cancers

MDPI

# Supplementary: AKT3 Is A Novel Regulator of Cancer-Associated Fibroblasts in Head and Neck Squamous Cell Carcinoma

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**Table S1.** Relationship between expression of CAF-specific markers and immune markers in 522 HNSCC patients from TCGA database.

| Genes | *FAP* | | *COL11A1* | | *PDGFRB* | | *POSTN* | |
|---|---|---|---|---|---|---|---|---|
| | ***r*-value** | ***p*-value** | ***r*-value** | ***p*-value** | ***r*-value** | ***p*-value** | ***r*-value** | ***p*-value** |
| *CD68* | 0.292 | <0.001 | 0.233 | <0.001 | 0.302 | <0.001 | 0.288 | <0.001 |
| *CD163* | 0.383 | <0.001 | 0.342 | <0.001 | 0.481 | <0.001 | 0.432 | <0.001 |
| *MRC1* | 0.500 | <0.001 | 0.371 | <0.001 | 0.542 | <0.001 | 0.482 | <0.001 |
| *CD3E* | −0.016 | 0.712 | −0.040 | 0.362 | 0.168 | <0.001 | 0.021 | 0.633 |
| *CD4* | 0.237 | <0.001 | 0.235 | <0.001 | 0.406 | <0.001 | 0.323 | <0.001 |
| *FOXP3* | 0.181 | <0.001 | 0.172 | <0.001 | 0.417 | <0.001 | 0.247 | <0.001 |
| *CD8A* | −0.090 | 0.039 | −0.074 | 0.090 | 0.073 | 0.097 | −0.045 | 0.300 |
| *CD19* | −0.168 | <0.001 | −0.168 | <0.001 | 0.051 | 0.242 | −0.083 | 0.059 |
| *CD14* | 0.329 | <0.001 | 0.189 | <0.001 | 0.325 | <0.001 | 0.262 | <0.001 |
| *IFNG* | −0.132 | 0.003 | −0.142 | 0.001 | −0.072 | 0.099 | −0.155 | <0.001 |
| *GZMB* | −0.117 | 0.007 | −0.187 | <0.001 | −0.045 | 0.302 | −0.162 | <0.001 |
| *IL10* | 0.401 | <0.001 | 0.210 | <0.001 | 0.504 | <0.001 | 0.358 | <0.001 |
| *TGFB1* | 0.429 | <0.001 | 0.234 | <0.001 | 0.259 | <0.001 | 0.252 | <0.001 |
| *IL6* | 0.304 | <0.001 | 0.165 | <0.001 | 0.306 | <0.001 | 0.223 | <0.001 |
| *CXCL8* | 0.108 | 0.013 | 0.007 | 0.873 | 0.077 | 0.080 | 0.014 | 0.741 |
| *CSF1* | 0.309 | <0.001 | 0.263 | <0.001 | 0.438 | <0.001 | 0.326 | <0.001 |
| *CSF2* | 0.354 | <0.001 | 0.010 | 0.816 | 0.159 | <0.001 | 0.077 | 0.081 |

Abbreviations: CAF, cancer-associated fibroblast; HNSCC, head and neck squamous cell carcinoma; TCGA, The Cancer Genome Atlas.

**Table S2.** Gene Set Enrichment Anaylisis of hallmark gene sets and C2 canonical pathways up-regulated in CAFs (FDR <0.10).

| Gene Set | NES | FDR |
|---|---|---|
| HALLMARK_PROTEIN_SECRETION | 2.28 | 0 |
| REACTOME_CELL_EXTRACELLULAR_MATRIX_INTERACTIONS | 2.18 | 0.034 |
| REACTOME_EPHB_MEDIATED_FORWARD_SIGNALING | 2.09 | 0.055 |
| REACTOME_TRANSLOCATION_OF_SLC2A4_GLUT4_TO_THE_PLASMA_MEMBRANE | 2.08 | 0.041 |
| REACTOME_RHO_GTPASES_ACTIVATE_FORMINS | 2.04 | 0.048 |
| REACTOME_RESOLUTION_OF_SISTER_CHROMATID_COHESION | 2.04 | 0.041 |
| REACTOME_MITOTIC_SPINDLE_CHECKPOINT | 2.01 | 0.045 |
| REACTOME_RHO_GTPASES_ACTIVATE_PAKS | 2.01 | 0.039 |
| REACTOME_ANTIGEN_PRESENTATION:_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC | 2 | 0.035 |
| PID_INTEGRIN_A4B1_PATHWAY | 2 | 0.034 |
| REACTOME_RHO_GTPASE_EFFECTORS | 1.99 | 0.032 |
| REACTOME_RHO_GTPASES_ACTIVATE_ROCKS | 1.99 | 0.03 |
| REACTOME_SMOOTH_MUSCLE_CONTRACTION | 1.99 | 0.028 |
| REACTOME_MITOTIC_METAPHASE_AND_ANAPHASE | 1.92 | 0.053 |
| REACTOME_VXPX_CARGO_TARGETING_TO_CILIUM | 1.92 | 0.051 |
| REACTOME_GOLGI_TO_ER_RETROGRADE_TRANSPORT | 1.9 | 0.054 |
| KEGG_FOCAL_ADHESION | 1.89 | 0.058 |

