CRISPR/Cas9-based *in vivo* identification of EZH2 as a therapeutic target in desmoid tumors

Kris Vleminckx
Developmental Biology Lab – University of Ghent
Modeling desmoid tumors in *Xenopus tropicalis*

*In vivo* dependency factor identification

The SET domain of EZH2 is an essential driver in desmoid tumorigenesis
Why *Xenopus tropicalis*?

- Vertebrate – tetrapod (aquatic)
- High number offspring (> 1000 eggs)
- External embryonic development
- Embryos have a large size
- Embryogenesis proceeds rapidly

= *X. laevis* and zebrafish

- *Xenopus tropicalis* is true diploid
- High synteny of genome
- Targeted injections are possible

>< *X. laevis* and zebrafish
Outline

Modeling desmoid tumors in *Xenopus tropicalis*

*In vivo* dependency factor identification

The SET domain of EZH2 is an essential driver in desmoid tumorigenesis
**Desmoid tumor**

Tumor of mesenchymal origin

Arise in deep muscle fascia, aponeurosis, and tendons

Driven by Wnt signaling hyperactivation (*apc* or *beta-catenin* mutations)
*apc* crispants develop desmoid tumors

**apc CRISPR/Cas9**

Targeted = lethality ↓

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95% injected animals 6 weeks old

Positive selection

Bi-allelic truncated *apc* protein hyperactivates the Wnt pathway
Modeling desmoid tumors in *Xenopus tropicalis*

*In vivo* dependency factor identification

The SET domain of EZH2 is an essential driver in desmoid tumorigenesis
Treatment modalities for desmoid tumors are inadequate

Aggressive treatments

- Surgery
- Radiotherapy
- Classical chemotherapy

Extremely high recurrence rate
90% within 2 years

While patient survival is high (>90% over 10 years)
Chronic pain affects quality of life

Search for novel targets for targeted molecular treatment

Dependency Factors
Searching for potential dependency factors

Microarray
8 desmoid tumors
22 ≠ fibrous lesions

RNA-seq
7 desmoid tumors
9 ≠ fibrous lesions

Genes identified in both datasets with FDR <1% and with contrast (fold change) > 2 in desmoid tumors compared to other lesions

LOX
ADAM-12
MDK
HMMR
WISP-1
PYCR1

NUAK1
FAP-α
PCLAF
EZH2
CREB3L1

Prof. Matt van de Rijn
Joanna Przybyl
Stanford University
Dependency factor screen – Dropout CRISPR/Cas9

CRISPR library

Negative selection

Dependency factor

No dependency factor

No selection
CRISPR-based *in vivo* identification of dependency factors

- **apc + Not a dependency factor**
  - No editing
  - Heterozygous editing
  - Homozygous editing
CRISPR-based *in vivo* identification of dependency factors

\[ \text{apc} + \text{dependency factor} \]

- No editing
- Heterozygous editing
- Homozygous editing
EZH2 and CREB3L1 as dependency factors in desmoid tumors

**CRISPR/Cas9**

apc + potential dependency factor

Dissect tumor(s)

Targeted amplicon sequencing of potential dependency factor

Percentage of tumors exhibiting **homozygous frameshift** mutations
Outline

Modeling desmoid tumors in *Xenopus tropicalis*

*In vivo* dependency factor identification

The SET domain of EZH2 is an essential driver in desmoid tumorigenesis
Which EZH2 domain underlies the negative selection pressure

Polycomb Repressive Complex 2

Heterozygous editing

Homozygous editing

Homozygous in-frame editing

apc + ezh2^{CXC}
SET domain of EZH2 as therapeutic target in desmoid tumors

Polycomb Repressive Complex 2

No editing
Heterozygous editing
Homozygous editing
Homozygous in-frame editing

apc + ezh2^{SET}
Take-Home

*apc* crispants develop **desmoid tumors**

Methodology cancer *in vivo* **dependency factor identification** by multiplex CRISPR/Cas9

SET domain of **EZH2 as new therapeutic target** in desmoid tumor disease

Targetable by compounds already in clinical trials for other indications
Chemical inhibition of EZH2 by GSK126 in established DTs

Currently repeated with other EZH2 inhibitor tazemetostat (EPZ-6438)

Oral drug (currently in Phase II for other cancers)

Histological evaluation expected in October.
### GSK126 in human desmoid tumor cells (R. Poon and B. Alman)

#### wk3

**Summary**

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<th></th>
<th>Normal</th>
<th>AF</th>
<th>0.1% DMSO</th>
<th>1 uM</th>
<th>0.1% DMSO</th>
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<td>180T</td>
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<td>Ttest (treated AF to treated Normal)</td>
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#### Ave % Viability of Normal and AF cells treated by 1 uM GSK126

![Graph showing cell viability of Normal and AF cells after GSK126 treatment for 7 days]
Experiments planned in remainder of third year

1. further testing the EZH2 inhibitors (GSK126 and EPZ-6438)

2. initiate experiments with the protease inhibitors AEBSF and PF429242 (interfere with the proteolytic activation of CREB3L1/Oasis)

3. Desmoid tumor allografting in *Xenopus*
Unit Developmental Biology
Suzan Demuynck
Dionysia Dimitrakopoulou
Dieter Tulkens
Marjolein Carron
Prof. Kris Vleminckx

Ex-members
Dr. Tom Van Nieuwenhuysen
Dr. Rivka Noelanders
Dr. Hong Thi Tran

UZ Gent
pathology department
Dr. David Creytens

Stanford Clinical pathology
Prof. Matt van de Rijn
Joanna Przybyl

Hospital for Sick Children
Prof. Benjamin Alman
Raymond Poon