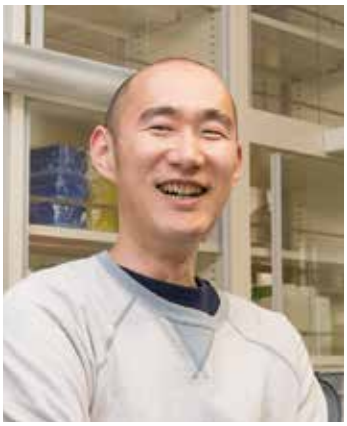

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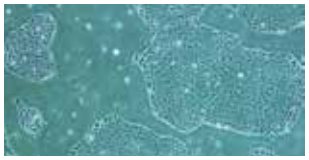
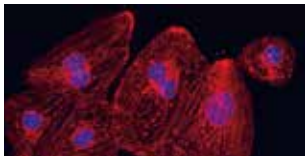
Project Leader **Yuichiro Miyaoka** Regenerative Medicine Project

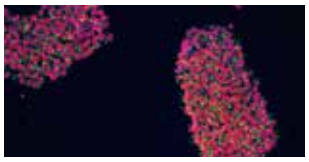
Genome Editing in Human iPS Cells: To study and cure genetic disorders

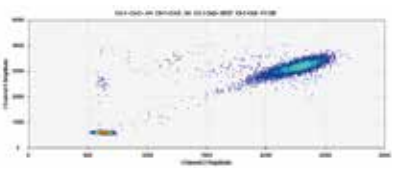
Genome editing technology allows us to rewrite the genetic information in virtually any species and any cell type including human cells. To study the pathogenesis of human diseases at the molecular level, and to develop new therapies using genome editing, we need appropriate human cellular models. Our focus is on human iPS (induced pluripotent stem) cells, a type of pluripotent stem cell that can be generated from patients' cells by introduction of specific transcription factors, and differentiated into other cell types. Our goal is to use genome editing of iPS cells to both model human diseases, and develop new therapies.





"Our goal is to develop methods to precisely and efficiently edit the genome in human iPS cells to allow us to develop disease models using human cells, and develop new therapies for these diseases."



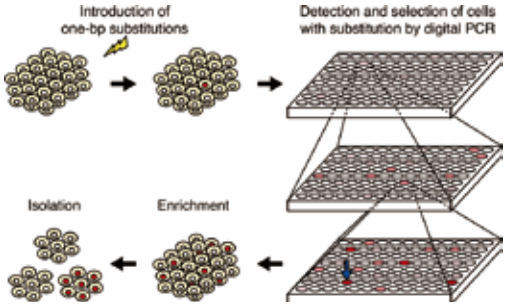


We developed an efficient method to isolate iPS cell lines containing a single nucleotide substitution. The nucleotide substitution is created by genome editing based on digital PCR, and isolation is accomplished by repeated limited dilutions in the absence of selection markers (Miyaoka, Nat. Methods 2014). Using this method, we are analyzing the pathogenesis of cardiomyopathy caused by point mutations of RBM20 (RNA-binding motif protein 20) in isogenic cardiomyocytes derived from genome-edited iPS cells. We are also improving the precision and efficiency of genome editing technology, and developing new therapies based on correcting mutations in iPS cells from patients. In addition, we are developing a strategy to directly edit the genome in cells in the human body.

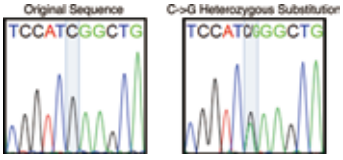
Regenerative Medicine

Changing a Single DNA Base-pair out of Three Billion

Single point mutations are often responsible for genetic disorders. Thus, the development of techniques to generate single point mutations is important for both modeling and curing diseases. However, thus far, it had been difficult to make specific single base-pair (bp) substitutions in the 3 billion-bp human genome. We have developed a method for isolating iPS cells with single-bp substitutions by combining genome editing, and serial limited dilutions using digital PCR .



Isolation of iPS cells with single nucleotide substitution

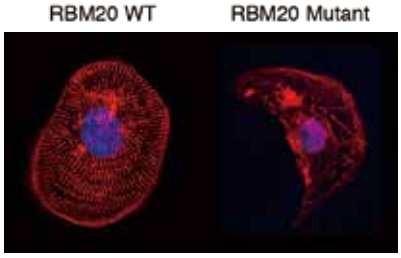


Isolated iPS cells with C→G substitution

Using this method, we can efficiently introduce single-bp substitutions at any location in the genome, allowing us to develop iPS cell-based disease models and transplantation therapies.

Heart Failure in a Dish

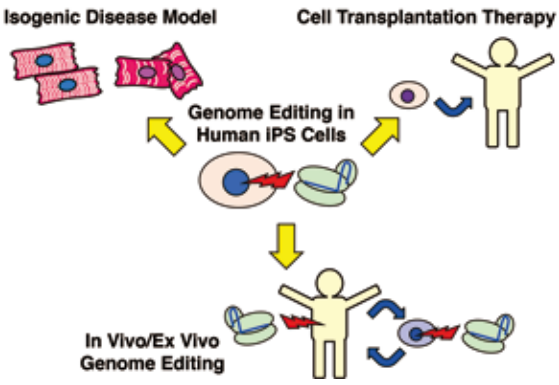
By editing the genome of iPS cells, we can study pathogenic mechanisms of genetic disorders in any cell type in a dish. For example, a point mutation in RBM20 (a cardiomyopathy mutation) introduced into iPS cells caused abnormal sarcomere structures (a functional unit of muscle contraction visualized as red stripes), when these cells were differentiated into cardiomyocytes. These cells can serve as a platform for drug screening.



Sarcomere (red) and nucleus (blue) in iPS cell-derived cardiomyocytes

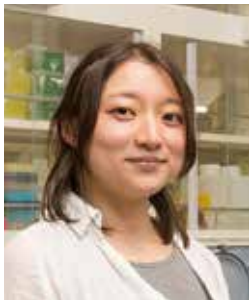
Development of Precise Ways to Edit the Genome

Current genome editing tools including CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats/CRISPR associated protein 9) have revolutionized our ability to modify the genetic information in cells. However, these tools still need to be improved for accuracy and efficiency when used in therapies. Therefore, we are developing a more precise and efficient way to edit the genome by modifying the Cas9 nuclease, and the guide RNA that directs Cas9 to the target regions. These improvements are necessary for further development of genome editing-based therapies.

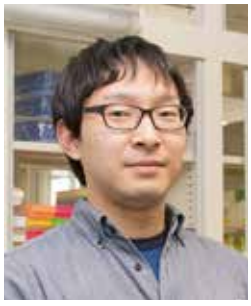


Genome editing in iPS cells to study and cure disease

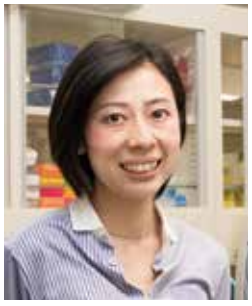
Members



Tomoko Kato-Inui



Gou Takahashi



Szuyin Hsu

Regenerative Medicine