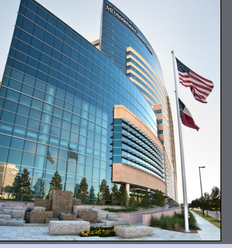

The Role of *HNRNPC::RARG* in APL-like AML

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Background

- Acute promyelocytic leukemia (**APL**) is a subtype of AML characterized by t(15;17)(q24.1, q21.1)/*PML::RARA*
- Standard of care typically involves **ATRA** and **ATO**
- Recent cases have been identified that morphologically resemble APL but are **resistant** to ATRA and ATO
- These cases involve ***RARG*** instead of *RARA*, with a myriad of fusion partners, like ***HNRNPC***, a reader protein of m6A involved in pre-mRNA processing
- Identification and treatment are urgent due to disseminated intravascular coagulopathy (**DIC**)

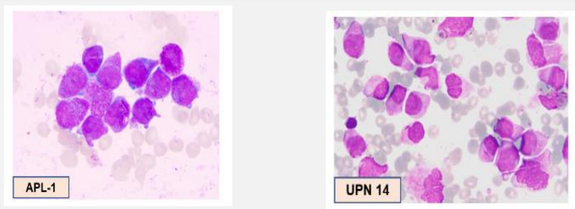


Figure 1. APL (left) and "APL-like" AML (right).

Hypothesis

HNRNPC::RARG rearrangement plays a significant role in the transformation of "APL-like" leukemia and may serve as a marker for targeted therapy

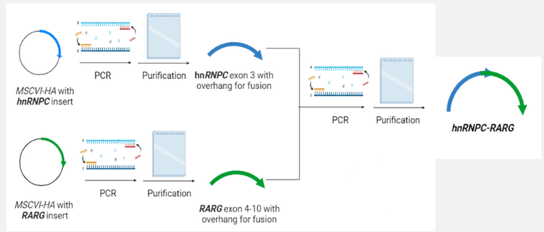
Figure 2. ***HNRNPC::RARG*** fusion (**exon 3** *HNRNPC* CDS, **exons 4-10** *RARG* CDS)

Methods

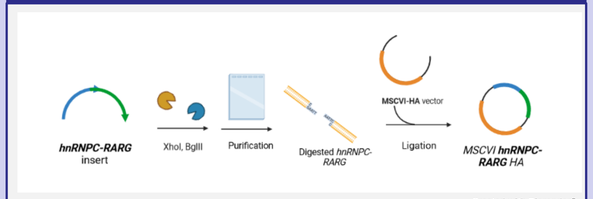
Recombinant cloning

- *HNRNPC* and *RARG* were cloned separately into *MSCVI* vectors previously
- **Exon 3** of *HNRNPC* CDS was then cloned with **exons 4-10** of *RARG* CDS into the **retroviral vector *MSCVI***
- **Fuse, insert, obtain**

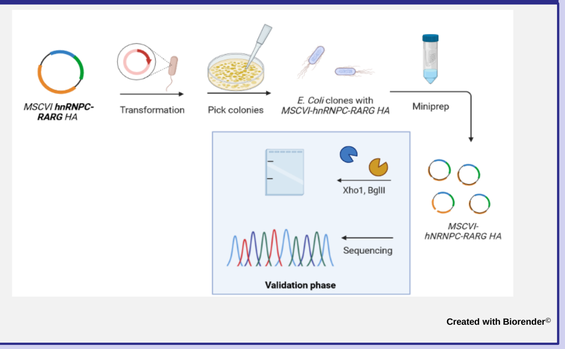
Fuse



Insert



Obtain



Results

- Primers designed for overlapping PCR were able to allow fusion and digestion by XhoI and BglII
- Gel images showed correct band sizes for PCR, overlapping PCR, digestion products, and a validation digestion
- *In-silico* product was generated using Benchling©
- MAAFT alignment against *in-silico* product confirmed correct *HNRNPC::RARG* fusion sequence without evidence of mutation

a) 5' ctttatccagccctcac

5' TGCTCTGTGTCTCCACCGctaaaacctggccagcaatc

b) 5' gattgctggccaggttttagCGGTGGAGACACAGAGCAC

5' AGACTGCCTTGGGAAAAGC

Figure 3. Primers used for **a)** *HNRNPC* exon 3 **b**) *RARG* exons 4-10; **BLUE** anneals to *RARG,* **red** anneals to *HNRNPC*

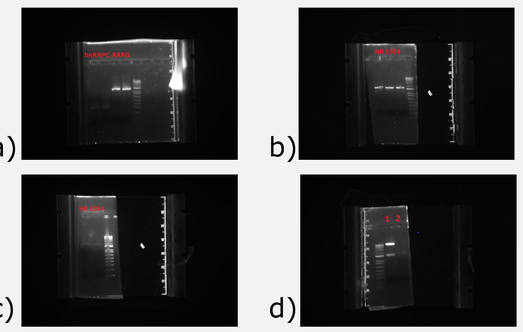


Figure 4. Gel images: **a)** PCR of *HNRNPC* exon 3, *RARG* exons 4-10; **b)** overlapping PCR to make *HNRNPC-RARG,* **c)** digestion of *HNRNPC-RARG*, **d)** validation digestion

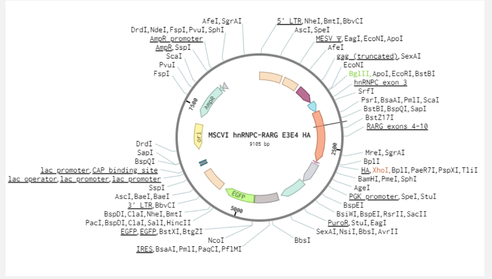


Figure 5. *MSCVI-HNRNPC-RARG HA*; created with Benchling©

Figure 6. MAAFT alignment of sequencing results; mismatches were determined to be artifacts

Future directions

- Obtain **retrovirus** via **co-transfection** with packaging plasmids in 293-T cells
- Adapt a **mouse model**, transplanting **infected** HSCs into mice
- Other fusions (e.g. *HNRNPCL*, *CPSF6*)

References

Zhu HH, Qin YZ, Hu J, Zhang ZL, Huang JY, et al. Acute myeloid leukemia with RARG rearrangement. (Under review)

He L, Li H, Wu A, Peng Y, Shu G, et al. Functions of N6-methyladenosine and its role in cancer. Molecular Cancer. 2019; 18:176.