGGA | Forward | 58.29 oC |
| Kras\_c61\_R | ATGGCAAATACACAAAGAAAGC | Reverse | 100 bps | 56.94 oC | 60-54oC | 30s |
| Kras\_c146\_F | CTTTTTATGTATTTCAGGGTGTTGA | Forward | 194 bps | 57.31 oC | 60-54oC | 30s | Semi-nested PCR for Kras codon 146 mutations |
| Kras\_c146\_R | AGTGTAATGTACAAAAATTACCACTTG | Reverse | 55.86 oC |
| Nras\_ex2\_F\_ex | TTGCATTCCCTGTGGTTTTT | Forward | 252 bps | 58.86 oC | 60-54oC | 30s | Nested PCR for Nras exon 2 in genomic DNA |
| Nras\_ex2\_R\_ex | CCTGTAGAGGTTAATATCCGCAAA | Reverse | 59.39 oC |
| Nras\_ex2\_F\_in | CACCCCCAGGATTCTTACAG | Forward | 172 bps | 57.89 oC | 60-54oC | 30s |
| Nras\_ex2\_R\_in | TCCGCAAATGACTTGCTATT | Reverse | 56.90 oC |
| MEK1\_ex2\_F\_ex | TTGACTTGTGCTCCCCACTT | Forward | 250 bps | 59.13 oC | 60-54oC | 30s | Nested PCR for MEK1 exon 2 in genomic DNA |
| MEK1\_ex2\_R\_ex | AGGCAAACTCACCTTTCTGG | Reverse | 57.82 oC |
| MEK1\_ex2\_F\_in | GGAACAGGACCAACTTGGAG | Forward | 189 bps | 58.00 oC | 60-54oC | 30s |
| MEK1\_ex2\_R\_in | TTGTGGGAGACCTTGAACAC | Reverse | 56.94 oC |
| Braf\_ex11\_outF | TCCCTCTCAGGCATAAGGTAA | Forward | 313 bps | 57.35 oC | 60-54oC | 30s | Nested PCR for Braf exon 11 in genomic DNA |
| Braf\_ex11\_outR | CGAACAGTGAATATTTCCTTTGAT | Reverse | 57.30 oC |
| Braf\_ex11\_inF\* | TTTTCTGTTTGGCTTGACTTGA | Forward | 192 bps | 58.46 oC | 60-54oC | 30s |
| Braf\_ex11\_inR | TGTCACAATGTCACCACATTACA | Reverse | 58.25 oC |

con't Table S1 - List of primers for PCR and PCR conditions for direct PCR and sequencing experiments

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Primer Name | Sequence | Direction | Size | Tm | Ta | Ext. | Description |
| Braf\_ex15\_outF | TCATAATGCTTGCTCTGATAGGA | Forward | 224 bps | 57.59 oC | 60-54oC | 30s | Semi-nested PCR for Braf exon 15 in genomic DNA |
| Braf\_ex15\_outR | GGCCAAAAATTTAATCAGTGGA | Reverse | 58.82 oC |
| Braf\_ex15\_inR\* | TGGAAAAATAGCCTCAATTCTTA | Reverse | 206 bps | 55.90 oC | 60-54oC | 30s |
| PIK3CA\_ex9\_exF\* | CTGTGAATCCAGAGGGGAAA | Forward | 248 bps | 58.50 oC | 60-54oC | 45s | Semi-nested PCR for PIK3CA exon 9 genomic DNA |
| SF-PIK3CA\_ex9Rout | ACATGCTGAGATCAGCCAAAT | Reverse | 58.20 oC |
| SF-PIK3CA\_ex9Fin\* | GACAAAGAACAGCTCAAAGCAA | Forward | 168 bps | 58.23 oC | 60-54oC | 30s |
| PIK3CA-20-2F   | TATTCGACAGCATGCCAATC | Forward | 312 bps | 58.11 oC | 60-54oC | 45s | Semi-nested PCR for PIK3CA exon 20 genomic DNA |
| PIK3CA-20-2R  | GGTCTTTGCCTGCTGAGAGT | Reverse | 58.05 oC |
| PIK3CA-20-2F\*  | TTGCATACATTCGAAAGACC   | Forward | 226 bps | 54.73 oC | 60-54oC | 30s |
| MET\_int13\_outF | CGTCGATTCTTGTGTGCTGT | Forward | 326 bps267 bps | 58.31 oC | 60-54oC60-54 oC | 45s30s | MET exon14 and exon boundaries in genomic DNA |
| MET\_int13\_inF\* | CCATGAGTTCTGGGCACTG | Forward | 58.60 oC |
| MET\_int14\_outR | TGTCACAACCCACTGAGGTA | Reverse | 202 bps | 55.82 oC | 54oC | 30s |
| (1) Primer marked with asterisk (\*) was used for sanger sequencing(2) Tm: Melting Temperature(3) Ta: Annealing Temperature(4) Ext.: Extension Time (s= second) |

Table S2 Clinical characteristics and driver mutation status of 5 patients with lung adenosquamous carcinoma.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patient No. | Gender | Age | Stage | Smoking status | Mutations |
| 1 | Male | 61 | IA | NS | ALK translocation |
| 2 | Female | 71 | IB | NS | EGFR exon 19 deletion |
| 3 | Male | 69 | IIA | ES | Wild-type |
| 4 | Male | 67 | IIIA | ES | Wild-type |
| 5 | Male | 74 | IIIB | NS | Wild-type |
| NS: Never-Smoker; ES: Ever-smoker |

Table S3 Correlation between mutation status and TTF-1 expression

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Status | TTF-1 negative | TTF-1 positive | p value | Total |
| EGFR Mutation | Wild-type | 8 (10.0) | 72 (90.0) | 0.021\* | 80 |
| Mutant | 0 (0.0) | 58 (100.0) | 58 |
| KRAS Mutation | Wild-type | 4 (3.3) | 118 (96.7) | 0.007\*\* | 122 |
| Mutant | 4 (25.0) | 12 (75.0) | 16 |
| ALK | Wild-type | 7 (5.4) | 122 (94.6) | 0.425 | 129 |
| Mutant | 1 (11.1) | 8 (88.9) | 9 |
| MET Mutation | Wild-type | 8 (6.1) | 124 (93.9) | 1.000 | 132 |
| Mutant | 0 (0.0) | 6 (100.0) | 6 |
| MET amplification | Negative | 6 (4.5) | 126 (95.5) | 0.102 | 132 |
| Positive | 1 (50.0) | 1 (50.0) | 2 |
| MET copy number  | < 5 | 7 (5.4) | 122 (94.6) | 1.000 | 129 |
| >= 5 | 0 (0.0) | 5 (100.0) | 5 |
| Fusion gene | Negative | 7 (5.6) | 119 (94.4) | 0.527 | 126 |
| Positive | 1 (8.3) | 11 (91.7) | 12 |
| Any Drivers | Negative | 2 (4.8) | 40 (95.2) | 1.000 | 42 |
| Positive | 6 (6.3) | 90 (93.7) | 96 |