Targeting SWI/SNF/Hippo pathway in Squamous Cancers

Srinivas Vinod Saladi Ph.D.
Chabner Colloquium, 2019
Squamous cell carcinoma genomic landscape: largely tumor suppressors

**Squamous NSCLC**

**Head & Neck SCC**
Lung adenocarcinoma genotypes: many potential therapeutic opportunities

- KRAS: 23%
- EGFR: 15%
- TP53: 5%
- IDH1: <1%
- NRAS: 1%
- BRAF: 2%
- HER2: 2%
- PIK3CA: 4%
- ALK: 3%
- CTNNB1: 2%
- AKT: 1%
- ROS1: 1.5%
- No Mutation: ~40%
Excision of p63 in SCC *in vivo* results in rapid tumor regression.
p63 regulates genes by association with distinct epigenetic cofactors/complexes

Cistrome analysis
p63 ChIP-Seq (JHU029)

Factors
- TP53
- TCFP2L1
- NRF1
- TFCP2
- SMARCC1
- FOS
- E2F1
- BCL11A
- FOSL2
- SMARCA4
- FOSL1
- BACH2
- SMARCB1
- TRIM28
- RUNX1


(p63)
p63 regulates genes by association with distinct epigenetic cofactors/complexes

**Cistrome analysis**

p63 ChIP-Seq (JHU029)

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**Glycerol Gradient**

- Input
- Empty
- p63
- ACTL6A

**IP: FLAG, HA**

- GFP
- ΔNp63α
- ΔNp63α-FLAG-HA
- ΔNp63α-

**ΔNp63α-FLAG/HA**

- DMAP1
- RUVBL1
- RUVBL2
- ACTL6A

**GFP-FLAG/HA**
SWI/SNF-ATP – dependent chromatin remodeling enzymes are highly heterogeneous.
SWI/SNF complexes control nucleosome position

**Core subunits**

**BAF & PBAF subunits**

**BAF**
- BAF-specific subunits
- BAF & PBAF subunits

**PBAF**
- PBAF-specific subunits

**SWI/SNF complexes control nucleosome position**

**BAF complexes**
- Homo sapiens, Mus musculus

**PBAF complexes**
- Homo sapiens, Mus musculus

**SWI/SNF binding at specific loci**

**ATP**

**ADP**

**Disruption of DNA-histone contacts**

**Eviction**

**Sliding**
Transcriptional regulation

- **Compact**
  - NO transcription factor accessibility
- **Open**
  - Transcription factor accessibility

Chromatin (in highly condensed form)

Unwinding of Chromatin

SWI/SNF (ATP dependant)

Binding of Transcription Factors

Transcription of Genes

- Proliferation
- Cell migration
- Retinoblastoma pathway
- Immune response
- Embryonic stem cell and differentiation programs
- Polycomb silencing

SWI/SNF complex mediated Transcriptional Regulation
**Functions of SWI/SNF**

SWI/SNF enzymes play a key role in transcriptional regulation, replication, DNA repair, and cell cycle control.

SWI/SNF enzymes are critical for cellular differentiation and embryonic development.

SWI/SNF subunits are mutated or deleted in many forms of cancer and one of the subunits BAF47 may act as tumor suppressor.

Gastric carcinomas with lymph node metastasis over-express BRG1 and are depleted of BRM expression.

BRG1 expression is high in invasive forms of prostate cancer.
SWI/SNF complex altered in multiple cancers

**BAF/PBAF subunits**

<table>
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<tr>
<th>Subunit</th>
<th>Mutations in cBioPortal</th>
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<tr>
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<td>SMARCA4</td>
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<td>ARID1B</td>
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**mSWI/SNF complex (BAF)**

- Leukemia-T-ALL
- Colorectal cancer
- Synovial sarcoma
- Malignant rhabdoid tumors
- Epithiloid sarcoma
- Medulloblastoma
- Breast cancer
- Lung cancer
- Squamous cell carcinoma
- Head and neck cancer

