

Abstract:

Most genome disorders cause severe symptoms and are usually incurable. Recent, rapid development of programmable nucleases (PNs) brought new possibilities for the treatment of many diseases, such as genetic disorders, infectious diseases or cancer. PNs are enzymes, which enable site specific DNA cleavage that can lead to targeted modification of desired genomic loci. They are composed of separable non-specific cleavage domain and DNA-binding domain. The DNA binding domain is in the form of modular DNA-binding proteins or complementarity-based pairing of the oligonucleotide. The non-specific cleavage domain mediates DSB stimulation, which is necessary for further genome editing. Development of zinc finger nucleases (ZFNs) followed by transcription activator-like effector nucleases (TALENs) enabled the first therapeutic approaches based on targeted manipulation of human genome. The clustered regularly interspaced short palindromic repeats (CRISPR)-Cas technology brought further simplification to the method and broadened the availability of PN-based toolkits. This thesis will provide a summary of the recent developments, application of PNs in the therapy of human patients and potential obstacles preventing their implementation in clinics.