| | | |
|---|---|---|
| PID_RHOA_PATHWAY | 1.88 | 0.06 |
| PID_CDC42_PATHWAY | 1.88 | 0.059 |
| KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION | 1.87 | 0.06 |
| SIG_REGULATION_OF_THE_ACTIN_CYTOSKELETON_BY_RHO_GTPASES | 1.87 | 0.057 |
| REACTOME_SYNDECAN_INTERACTIONS | 1.86 | 0.059 |
| BIOCARTA_INTEGRIN_PATHWAY | 1.85 | 0.061 |
| REACTOME_SYNTHESIS_OF_PIPS_AT_THE_EARLY_ENDOSOME_MEMBRANE | 1.85 | 0.058 |
| KEGG_SMALL_CELL_LUNG_CANCER | 1.85 | 0.06 |
| REACTOME_EPH_EPHRIN_SIGNALING | 1.81 | 0.078 |
| REACTOME_RHO_GTPASES_ACTIVATE_CIT | 1.8 | 0.086 |
| REACTOME_NON_INTEGRIN_MEMBRANE_ECM_INTERACTIONS | 1.79 | 0.085 |
| KEGG_REGULATION_OF_ACTIN_CYTOSKELETON | 1.79 | 0.084 |
| REACTOME_ATTENUATION_PHASE | 1.79 | 0.082 |
| REACTOME_HSP90_CHAPERONE_CYCLE_FOR_STEROID_HORMONE_RECEPTORS_SHR | 1.78 | 0.084 |
| REACTOME_COPI_DEPENDENT_GOLGI_TO_ER_RETROGRADE_TRAFFIC | 1.78 | 0.081 |
| HALLMARK_G2M_CHECKPOINT | 1.68 | 0.011 |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 1.68 | 0.007 |
| HALLMARK_MITOTIC_SPINDLE | 1.51 | 0.028 |
| HALLMARK_UV_RESPONSE_DN | 1.5 | 0.023 |
| HALLMARK_APICAL_JUNCTION | 1.49 | 0.02 |
| HALLMARK_E2F_TARGETS | 1.44 | 0.025 |
| HALLMARK_MTORC1_SIGNALING | 1.42 | 0.027 |
| HALLMARK_ANDROGEN_RESPONSE | 1.38 | 0.031 |
| HALLMARK_MYC_TARGETS_V1 | 1.26 | 0.081 |

Abbreviations: CAFs, cancer-associated fibroblasts; NES, normalized enrichment score; FDR, false discovery rate.

**Table S3.** Gene Set Enrichment Anaylisis of hallmark gene sets and C2 canonical pathways down-regulated in CAFs (FDR <0.10).

