Codon swapping of zinc finger nucleases confers expression in primary cells and in vivo from a single lentiviral vector

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Résumé en anglais	**BACKGROUND:** Zinc finger nucleases (ZFNs) are promising tools for genome editing for biotechnological as well as therapeutic purposes. Delivery remains a major issue impeding targeted genome modification. Lentiviral vectors are highly efficient for delivering transgenes into cell lines, primary cells and into organs, such as the liver. However, the reverse transcription of lentiviral vectors leads to recombination of homologous sequences, as found between and within ZFN monomers. **METHODS:** We used a codon swapping strategy to both drastically disrupt sequence identity between ZFN monomers and to reduce sequence repeats within a monomer sequence. We constructed lentiviral vectors encoding codon-swapped ZFNs or unmodified ZFNs from a single mRNA transcript. Cell lines, primary hepatocytes and newborn rats were used to evaluate the efficacy of integrative-competent (ICLV) and integrative-deficient (IDLV) lentiviral vectors to deliver ZFNs into target cells. **RESULTS:** We reduced total identity between ZFN monomers from 90.9% to 61.4% and showed that a single ICLV allowed efficient expression of functional ZFNs targeting the rat UGT1A1 gene after codon-swapping, leading to much higher ZFN activity in cell lines (up to 7-fold increase compared to unmodified ZFNs and 60% activity in C6 cells), as compared to plasmid transfection or a single ICLV encoding unmodified ZFN monomers. Off-target analysis located several active sites for the 5-finger UGT1A1-ZFNs. Furthermore, we reported for the first time successful ZFN-induced targeted DNA double-strand breaks in primary cells (hepatocytes) and in vivo (liver) after delivery of a single IDLV encoding two ZFNs. **CONCLUSION:** These results demonstrate that a codon-swapping approach allowed a single lentiviral vector to efficiently express ZFNs and should stimulate the use of this viral platform for ZFN-mediated genome editing of primary cells, for both ex vivo or in vivo applications.
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