Characterizing the role of chromosome 3 copy-loss in driving late-stage Uveal Melanoma

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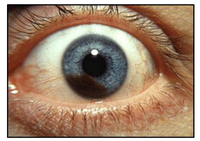
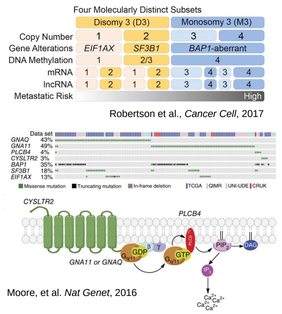
Introduction

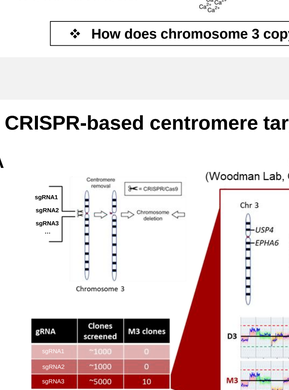
- **Most common primary intraocular tumor in adults:** ~5 cases per million per year in US
- **Around 50% patients develop metastatic disease predominantly to the liver:**
  - No effective therapy: median OS 10.2 months
  - 2022 Breakthrough Therapy Designation to the first-in-class bispecific fusion protein tebentafusp
- **Malignant transformation is suggested to be based on a combination of two main events:**
  - Activation of the Gαq/11 pathway
  - "**BSE**" event: inactivation of ***BAP1***, or mutations in ***SF3B1*** or ***EIF1AX***
- **Disease progression driven by chromosome-level aberrations (~97% chromosome 3 copy-loss)**



Melanoma of the iris



Robertson et al., *Cancer Cell*, 2017



Moore, et al. *Nat Genet*, 2016

- Chromosome 3 copy-loss is associated with metastasis (liver), and other late-stage disease phenotypes such as depigmentation
- The mechanistic role of chromosome 3 copy-loss (**Monosomy 3**) is unknown and often conflated with *BAP1* LOF mutations or deletions
- **Modeling** and **characterizing Monosomy 3** provides an opportunity to:
  - Expand on limited model availability in the field
  - Inform on the biology of chromosome 3 copy-loss
  - Identify potential therapeutic vulnerabilities

❖ **How does chromosome 3 copy-loss provide fitness/metastatic advantage in uveal melanoma?**

Results

CRISPR-based centromere targeting successfully generates M3 isogenic clones

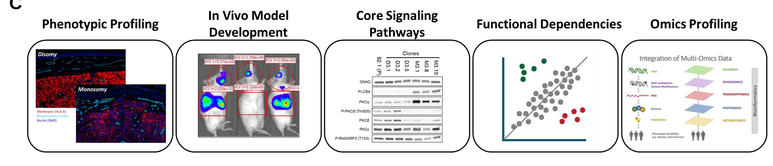


Figure 1: (A) Generation of Monosomy 3 isogenic clones using CRISPR-based centromere targeting. Clonal selection by qPCR and validation via karyotyping. **(B)** PCA of Parental model and isogenic clones (Disomy 3 and Monosomy 3). **(C)** Comprehensive characterization strategy to inform on the biology of uveal melanoma.

Monosomy 3 Isogenic Clones Recapitulate Late-Stage Uveal Melanoma Metastatic Phenotypes

