The Functional GRHL3-FLG Axis Predicts Targeted Therapy Response in Head and Neck Cancer

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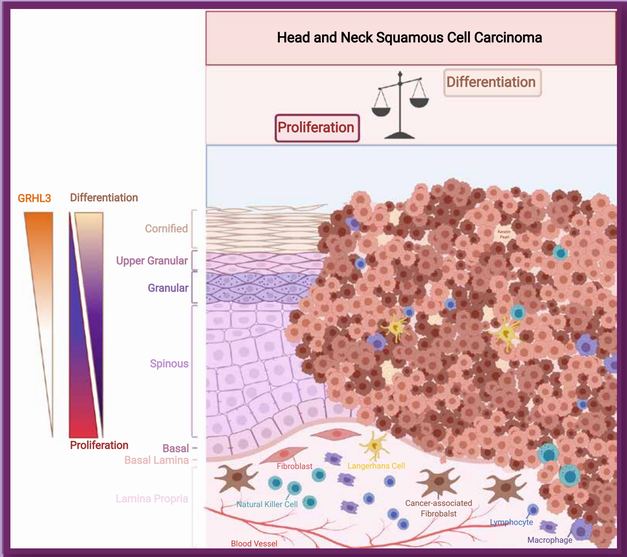

Introduction:

Head and Neck Cancer

- A **heterogenous** disease harbouring the most frequent hotsopt mtuations in the **differentiation** genes
- Disruption of **differentiation** acts as a **primary driver** of HNSCC and correlates with a poor patient's prognosis

GRHL3

- A highly conserved **transcription factor** for oral epithelial development and homeostasis maintenance
- Loss of ***GRHL3*** induces HNSCC

Therapeutic Strategy

- Conventional treatment: **non-selective**
- Targeted therapy: FDA **Only** approved **Cetuximab** having a low response rate with considerable toxicity

Hypothesis:

FUNCTIONAL DIFFERENTIATION CAN SERVE AS A MOLECULAR VULNERABILITY AND/OR A PREDICTOR OF TARGETED THERAPY RESPONSE IN HNSCC

Methods:

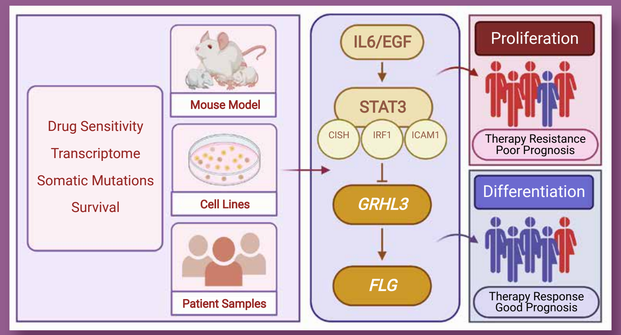

Figure: Schematic diagram of the functional GRHL3-FLG differentiation axis underlying response to therapy in HNSCC.

While high tumour **STAT3** activity is linked to targeted therapy resistance (upper panel), functional ***GRHL3-FLG*** signaling (lower panel) maintains **differentiation** potential in tumours, acts as a predictor of therapeutic response, and results in improved survival of HNSCC patients with **high *GRHL3 and* wild-type *FLG***.

Multi-omic Approach

Whole-genome and whole-transcriptome sequencing with drug sensitivity screening were employed in tumours from a **spontaneous HNSCC murine model, HNSCC patient's tumours, and established human cell lines** to reveal potential predictive bioarkers for targeted therapies.

CRISPR-Cas9-mediated Gene Manipulation

CRISPR-Cas9-mediated gene **knockout and activation** validated candidate predictors in HNSCC cell lines with inhibitors of the PI3K/mTOR, c-MYC and STAT3 pathways, the key signalling in HNSCC oncogenesis.

Conclusions:

*Functional differentiation (**GRHL3^{hi}-FLG^{wt}**):*

- provides the **first example** of differentiation-dependent therapy response
- establishes a rationale for clinical investigation of **differentiation-paired targeted therapy** that may **improve outcomes** in HNSCC and other heterogeneous cancers.