| Gene Set | NES | FDR |
|---|---|---|
| KEGG_RIBOSOME | −2.54 | 0 |
| REACTOME_NONSENSE_MEDIATED_DECAY_NMD_INDEPENDENT_OF_THE_EXON_JUNCTION_COMPLEX_EJC | −2.42 | 0 |
| REACTOME_SELENOAMINO_ACID_METABOLISM | −2.32 | 0 |
| REACTOME_NONSENSE_MEDIATED_DECAY_NMD | −2.29 | 0 |
| REACTOME_EUKARYOTIC_TRANSLATION_INITIATION | −2.24 | 0 |
| REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE | −2.15 | 0 |
| REACTOME_BETA_DEFENSINS | −2.05 | 0.001 |
| KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450 | −2 | 0.002 |
| REACTOME_COMPLEMENT_CASCADE | −1.96 | 0.003 |
| REACTOME_REGULATION_OF_EXPRESSION_OF_SLITS_AND_ROBOS | −1.94 | 0.004 |
| REACTOME_INFLUENZA_INFECTION | −1.93 | 0.005 |
| BIOCARTA_COMP_PATHWAY | −1.92 | 0.005 |
| REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S | −1.91 | 0.005 |
| REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_27_HYDROXYCHOLESTEROL | −1.87 | 0.009 |
| REACTOME_RA_BIOSYNTHESIS_PATHWAY | −1.84 | 0.013 |
| KEGG_DRUG_METABOLISM_CYTOCHROME_P450 | −1.84 | 0.013 |
| KEGG_RETINOL_METABOLISM | −1.83 | 0.014 |
| BIOCARTA_CLASSIC_PATHWAY | −1.82 | 0.015 |
| REACTOME_RRNA_PROCESSING_IN_THE_NUCLEUS_AND_CYTOSOL | −1.81 | 0.017 |
| REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL | −1.8 | 0.021 |
| REACTOME_FOXO_MEDIATED_TRANSCRIPTION_OF_OXIDATIVE_STRESS_METABOLIC_AND_NEURONAL_GENES | −1.8 | 0.02 |
| REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT | −1.8 | 0.019 |
| REACTOME_DEFENSINS | −1.79 | 0.02 |
| REACTOME_DIGESTION_AND_ABSORPTION | −1.78 | 0.022 |

| | | |
|---|---|---|
| KEGG_LINOLEIC_ACID_METABOLISM | −1.78 | 0.022 |
| KEGG_ARACHIDONIC_ACID_METABOLISM | −1.77 | 0.022 |
| REACTOME_PHASE_I_FUNCTIONALIZATION_OF_COMPOUNDS | −1.76 | 0.026 |
| REACTOME_RRNA_PROCESSING | −1.75 | 0.03 |
| KEGG_COMPLEMENT_AND_COAGULATION_CASCADES | −1.74 | 0.033 |
| KEGG_STEROID_HORMONE_BIOSYNTHESIS | −1.73 | 0.036 |
| REACTOME_ARACHIDONIC_ACID_METABOLISM | −1.72 | 0.037 |
| REACTOME_INTERLEUKIN_20_FAMILY_SIGNALING | −1.72 | 0.037 |
| REACTOME_BIOSYNTHESIS_OF_SPECIALIZED_PRORESOLVING_MEDIATORS_SPMS | −1.72 | 0.038 |
| REACTOME_PHASE_2_PLATEAU_PHASE | −1.71 | 0.038 |
| REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES | −1.69 | 0.051 |
| REACTOME_SIGNALING_BY_ROBO_RECEPTORS | −1.67 | 0.061 |
| REACTOME_TERMINATION_OF_O_GLYCAN_BIOSYNTHESIS | −1.67 | 0.061 |
| REACTOME_DIGESTION | −1.66 | 0.065 |
| REACTOME_INTERLEUKIN_10_SIGNALING | −1.66 | 0.065 |
| REACTOME_ANTIMICROBIAL_PEPTIDES | −1.66 | 0.066 |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | −1.64 | 0.028 |
| REACTOME_ACYL_CHAIN_REMODELLING_OF_PC | −1.63 | 0.087 |
| HALLMARK_BILE_ACID_METABOLISM | −1.61 | 0.021 |

Abbreviations: CAFs, cancer-associated fibroblasts; NES, normalized enrichment score; FDR, false discovery rate.

