Validation of Candidate Drivers of Osteosarcomagenesis with High-Throughput In Vitro Screening

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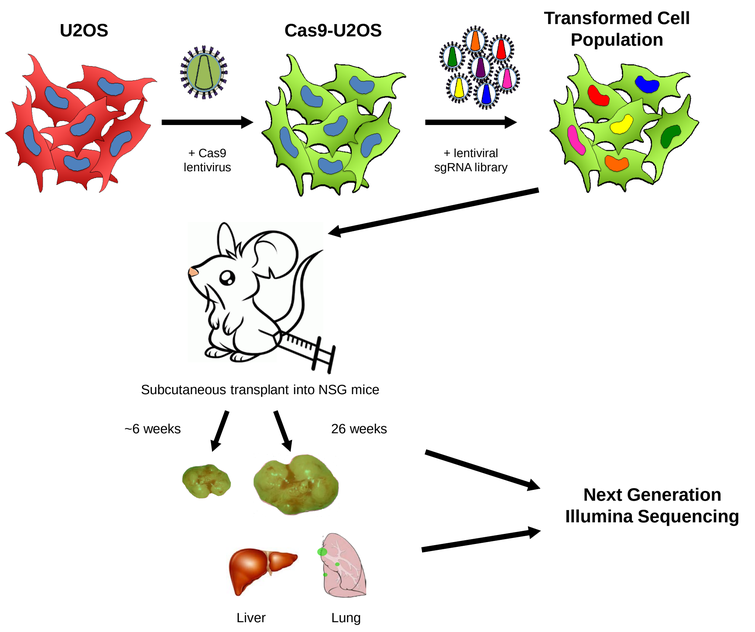
Background

Osteosarcomas (OS) are rare, aggressive bone cancers with a peak prevalence in late adolescence. OS, particularly metastatic disease, has a very poor prognosis and progress in developing new therapies has been slow due, in part, to a paucity of information on targetable genomic events responsible for its development. Isolating genetic mutations that drive the development and progression of OS is complicated by the genomic heterogeneity of the disease, which is almost universally characterized by chromothripsis and genomic instability. Previously, our lab has completed a forward genetic screen for drivers of osteosarcomagenesis in mice using an inducible *Sleeping Beauty* system. To evaluate these drivers and identify new ones, we chose to conduct a CRISPR/Cas9 screen in a human cell line. Using a CRISPR library that can target the entire genome allows us to select for the development of specific aggressive cancer phenotypes such as metastasis and treatment resistance.

Design

We chose a poorly metastatic, p53 wildtype, OS cell line (U2OS) for these studies. A previously generated lentiviral CRISPR library representing ~3x genome coverage was used at a low multiplicity of infection to ensure a single gRNA per cell. The constitutive expression of the CRISPR/Cas9 system will generate a diverse population of transform cells, each carrying a unique mutation. When these cells are subsequently xenographed into either the flank or tail vein of NRG mice, they were exposed to a tumorigenic bottle neck and cells where critical TSGs had been disrupted should experience selective outgrowth to form a tumor mass.



