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CRISPR/Cas9-mediated cleavage of protospacer with DNA damage

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Motivation and Aim: A CRISPR/Cas9 system widely uses in the genome editing. The main principle of genome editing is the formation of double-strand breaks in DNA and following DNA reparation. A Cas9/sgRNA complex is targeted to the protospacer in DNA by PAM recognition and R-loop formation. It was shown that mismatches in the sequence of a protospacer influence negatively on the DNA binding and cleavage by the Cas9/sgRNA complex [1]. However, an influence of the DNA damage on cleavage efficiency by the Cas9/sgRNA previously wasn't studied. Thereby we decided to investigate the Cas9/sgRNA cleavage of duplexes and plasmids with point DNA lesions in PAM and the protospacer sequence.
Methods: To detect the DNA cleavage product *in vitro*, we've obtained radiolabeled duplexes with substitutions such as 8-oxo-2′-deoxyguanosine (oxoG), uridine (U) and tetrahydrofuran abasic site (F). These substitutions contained both in PAM (5′-TGG-3′) and the protospacer sequence. In addition, plasmids with the same substitutions as in duplexes have been used as substrates.
Results: Surprisingly, any substitutions in PAM sequence (5′-TGG-3′) of guanosine on the tetrahydrofuran abasic site or 8-oxo-2′-deoxyguanosine led to cleavage resistance for duplex substrates. The opposite situation was observed when substrates were plasmids. 8-Oxo-2′-deoxyguanosine in the second and third positions of PAM partially reduced of cleavage efficiency while tetrahydrofuran abasic sites in the same position didn't effect on activity of the Cas9/sgRNA complex. The cleavage efficiency of duplexes with substitutions at the protospacer positions upstream of PAM of non-target strand decreases in the row (8G → F) – (8G → oxoG) – (16C → U) – (14C → U).
Conclusion: Our results have shown that the DNA cleavage by the Cas9/sgRNA complex depends not only on the type of DNA damage, but also on the type of substrate.

References

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