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The role of DNA repair in active DNA demethylation is studed by the construct based on the CRISPR/Cas9 system

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***Abstract* — Recently published data have demonstrated the possibility of using CRISPR / Cas9-based systems that direct intracellular DNA repair systems to consolidate changes in the genome and epigenome. We develop an approach for assessing the contribution of various DNA repair processes for integrity of DNA, and we proposed the mechanisms of active epigenetic DNA demethylation includes the participation of base excision repair and non-canonical mismatch repair system in this process.**

Keywords — CRISPR/Cas9, base excision repair, non-canonical mismatch repair, epigenetics, active DNA demethylation.

Motivation and Aim

Motivation

In mammalian cells the cytosine methylation of the CpG-rich regulatory regions of the individual genes forms the epigenetic landscape of the cell. During differentiation processes the molecular mechanism of local methylation status changes still remains a mystery. We develop the system based on CRISPR/Cas9 for investigation the molecular mechanism of the active DNA demethylation through DNA repair pathways: base excision repair and non-canonical mismatch repair.

Aim

Active DNA demethylation occurs via hydroxylation and/or deamination of 5-methylcytosine (5mC). The resulting 5mC derivatives are removed through the base excision DNA repair pathway [1] and cell needs the special mechanism for eliminate closely spaced toxic intermediates as single- and double-strand breaks. Recently, we demonstrated cooperative mechanism between the base excision repair and non-canonical mismatch repair pathways for local active DNA demethylation. [2]. This project aims to fundamental questions about the contribution of different DNA repair pathways into epigenetic DNA demethylation and also the development of technics for directed epigenome changes.

Methods

The phagemid DNA substrate containing defined mismatches and methylation mark was treated by mouse embryonic fibroblasts (MEF) cell-free extracts differing in the level of DNA repair activity followed by restriction analysis. For estimate of the contribution base excision repair and non-canonical mismatch repair systems in active DNA demethylation we initiated nick-formation by CRISPR/Cas9 system.

Results

The presence of multiple mispairs and nick-formation by CRISPR/Cas9 system promote proficient ncMMR resulting DNA demethylation in the internal region. In addition, we found that MSH2 protein is not required for the BER/Cas9-dependent removal of tandem oxidative mismatches.

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