**Table S4.** Primers used for qRT-PCR.

| Gene | Forward Primer | Reverse Primer |
|---|---|---|
| *ARG1* | 5′-AAAGGCTGGTCTGCTTGAGAA-3′ | 5′-GTCATTAGGGATGTCAGCAAAGG-3′ |
| *IL10* | 5′-GAGATGCCTTCAGCAGAGTGAAGA-3′ | 5′-AGGCTTGGCAACCCAGGTAAC-3′ |
| *TGFB1* | 5′-AGCGACTCGCCAGAGTGGTTA-3′ | 5′-GCAGTGTGTTATCCCTGCTGTCA-3′ |
| *VEGFA* | 5′-ACTTCCCCAAATCACTGTGG-3′ | 5′-GTCACTCACTTTGCCCCTGT-3′ |
| *TNF* | 5′-TGCTTGTTCCTCAGCCTCTT-3′ | 5′-CAGAGGGCTGATTAGAGAGAGGT-3′ |
| *IL1B* | 5′-TCGCCAGTGAAATGATGGCTTA-3′ | 5′-GTCCATGGCCACAACAACTGA-3′ |
| *IL6* | 5′-AAGCCAGAGCTGTGCAGA TGAGTA-3′ | 5′-TGTCCTGCAGCCACT GGTTC-3′ |
| *CXCL8* | 5′-GTGCAGAGG GTTGTGGAGAAGTTT-3′ | 5′-TCACTGG CATCTTCACTGATTCTTG-3′ |
| *NOS2* | 5′-GCCAAGCTGAAATTGAATGAGGA-′3 | 5′-TTCTGTGCCGGCAGCTTTAAC-3′ |
| *IL12B* | 5′-GGAGCGAATGGGCATCTGT-3′ | 5′-TGGGTCTATTCCGTTGTGTCTTT-3′ |
| *CXCL12* | 5′-GAGCCAACGTCAAGCATCTCAA-3′ | 5′-TTAGCTTCGGGTCAATGCACAC-3′ |
| *CCL2* | 5′-CTTCTGTGCCTGCTGCTCATA-3′ | 5′-CTTTGGGACACTTGCTGCTG-3′ |
| *ACTA2* | 5′-ATTGCCGACCGAATGCAGA-3′ | 5′-ATGGAGCCACCGATCCAGAC-3′ |
| *CD274* | 5′-CAATGTGACCAGCACACTGAGAA-3′ | 5′-GGCATAATAAGATGGCTCCCAGAA-3′ |
| *PDCD1LG2* | 5′-TGGAATTGCAGCTTCACCAGATAG-3′ | 5′-GGCTGTTATTGCTCCAAGGTTCA-3′ |
| *GAPDH* | 5′-GCACCGTCAAGGCTGAGAAC-3′ | 5′-ATGGTGGTGAAGACGCCAGT-3′ |

**Table S5.** Characteristics of 72 HNSCC patients.

| | **No. (%)** |
|---|---|
| Age, years | |
| - median | 68 |
| - range | 33–92 |
| Gender | |
| - Male | 45 (63) |
| - Female | 27 (37) |
| Differentiation | |
| - Well/moderate | 62 (86) |
| - Poorly | 10 (14) |
| Lymphatic invasion | 33 (46) |

| | |
|---|---|
| Vascular invasion | 23 (32) |
| T factor | |
| - T1-2 | 63 (88) |
| - T3-4 | 9 (12) |
| N factor | |
| - N0 | 48 (67) |
| - N1-3 | 24 (33) |
| TNM stage | |
| - I-II | 47 (65) |
| - III-IV | 25 (35) |

HNSCC, head and neck squamous cell carcinoma.

**Table S6.** Relationships between AKT3 expression and clinical parameters in 72 HNSCC patients.

| Variable | AKT3 in CAFs | | |
|---|---|---|---|
| | Negative | Positive | *p*-value |
| Age (years) | | | |
| - <71 | 24 | 17 | 1.00 |
| - ≥71 | 19 | 12 | |
| Gender | | | |
| - Male | 23 | 22 | 0.08 |
| - Female | 20 | 7 | |
| Differentiation | | | |
| - Well/moderate | 36 | 26 | 0.71 |
| - Poorly | 7 | 3 | |
| Lymphatic invasion | | | |
| - Negative | 25 | 14 | 0.41 |
| - Positive | 18 | 15 | |
| Vascular invasion | | | |
| - Negative | 32 | 17 | 0.16 |
| - Positive | 11 | 12 | |
| T factor | | | |
| - T1-2 | 40 | 23 | 0.14 |
| - T3-4 | 3 | 6 | |
| N factor | | | |
| - N0 | 31 | 17 | 0.23 |
| - N1-3 | 12 | 12 | |
| TNM stage | | | |
| - I-II | 33 | 14 | 0.43 |
| - III-IV | 10 | 15 | |

Abbreviations: CAF, cancer-associated fibroblast; HNSCC, head and neck squamous cell carcinoma.

**Table S7.** Antibodies used for immunohistochemistry.

| Antibody | Clone | Dilution | Company |
|---|---|---|---|
| AKT3 | Polyclonal | 1:250 | Sigma-Aldrich |
| PIK3CA | Polyclonal | 1:100 | Sigma-Aldrich |
| αSMA | 1A4 | 1:20 | R&D Systems |
| CD68 | PG-M1 | Ready-to-use | Dako |
| CD163 | 10D6 | 1:200 | Leica Biosystems |
| CD1a | O10 | Ready-to-use | Beckman Coulter |
| CD3 | Polyclonal | Ready-to-use | Dako |
| CD56 | 123.C3.D5 | Ready-to-use | Thermo Fisher Scientific |