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KNOCK-OUT OF THE HUMAN ENDOGENOUS RETROVIRUS ERV3 USING CRISPR/CAS9 TECHNOLOGY

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Objectives: Human endogenous retroviruses (ERV) are retroelements which integrated into the human genome. ERV3 is an endogenous retrovirus which is deregulated in many diseases, including cancer. It is expressed in the syncytiotrophoblast of the human placenta and thought to be involved in cell cycle regulation. So far little is known about its functional role during human placental development and carcinogenesis. To investigate the functional significance of ERV3 we aimed to induce an

ERV3 knock-out in the human choriocarcinoma cell-line Jeg-3 using CRISPR/Cas9 technology.
Methods: The CRISPR-associated protein 9 nuclease (Cas9) from Streptococcus pyogenes is part of the bacterial immune mechanism used to protect the bacteria from foreign nucleic acids. By expression of a guide RNA (gRNA) the nuclease is directed to the target sequence and consequently is able to induce sequence specific double strand breaks.
A specific gRNA was cloned into the pSPCas9(BB)-2A-GFP plasmid expressing Cas9 from S. pyogenes and GFP. Jeg-3 cells were transfected with the construct and single cell clones were isolated by fluorescence activated cell sorting (FACS). Mutations within the ERV3 gene were detected by sequencing. Knock-out of the ERV3 protein was analyzed using western blot.
Results: Transfection of Jeg-3 cells with the CRISPR/Cas9 plasmid induced different ERV3 mutations. We were able to isolate six Jeg-3 clones with deletions from 1 to 186 nucleotides and one clone with a heterozygote point mutation. Two of these mutations induced a frameshift resulting in a loss of ERV3 protein expression.
Conclusions: Using CRISPR/Cas9 technology we were able to induce different mutations within the ERV3 gene in Jeg-3 cells. A stable knock-out of ERV3 in Jeg-3 cells might help to investigate its functional significance for cancer progression, proliferation or cell fusion. Functional assays using the ERV3 knock-out cell-line will give insights into the impact of ERVs for cancer and placental development.