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CRISPR/Cas9-mediated generation of HADHA KO PANC-1 cells to study the role of HADHA in pancreatic cancer stemness

Mauro Voi[1], A. Dezhgir[1], G. Siragusa[2], A. Ruggiero[1], D. Cecconi[2], D. Zipeto[1]
[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona, Italy
[2]Department of Biotechnology, University of Verona, Verona, Italy

Background. The CRISPR/Cas9 technique was applied to generate HADHA KO PANC-1 cells to depict the role of HADHA in stemness of pancreatic cancer stem cells (PCSCs).
Methods. Two different gRNAs targeting exon 1 of the HADHA gene were designed and cloned into Cas9-expressing plasmids. Parental PANC-1 (P) cells were transfected with Cas9/gRNA plasmids to generate HADHA-KO cells. Individual clones were isolated and analysed by Western Blot and Sanger sequencing of the edited region. To obtain PCSCs, P cells were cultured in a specific “stem selective medium.” Tumoursphere formation was imaged after 5,10,15 days using the EVOS FL Imaging System.
Results. Different transfection protocols were tested on PANC-1 cells, with Lipofectamine3000 giving the best results of transfection efficiency, albeit moderate. We identified a clone (C8E12) showing the absence of the HADHA protein by Western Blot. Sanger sequencing revealed a heterozygous editing on the gRNA-targeted region, with a 124-bp and a 78-bp deletion on the two different alleles. Induction of dedifferentiation on the KO clone showed that HADHA knockout does not alter the stemness of the pancreatic cancer cell line: the KO clone is still able to grow in suspension and to remain undifferentiated. HADHA KO-PCSCs exhibited the formation of more compacted and denser tumourspheres than WT-PCSCs.
Conclusion. The low transfection efficiency on PANC-1 cells led to the identification of a single HADHA KO clone after the application of the CRISPR/Cas9 technique. The effect of the absence of HADHA expression was evaluated on PCSC morphology. KO cells retain the ability to form spheres, although morphologically they appear more compact and show denser structures than their WT counterpart. However, the effects of HADHA knockout on PCSC proliferation, chemoresistance, and tumour migration should be further investigated to understand its role in PCSCs biology and its potential as therapeutic target in pancreatic cancer.