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Validation of strategies enhancing homology-directed repair pathway

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Statement of the Problem: Repertoire of genomic alterations via CRISPR/Cas9 genome editing depends on the efficiency of dsDNA breaks and the subsequent DNA repair events, which consists of two distinct pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). HDR permits precise genome modifications which can be used to mimic disease relevant genotypes or to integrate functional domains of interest into the genome. However, the efficiency of HDR via genome editing remains quite low, mostly ranging from 0.1~10%. Although several strategies have been claimed to enhance HDR efficiency, there are still concerns about variability and reproducibility.

Strategies: Relevant literature reporting HDR enhancement has been extensively surveyed. We hypothesized 1) high NHEJ event without a donor DNA also increase the chance of HDR in the presence of a donor. 2)HDR may increases by antagonizing NHEJ since NHEJ and HDR compete for the repair of limited number of dsDNA breaks. Combinations of modifications of gRNA and donor DNA, use of chemical inhibitors and targeted knock down have been examined.

Findings: Temporal delivery of ribonucleoprotein (RNP) and chemical modifications of donor DNA increased HDR efficiency. Optimized conditions with KU0060648 or NU7441, DNA-PK inhibitors, reproducibly increase the HDR efficiency up to 50% in several different cell lines.

Conclusion & Significance: Enhancement of HDR up to 50% has been achieved using combinations of different strategies. Our findings provide useful guideline to improve the efficiency of precise genome modifications in human cells.

Biography

Sungtae Kim is a scientific investigator and has his expertise in stem cells, CRISPR/Cas9 genome editing, genomics and disease modeling. He has been implementing cutting-edge technologies and platforms to establish cellular disease models and for target validation studies. He has lead genome engineering tasks at GSK in multiple cell lines and primary cells by introducing novel strategies/technologies to the group and created new technological platforms for drug discovery in higher throughput manners. He is the first line leader in genome editing who cross-trains scientists in several discovery performance units, enjoys disseminating updates in science, organizes meetings for scientific discussion and established himself as a go-to person in this area. He is actively involved in several target identification studies and attempts to bridge genome editing strategies with advanced cellular modeling for better assessment of targets.

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