**Polybromo-containing BAF (PBAF)**

- Head and neck cancer
- Clear cell renal carcinoma
- Head and neck cancer

**In-frame**

**Splice**

**Missense**

**Truncating**

Kadoch et al. Human Cancer 2015
Kadoch et al. Biochemistry 2016
SWI/SNF complex widely considered as tumor suppressor
ACTL6A (Baf53a) subunit of SWI/SNF complex is amplified in multiple cancers
ACTL6A (Baf53a) subunit of SWI/SNF complex is amplified in multiple cancers

ACTL6A is required for maintaining the progenitor state in epithelium

(Bao X et al., Cell Stem Cell, 2013)
ACTL6A is co-amplified with p63 and confers poor prognosis in HNSCC.
ACTL6A is co-amplified with p63 and confers poor prognosis in HNSCC

Genetic Alteration

<table>
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<tr>
<th>3q26</th>
<th>Amplification</th>
<th>Missense Mutation</th>
<th>Truncating Mutation</th>
<th>Inframe Mutation</th>
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<td>TP63</td>
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(n=530)

TCGA HNSCC Kaplan-Meier Survival for ACTL6A Expression

Kaplan-Meier log-rank p-value = 0.0307

- < 20% Quantile (1173.7) (99 Samples)
- > 20% Quantile (1173.7) (395 Samples)
ACTL6A is required for tumor progression in vivo

FaDu
Dox inducible
shACTL6A
Inject Cells (Day 0)

Tumors
(Day 8)

Harvest Tumors
(Day 20)

Tumor Volume (X100mm³)

- Dox
+ Dox

shACTL6A
Dox
- #1
+ #1
+ #2

****
***

- Dox + Dox + Dox

- Dox + Dox + Dox
ACTL6A is required for tumor progression \textit{in vivo}
ACTL6A promotes transformation of mouse keratinocytes via activation of YAP
ACTL6A promotes transformation of mouse keratinocytes via activation of YAP

ACTL6A

YAP1
ACTL6A promotes Tumorigenesis in HNSCC

Cancer Cell, 2017
ACTL6A inhibits SMARCA4 (BRG1) and correlates to high H3K27me3 (EZH2) at its target genes
SWI/SNF antagonistic to PRC2 complex


Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation.

Wilson BG¹, Wang X, Shen X, McKenna ES, Lemieux ME, Cho YJ, Koellhoffer EC, Pomeroy SL, Orkin SH, Roberts CW.

Author information

ARID1B is a specific vulnerability in ARID1A-mutant cancers.


SWI/SNF antagonizes PRC2 complex

Highly dependent on EZH2 activity

Poly Comb Repressor (PRC) complex

Herviou et al. Oncotarget - 2016
SWI/SNF complex subunits alterations renders cells sensitive to EZH2 inhibitors

Stem or Progenitor Cells

**Highly dependent on EZH2 activity**

ACTL6A

SWI/SNF

INI1

SMARCA4

SMARCA2?

ARID1A?

↓↓↓ PRC2 target genes

↑↑ Stem cell programs

Oncogenic Transformation

**Clinical Compound**

Tazemetostat (EPZ-6438)

IC50 < 4 nM

Selectivity > 20,000-fold over all other HMTs tested (100-fold for EZH1)

Hyper-repression of PRC2 targets

Potentiation of stem cell programs
ACTL6A expression correlates strongly with EZH2 expression

Pearson: 0.35
Spearman: 0.38
EZH2 in HNSCC patients

PDX (frozen)  HNSCC (FFPE)

Scale bar represents 50μM

(PDX samples from Sara Pai)
ACTL6A cooperates with EZH2 to repress differentiation programs

