Analysis of ultra-deep targeted sequencing reveals mutation burden is associated with gender and clinical outcome in lung adenocarcinoma

Supplementary Materials

Supplementary Table S1: The gene list and number of samples with genetic alterations

| Gene Name | No. of samples with genetic alterations | Percentage (%) |
|-----------|----------------------------------------|-----------------|
| TP53      | 148                                    | 44.2            |
| EGFR      | 134                                    | 40.0            |
| LRP1B     | 62                                     | 18.5            |
| KRAS      | 36                                     | 10.7            |
| CDC27     | 35                                     | 10.4            |
| AGGF1     | 30                                     | 9.0             |
| PTPRD     | 24                                     | 7.2             |
| APC       | 18                                     | 5.4             |
| RHPN2     | 16                                     | 4.8             |
| PIK3CA    | 16                                     | 4.8             |
| PARP4     | 15                                     | 4.5             |
| STK11     | 15                                     | 4.5             |
| GLI3      | 15                                     | 4.5             |
| KEAP1     | 14                                     | 4.2             |
| BRAF      | 13                                     | 3.9             |
| ITIH5     | 12                                     | 3.6             |
| ATF71P    | 12                                     | 3.6             |
| FLT1      | 11                                     | 3.3             |
| IQGAP3    | 11                                     | 3.3             |
| MET       | 10                                     | 3.0             |
| NASP      | 8                                      | 2.4             |
| MRC2      | 8                                      | 2.4             |
| ERBB2     | 8                                      | 2.4             |
| CFTR      | 7                                      | 2.1             |
| SMAD2     | 6                                      | 1.8             |
| CRIPAK    | 6                                      | 1.8             |
| TERT      | 6                                      | 1.8             |
| CDKN2A    | 6                                      | 1.8             |
| CHEK2     | 6                                      | 1.8             |
| SUSD2     | 6                                      | 1.8             |
| RIN3      | 5                                      | 1.5             |
| YES1      | 4                                      | 1.2             |
| TMC6      | 4                                      | 1.2             |
| MECP2     | 3                                      | 0.9             |
| Gene     | Value | Score |
|----------|-------|-------|
| EBF3     | 3     | 0.9   |
| FSCN1    | 3     | 0.9   |
| PARP1    | 3     | 0.9   |
| EML4     | 3     | 0.9   |
| MAFB     | 2     | 0.6   |
| APOL1    | 2     | 0.6   |
| TBP      | 1     | 0.3   |
| VEGFA    | 1     | 0.3   |
| MAP2K1   | 1     | 0.3   |
| NRAS     | 1     | 0.3   |
| FADD     | 0     | 0.0   |
| NKX2-1   | 0     | 0.0   |
| TLX3     | 0     | 0.0   |
| CCNE2    | 0     | 0.0   |
| AKT1     | 0     | 0.0   |
| RANBP10  | 0     | 0.0   |
| KDM5B    | 0     | 0.0   |
Supplementary Table S2: Univariate and multivariate analysis with negative binomial regression comparing the counts of missense mutation by variables in patients with lung adenocarcinoma ($n = 335$)

| No. of patients | No. of missense Median (Range) | Univariate analysis | Multivariate analysis |
|-----------------|---------------------------------|--------------------|----------------------|
| Gender          |                                 | $p$ value          | Male-to-Female Ratio (95% CI Ratio) | $p$ value | Male-to-Female Ratio (95% CI Ratio) |
| Female          | 152                             | (reference)        | (reference)          |           |                                   |
| Male            | 183                             | 2 (0–28)           | 3.49E-05             | 1.565 (1.267–1.936) | 0.000896 | 1.422(1.093–1.852) |
| Age             |                                 |                    |                      |           |                                   |
| < 65            | 228                             | 1 (0–15)           | (reference)          | (reference) |                                   |
| ≥ 65            | 107                             | 2 (0–28)           | 0.701                |           | 0.997                              |
| Smoking         |                                 |                    |                      |           |                                   |
| No              | 199                             | 1 (0–28)           | (reference)          | (reference) |                                   |
| Yes             | 105                             | 2 (0–15)           | 0.0044               |           | 0.303                              |
| NA              | 31                              |                    |                      |           |                                   |
| Stage           |                                 |                    |                      |           |                                   |
| I               | 82                              | 2 (0–28)           | (reference)          | (reference) |                                   |
| II              | 69                              | 1 (0–11)           | 0.205                |           | 0.587                              |
| III             | 154                             | 2 (0–7)            | 0.183                |           | 0.652                              |
| IV              | 29                              | 1 (0–15)           | 0.947                |           | 0.639                              |
| NA              | 1                               |                    |                      |           |                                   |

NA, not applicable.
Supplementary Table S3: Negative binomial regression comparing the counts of missense mutations in smokers and non-smokers with lung adenocarcinoma

|                  | No. of patients | No. of missense mutations | p value | Male to female ratio (95% CI ratio) |
|------------------|----------------|---------------------------|---------|-----------------------------------|
|                  |                | Median (range)            |         |                                   |
|                  | Female         | 128                       | 1 (0–11)| (reference)                       | 1.481 (1.099–1.996) |
| Non-smoker       | Male           | 71                        | 2 (0–28)| 9.90E-03                          |                         |
|                  | Female         | 9                         | 2 (1–4)| (reference)                       | 1.151 (0.643–2.127)   |
| Smoker           | Male           | 96                        | 2 (0–15)| 0.6429                            |                         |
Supplementary Table S4: Univariate and multivariate survival analysis of the patients with lung adenocarcinoma (n = 335)

|                        | Univariate analysis | Multivariate analysis |
|------------------------|---------------------|-----------------------|
|                        | HR  | 95% CI   | p value | HR  | 95% CI | p value |
| **Age**                |     |          |         |     |        |         |
| < 65 vs ≥ 65           | 0.87| 0.55~1.36| 0.538   | 1.19| 0.76~1.86| 0.452   |
| **Gender**             |     |          |         |     |        |         |
| Female vs Male         | 1.71| 1.12~2.61| 0.013   | 1.97| 1.19~3.26| 0.008   |
| **Smoking**            |     |          |         |     |        |         |
| No vs Yes              | 0.98| 0.62~1.57| 0.947   | 0.682| 0.40~1.17| 0.374   |
| **Stage**              |     |          | < 0.0001|     |        | < 0.0001|
| I                      |     |          | 1.93    |     | 1.36~6.27|
| II                     |     |          | 3.74    |     | 1.86~7.53|
| III                    |     |          | 9.15    |     | 4.02~20.8|
| IV                     |     |          | 2.45    |     | 1.19~5.02| 0.015   |

Supplementary Figure S1: Kaplan-Meier overall survival curves for patients with higher or lower burden of total mutations (A and B) or missense mutations (C and D) among non-smokers (A and C) and smokers (B and D) were shown.