Figures and figure supplements

NRG1 is a critical regulator of differentiation in TP63-driven squamous cell carcinoma

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Figure 1. TP63 regulates NRG1 in SCC. (A) Correlation of TP63 and NRG1 in LUSC (n = 223) and Esophageal (n = 263) patient samples in TCGA data set. Statistical significance was determined by two-tailed test and Pearson correlation (r) was determined. (B) Relative expression of TP63, NRG1α and NRG1β upon TP63 knockdown using siRNA in OE21 and KYSE-140 SCCs. (C) Relative expression of TP63 (all, TA and deltaN isoforms), NRG1α, NRG1β upon TP63 knockdown using siRNA in KYSE-180 SCC. Expression of TA and deltaN TP63 and upon TP63 knockdown by siRNA. siRNAs for TA-TP63 (#13), all isoform-TP63 (#14), deltaN-TP63 (#1, #2, #3) and non-target control (NTC#3, NTC#4). Relative quantitation by qPCR in B and C is mean with standard deviation relative to dharmafect transfection reagent control. (D) ChIP analysis of p63alpha and deltaNp63 binding −21 kb from the NRG1 transcriptional start site (TSS) in KYSE-180 SCC. IgG and primers amplifying the −21 kb region were used as controls. Data is represented as average Figure 1 continued on next page
Figure 1 continued

with standard error of mean from three independent experiments. One-way ANOVA test was used to determine the statistical significance in comparison to IgG. *p<0.05.

DOI: https://doi.org/10.7554/eLife.46551.002
Figure 2. Modest but significant growth inhibition of SCCs by anti-NRG1 treatment in vitro. (A) NRG1 and ERBB3 mRNA levels in head and neck squamous carcinoma (HNSC), lung squamous carcinoma (LUSC) and lung adenocarcinoma (LUAD) patient samples from TCGA. (B) In vitro growth of different SCC cell lines in the presence of anti-NRG1 or control anti-Ragweed antibody. Expression levels of NRG1, Erbb3, Erbb4 and TP63 (RPKM) for each cell line are shown below the respective in vitro growth data. Average growth is presented as mean with standard error of mean relative to anti-Ragweed from four independent experiments with more than three replicates in every experiment. Statistical significance was determined using t-test with * indicates p<0.05.

DOI: https://doi.org/10.7554/eLife.46551.003
Figure 3. Anti-NRG1 significantly inhibits the tumor growth in vivo of SCCs that express both NRG1 and ERBB3. Effect of anti-NRG1 or anti-Ragweed (control) antibodies in (A) FaDu (head and neck), HCC95 (lung) and KYSE-180 (esophagus) SCC cell lines in preclinical mouse xenograft models, N = 8–12 mice/group, (B) Lung SCC PDX models, n = 4 mice/group, and (C) Lgr5CreERT2;PtenloxP/lox;KrasLSL-G12D/+ skin GEMM, n = 7–8 mice/group. Statistical significance was determined by Log-rank test. Red arrow indicates the time of the final antibody dose. (D) H and E staining for GEMM skin at the end of study.

DOI: https://doi.org/10.7554/eLife.46551.004
Figure 3—figure supplement 1. Anti-NRG1 inhibits in vivo tumor growth in SCC models. Effect of anti-NRG1 or anti-Ragweed (control) antibodies in FaDu (head and neck), HCC95 (lung) and KYSE-180 (esophagus) SCC cell lines in preclinical mouse xenograft models, N = 8–12 mice/group. LUN#120, LUN#150 and LUN#331 are lung SCC PDX models, n = 4 mice/group. Percent change in tumor volume was determined at after one week from last antibody dose in Figure 3 with respect to initial tumor volume of respective animals.

DOI: https://doi.org/10.7554/eLife.46551.005
Figure 3—figure supplement 2. Anti-NRG1 does not inhibit the growth of ovarian models expressing NRG1 and ERBB3 receptor in vivo. (A) In vitro growth of ovarian cell lines in the presence of anti-NRG1 or anti-Ragweed (control) antibody. Expression of NRG1, Erbb3, Erbb4 and TP63 by RNAseq are shown below the respective in vitro growth of different cell lines. Average growth is presented as mean with standard error of mean relative to anti-Ragweed from four independent experiments with more than three replicates in every experiment. Statistical significance was determined using t-test with * indicates p<0.05. (B) Efficacy of anti-NRG1 or anti-Ragweed (control) antibody in ovarian PDX models, n = 8–12 mice/group. Red arrow indicates the last dose of antibody.

DOI: https://doi.org/10.7554/eLife.46551.006
Figure 4. Anti-NRG1 induces squamous differentiation and inhibits proliferation in SCCs. (A) Representative image of KRT10 (differentiation marker) staining of tumor from FaDu and HCC95 SCC models upon anti-NRG1 treatments compared to anti-Ragweed control. N = 5 mice/group. (B) Protein levels of apoptosis markers upon anti-NRG1 treatment in vivo in HCC95 SCC xenografts. phospho-ERBB3 level was used to assess inhibition of signaling. Expression of proliferation markers upon one and three doses of anti-NRG1 relative to anti-Ragweed treatment in vivo in HCC95 lung SCC by RNAseq. (C) Expression of lung basal cell differentiation markers after one or three doses of anti-NRG1 and one dose of anti-Ragweed treatment in HCC95 lung SCC xenograft tumors by RNAseq. N = 5 mice/group. Expression of (D) squamous differentiation markers and progenitor cell related factors as assessed by RNAseq.
Figure 4 continued

markers following three doses of anti-NRG1 relative to anti-Ragweed treatment in HCC95 lung SCC xenograft tumors by RNAseq. Average fold change relative to anti-ragweed from n = 5 mice/group.

DOI: https://doi.org/10.7554/eLife.46551.007
Figure 4—figure supplement 1. Anti-NRG1 induces differentiation in SCC. Representative image of H and E staining of KYSE-180, HCC95 and FaDu tumors upon anti-NRG1 treatments compared to anti-Ragweed control. N = 5 mice/group.
DOI: https://doi.org/10.7554/eLife.46551.008
Figure 4—figure supplement 2. Anti-NRG1 inhibits proliferation and induces differentiation in SCC. Expression of proliferation, differentiation and progenitor cell related markers following one dose of anti-NRG1 relative to anti-Ragweed treatment in HCC95 lung SCC xenograft tumors by RNAseq. Average fold change relative to anti-Ragweed from n = 5 mice/group.
DOI: https://doi.org/10.7554/eLife.46551.009
**Figure 4—figure supplement 3.** Anti-NRG1 increases differentiation markers in vitro in SCC. Relative expression of differentiation markers such as KRT1, KRT10, IVL and KRTDAP in FaDu, HCC95 and KYSE-180 cell lines after treatment with antibodies for 3 days in vitro.

DOI: https://doi.org/10.7554/eLife.46551.010