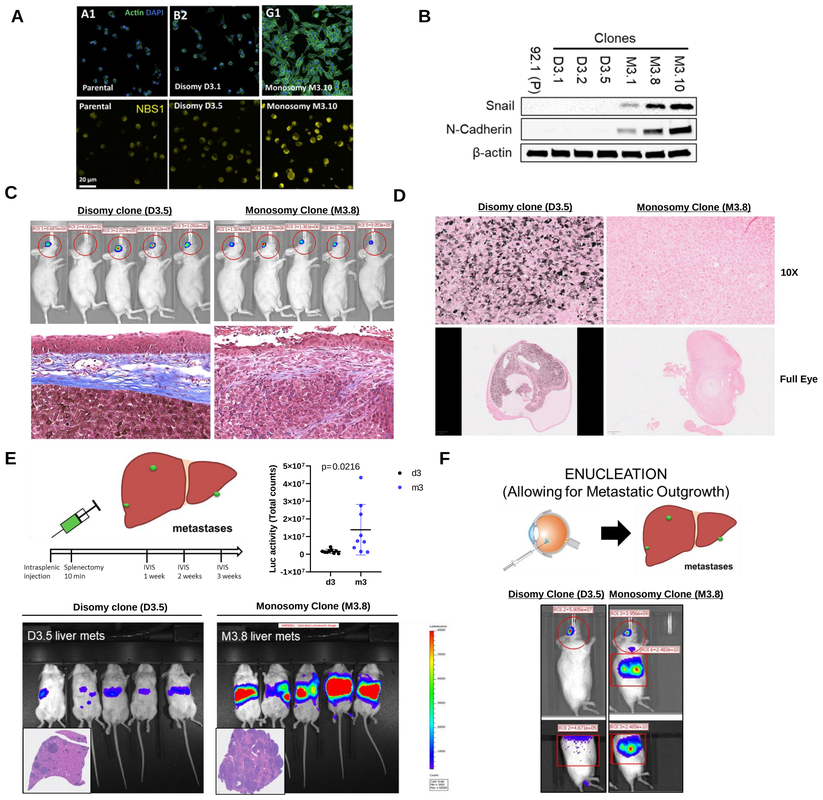


Figure 2: (A) Actin and DAPI staining for morphological characterization of isogenic models *in vitro*. **(B)** Immunoblotting of SNAIL and N-Cadherin across isogenic models. **(C)** Orthotopic modeling of select luciferated isogenic clones and subsequent IHC characterization outgrowing tumor (Masson's Trichrome staining of Collagen, Nuclei, and Cytoplasm). **(D)** Pigmentation differences between orthotopic Disomy 3 and Monosomy 3 isogenic clones (Fontana-Masson Strain for Melanin). **(E)** Intrasplenic injection assay of luciferated Disomy and Monosomy 3 isogenic clones (Day 27 Timepoint). **(F)** Development of a successful eye-to-liver metastasis model leveraging luciferated Disomy 3 and Monosomy 3 isogenic clones.

TRACTION Platform for the Integrative Analysis of D3 vs. M3 Isogenic Clones

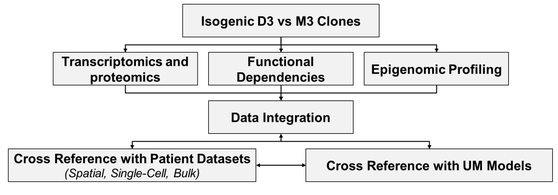


Figure 3: Overview of multiomic characterization platform implemented by TRACTION to characterize Disomy 3 and Monosomy 3 isogenic clones. Integrated data derived from the isogenic clones is continually cross referenced against other uveal melanoma models and patient datasets in order to characterize model artifacts and avenues of potential translation.

Chromosome 3 Copy-Loss Results in Global Transcriptomic, Chromatin-Level Restructuring, and Differential Motif Accessibility

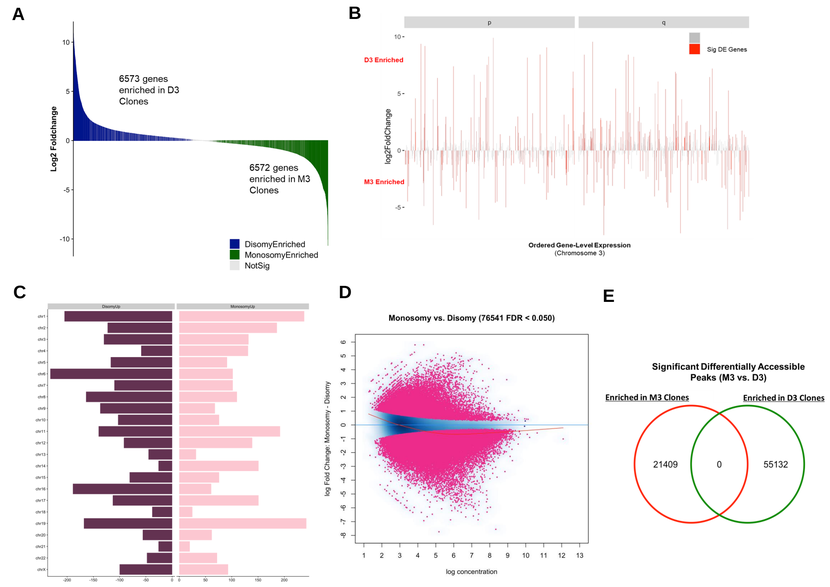


Figure 4: (A) Differential transcriptional enrichment in D3 vs. M3 clones. **(B)** Differential gene-level enrichment localized across Chromosome 3. **(C)** Chromosome-level differential gene enrichment. **(D)** ATACseq results highlighting differential peaks in D3 vs. M3 clones. **(E)** Venn diagram of differential peaks identified in D3 vs. M3 clones ($p < 0.05$).

Conclusions

- **First successful generation of isogenic Disomy 3 and Monosomy 3 clones allows to start understanding the nature of chromosome 3 copy-loss**
- **Ongoing characterization strategy to explore uveal melanoma biology ranges from phenotypic profiling, *in vivo* model development, identification and validation of context-specific dependencies and multiomic characterization**
- **Monosomy 3 isogenic clones recapitulate late-stage uveal melanoma phenotypes *in vitro* and *in vivo***
  - Monosomy 3 clones exhibit morphological alterations that correspond with a range of protein markers associated with increased mesenchymal properties, including SNAIL and N-Cadherin
  - Monosomy 3 isogenic clones invade the choroid layer at the edge of the eye compared to Disomy 3 models
  - Intrasplenic injections highlight increased liver seeding and outgrowth in Monosomy 3 clones
  - Developed first successful modeling of eye-to-liver metastases, highlighting liver-specific metastatic outgrowth
- **Chromosome 3 copy-loss induces significant transcriptional alterations via chromatin restructuring**

The TRACTION rare-tumor platform:

❖ Enables multiomic characterization and data integration for internally generated D3 and M3 isogenic clones, along with additional uveal melanoma models and patient datasets, **to work towards translatable findings.**
❖ Aims to characterize the biology of chromosome 3 copy-loss and **ultimately identify context-specific vulnerabilities for therapeutic intervention.**

Acknowledgements

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