**WWC1**

**S100A9**
ACTL6A cooperates with EZH2 and promotes H3K27me3 globally.

The figure shows a box plot comparing H3K27me3 log2 counts in 10Kb windows for ACTL6A loci and globally, between Control and shACTL6A conditions. The y-axis represents the log2 counts, normalized to input. The box plot on the left shows ACTL6A loci, while the right shows global counts. The boxes and whiskers indicate the distribution of log2 counts, with red indicating ACTL6aCTL_H3K27me3CTL_common_windows and blue indicating H3K27me3CTL_windows_only.
SWI/SNF complex subunits alterations renders cells sensitive to EZH2 inhibitors

Stem or Progenitor Cells

*Highly dependent on EZH2 activity*

SWI/SNF

PRC2

INI1

SMARCA4

SMARCA2?

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Oncogenic Transformation

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Hyper-repression of PRC2 targets

Potentiation of stem cell programs
EZH2 regulates differentiation program in SCC

HaCaT to FaDu: p = 0.043
HaCaT to SCC4: p = 0.013
High mutation load in HNSCC

ACTL6A correlates to high mutation load in HNSCC

Rho = 0.64  
$p = 1.4 \times 10^{-04}$

More Mutation Load

Better response to Immune check point inhibitors
ACTL6A correlates to high mutation load in HNSCC

- Total # mutations
- ACTL6A expression
  - Rho = 0.64
  - p = 1.4e-04

Comet Assay

EV

BRG1

Control

30 min post Cisplatin

2 hr post Cisplatin
High Mutation Load Predicts Better Clinical Outcome to Immune Check Point Inhibitors

Riaz N, Cell, 2017
High Mutation Load Predicts Better Clinical Outcome to Immune Check Point Inhibitors

Only 15-20% respond to Immune Check Point Inhibitors in HNSCC

ACTL6A mediated immune modulation in HNSCC

**HNSCC Cells**

BROWNE_INTERFERON_RESPONSIVE_GENES

Control/shACTL6A; HIGH  \(\rightarrow\)  LOW

**HNSCC Primary Tumors**

PD1_LIGATION_VS_CTRL_IN_ACT_TCELL_LINE_UP

Correlation with ACTL6A; HIGH  \(\rightarrow\)  LOW

**Repressed Programs**

Predicts PD1/PD-L1 response in HNSCC

- HLA-DRA
- CXCL-10
- STAT1/2
- PDL-2

**Cytotoxic T Cell Factors**

- Granzyme A, B

**HLA Components**

- HLA-B, C, E
- HLA-DRA, B6, B1; -DPA1, B1

**TNF Family Members**

- Fas Ligand
- Lymphotoxin Alpha, Beta
ACTL6A suppresses the InterferonG gene subset thereby evading the tumor immune response
Predictive Treatment Model for HNSCC patients: Epigenetic therapy coupled with Immunotherapy

EZH2

Immunologically Cold Tumors

EZH2i

Reactivation of Immune program

(Interferon gamma responsive genes,
T Cell responsive genes)

Immune Checkpoint Blockade

Better Clinical response

(Saladi SV, et al., Manuscript in prep)
EZH2 and DNMT inhibitors reactivate the Immune program.

**CXCL10**

**TLR3**

**STAT1**
Predictive Treatment Model for HNSCC patients: Epigenetic therapy coupled with Immunotherapy

Immunologically Cold Tumors

Reactivation of Immune program
(Interferon gamma responsive genes, T Cell responsive genes)

Better Clinical response

(Saladi SV, et al., Manuscript in prep)
**ACTL6A**, subunit of SWI/SNF complex is a driver oncogene in HNSCC

**Tumors**

ACTL6A, ACTL6A

ACTL6A

Smarca4/A2

Ezh2

↑H3K27Me3

**Differentiated Epithelium**

ACTL6A

Smarca4/A2

Ezh2

↓H3K27Me3

**Regenerative Proliferation**

Immune evasion

Differentiation

Differentiation
ACTL6A/YAP-dependent regenerative program in squamous carcinomas

GSEA analysis with BRD4 inhibition (JQ1 treatment) in all cancer cell line
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