Results:

*A novel differentiation **Grainyhead-like 3 - Filaggrin (GRHL3-FLG) axis** was identified as a **predictive biomarker** to targeted therapy response in HNSCC.*

- The loss of ***GRHL3*** potentiates **STAT3 activation** in mouse HNSCC. (***Fig.1***)
- A subset of HNSCC with **functional GRHL3-dependent differentiation** was the **most sensitive** to inhibitors of PI3K/mTOR, c-MYC and STAT3 signaling. (***Fig.2***)
- GRHL3 transcriptional **target gene FLG** was identified as a **novel tumour differentiation** gene and, more importantly, stratified HNSCC subsets as **treatment-resistant** based on their **FLG mutational** profile. (***Fig.2 & 3***)
- The **loss of FLG** in sensitive HNSCC cells resulted in a dramatic **resistance** to targeted therapies. (***Fig.3***)
- The **GRHL3^{hi}-FLG^{wt} signature** predicted a favourable patient's prognosis. (***Fig.4***)

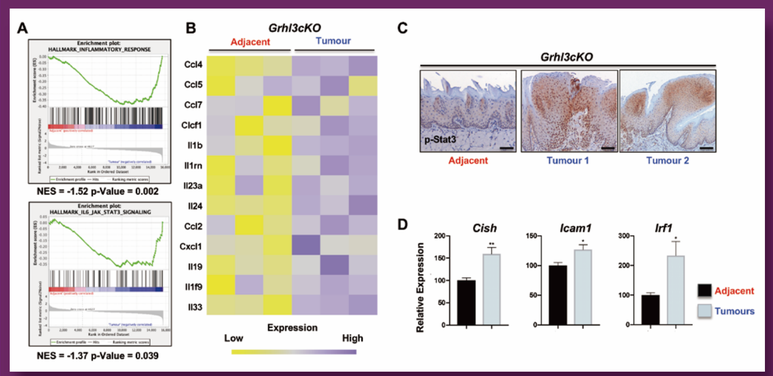

Figure 1: Loss of GRHL3 potentiates STAT3 activation in mouse HNSCC.
A) A significant enrichment for genes involved in the inflammatory response and STAT3 signaling is observed in Grhl3cKO tumours compared to normal adjacent tissues. B) Expression of cytokines/chemokines that promote STAT3 activation is shown as a heatmap for Grhl3cKO tumours and adjacent tissues. C) p-Stat3 IHC demonstrates hyperactive Stat3 in tumours but not in adjacent tissues from Grhl3cKO mice. D) Upregulation of the STAT3 target genes was shown by qPCR analyses for *Cish, Icam1 and Irf1* in tumours of Grhl3cKO mice.

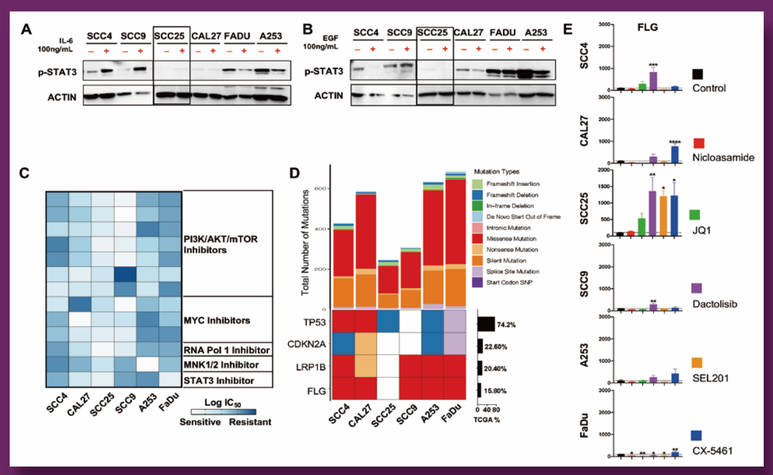

Figure 2: Mutational analysis and drug sensitivity of HNSCC cell lines.
WB analyses of STAT3 activation in cell lines treated with A) IL-6, or B) EGF. STAT3 activity is undetectable in SCC25 (boxed). C) Heatmap of drug sensitivity and resistance to inhibitors of STAT3, PI3K/AKT/mTOR, c-MYC in the HNSCC cell lines. D) The top panel shows the type and rate of mutations from the Cancer Cell Line Encyclopedia (CCLE) Cell Line mutation and COSMIC databases. The bottom panel depicts the most common somatic mutations in TCGA-HNSCC patients that were also present in the HNSCC cell lines. E) qPCR analysis of *FLG* in the HNSCC cell lines treated with the selected inhibitors.

