KaryoCreate (Karyotype CRISPR Engineered Aneuploidy Technology)

Efficient and less laborious isogenic model system to study aneuploidy in cancer and congenital syndromes.

Technology

The inventors have developed a method, referred to as KaryoCreate, of introducing aneuploidy into a cell through a system that enables generation of chromosome-specific aneuploidies by simultaneous expression of single guide (sg) RNA targeting chromosome-specific CENPA-binding α-satellite repeats (centromeric sequences) and non-catalytically active version of Cas9 (dCas9) fused to a mutant form of KNL1, a component of KMN protein complex responsible for kinetochore-microtubule assembly. Co-expression of the sgRNAs with KNL1Mut-dCas9 leads to mis-segregation and introduction of loss or gain of chromosomes in the cellular progeny with an average efficiency of 8% for gains and 12% for losses, which is tested and validated across 9 chromosomes. The inventors have developed unique and highly specific sgRNAs for 19 out of 24 chromosomes present in humans. The sgRNA sequence is complementary to the portion of the centromere where the kinetochore is assembled, positioning the mutated KNL1 proteins in the correct context to interfere with chromosome segregation, the specificity of which is predicted based on the latest human genome assembly.

Background

Chromosomal aneuploidy, characterized by the complete or partial gain or loss of chromosomes, is frequently observed in human cancers and congenital diseases. Congenital aneuploidy can be autosomal syndromes as well as due to ploidy changes in sex chromosomes. Aneuploidy occurs in more than 90% of human solid tumors and are usually chromosome specific. Despite its huge role in cancer, study of aneuploidy in cancers have been massively restricted due to lack of diligent study models. The only available model to study gain of chromosome functions, microcell-mediated chromosome transfer (MMCT), results in the addition of an exogenous chromosome to the cell and is laborious to perform. Methods developed to study loss of chromosome functions have a very low efficiency and utilize catalytically active Cas9, causing DNA double strand breaks and instigating genomic instability. Therefore, there is a colossal and urgent need for efficient model systems to study aneuploidy in cancers which will develop a better molecular understanding of chromosome ploidy burden in cancer, laying the foundation for development of more effective and novel therapeutic interventions for patients with cancer.

Applications

Technology ID

DAV06-02

Category

Life Sciences/Imaging

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- Model system for:
  - Congenital aneuploidy syndromes: Down, Edwards, Patau
  - Aneuploidy in sex chromosomes: To study Klinefelter and Turner syndromes
  - To understand chromosome specific changes in cancer: Generation of isogenic human cell lines with whole or partial chromosome gains and losses to understand the aneuploidy burden in cancer.
  - Novel drug testing in cancer: Aneuploidy tumor cell models can be used to test the efficacy of novel therapeutics developed against the cancer and the above congenital syndromes.
- Advanced understanding of molecular biology: Study of chromosome biology in addition to the study of centromere structure and function.

Advantages

- The only method available to generate isogenic cell lines with chromosome loss or gain without the drawback of exogenous chromosome addition or introduction of double strand breaks.
- Unique and highly specific sgRNAs for 19 out of 24 human chromosomes allowing for the for induction of whole or partial chromosome gains and losses in multiple chromosomes, thereby opening up a plethora of aneuploidy models that can be studied.
- Highly specific and multiple sgRNAs permits the visualization of chromosome mis-segregation by live cell imaging.
- Aneuploidy cell models can be developed in almost all cell types.

IP Status

Provisional patent application pending

References

1. Teresa Davoli et al.(April 18, 2023) , https://www.sciencedirect.com/science/article/abs/pii/S0092867423003264?via%3Dihub