Results

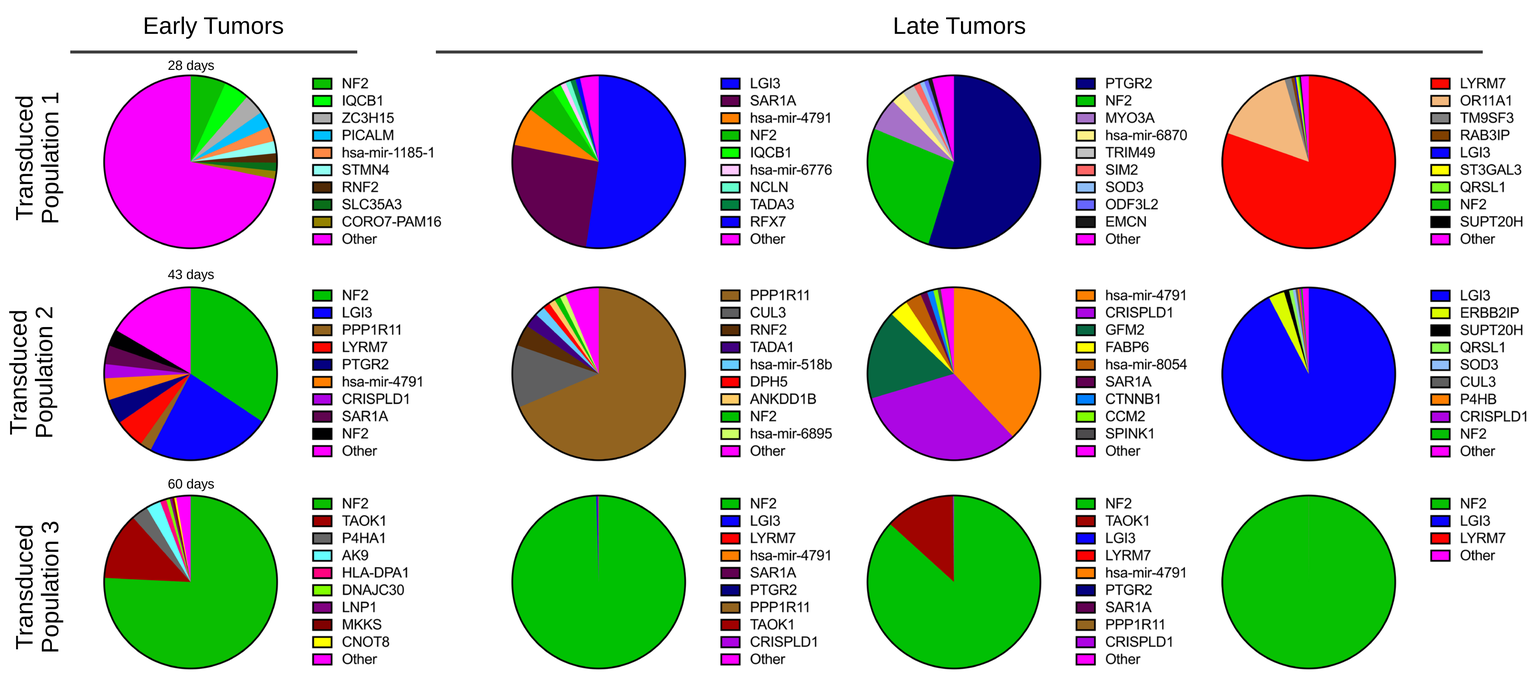


Figure 1: Sequencing of integrated gRNA in early and end time point tumors. Sequencing of the transduced cell populations collected at the early time point (when the tumor first became palpable), and endpoint (180 days). Three separate library transductions were preformed and $30_{E}{}^{6}$ cells were injected into four mice each. Top ten gRNAs as a percent of total reads for that sample are shown.

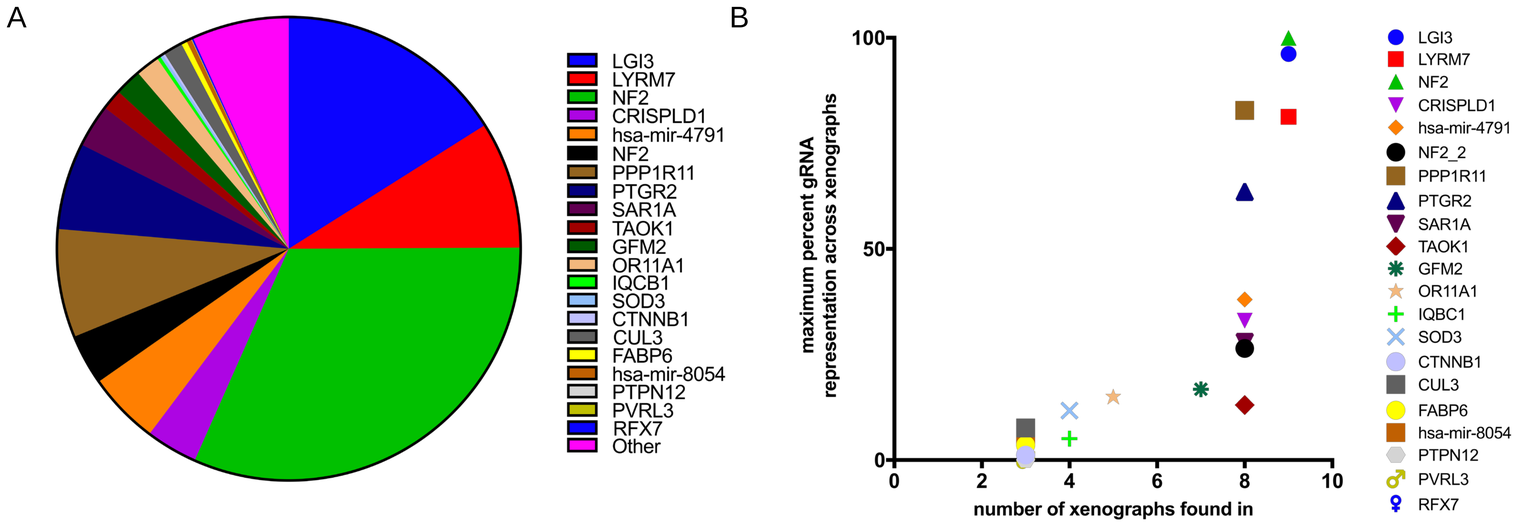


Figure 2: Characterization of top screen hits. (A) gRNA abundance as a percent of total reads from all late time point tumors (n=9) and (B) top gene hits mapped according to the the total number of late tumors they were identified in and the total percent of any tumor that gRNA comprised.

Conclusions

-There is a high variability in integrated gRNA between early and late tumors
-Transduction replicates accounted for high variability between early and late tumors, verifying the validity of top gRNA targets
-A subcutaneous model of tumor growth produces a strong selective pressure on edited cell populations

Future Work

- Sequence whole organ tissue from mice tail vein injected with GeCKO transduced cell populations for analysis of micrometastasic clones
- Generate secondary guides against top gene hits using Synthego chemically modified guide RNAs
- Validate transformed phenotype of individual gene knockouts in 2 additional OS cell lines both *in vitro* and *in vivo*
- Perform CRISPRa screen for proto-oncogenes in OS using newly validated library

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