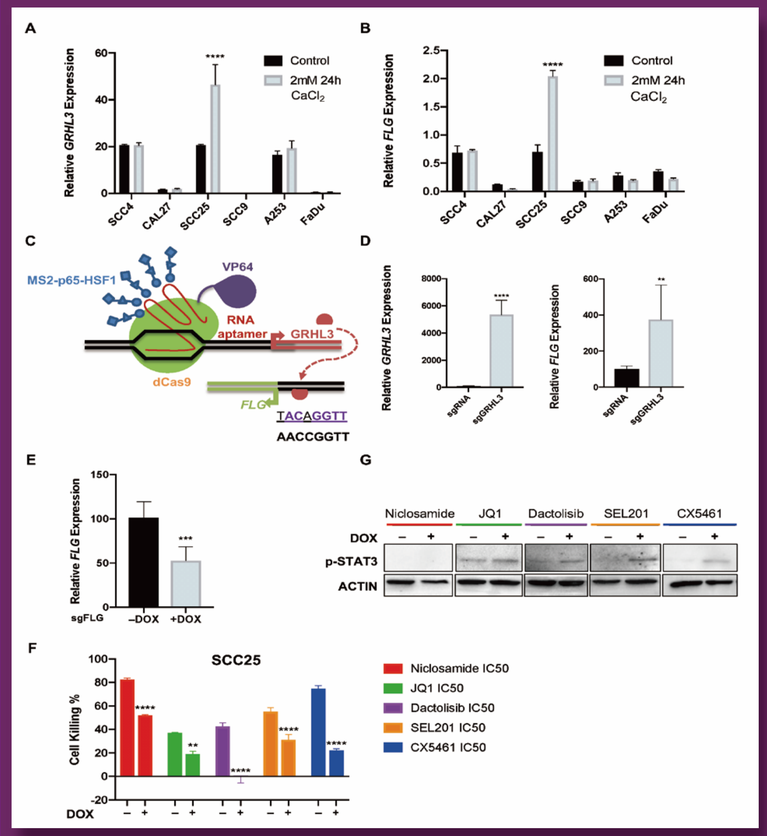

Figure 3: A functional GRHL3-FLG pathway promotes sensitivity to targeted therapy.
qPCR expression of A) GRHL3, and B) FLG in HNSCC cell lines with $CaCl_2$ shows significant induction only in SCC25. C) Illustration of the CRISPR-dCas9 GRHL3-activation SAM system with the potential GRHL3 binding site in FLG promoter. D) Significant induction of GRHL3 and FLG mRNA in 293T cells with overexpressed *GRHL3*. E) *FLG* expression of SCC25 cells transduced with an inducible CRISPR-Cas9 system to mediate FLG knock-down. Compared to control cells, downregulation of *FLG* (~50%) was achieved. F) Downregulation of *FLG* renders the treatment-sensitive SCC25 resistant to all small molecule inhibitors. G) WB analyses of p-STAT3 in SCC25 treated with the inhibitors at IC50 in the absence of FLG. p-STAT3 was increased in cells treated with c-MYC and PI3K/mTOR inhibitors and absent in those treated with Niclosamide.

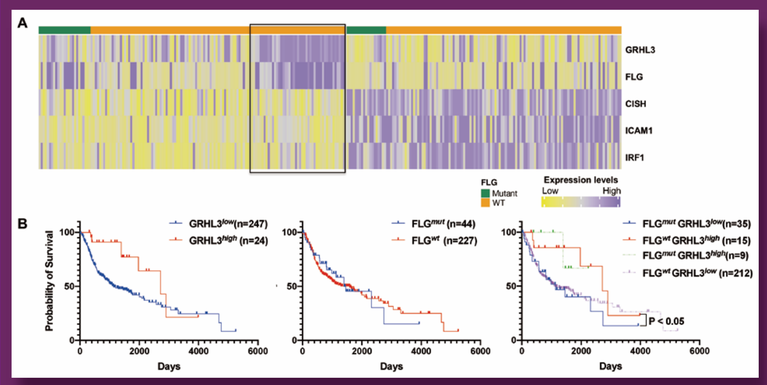

Figure 4: A GRHL3-FLG signature predicts favourable prognosis for HNSCC patients.
A) Unsupervised clustering of GRHL3, FLG and STAT3-target gene (*CISH, ICAM1 and IRF1*) expression. B) Kaplan-Meier survival analyses of HNSCC patients stratified based on their GRHL3 expression level and FLG mutation status. The difference in survival rates between patients with GRHL3^{high} and GRHL3^{low} was not significant, neither between FLG^{WT} and FLG^{Mut}. The survival of the cohort with GRHL3^{high} and FLG^{WT} was significantly superior to that of patients with GRHL3^{low} and FLG^{Mut} (P=0.04771, log-rank test).

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