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Investigating RUNX2 KO in B16 melanoma cells as a potential strategy to enhance in vivo tumor response to therapeutic approaches

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Abstract

Background: Cutaneous melanoma (CM) is a heterogeneous and highly metastatic disease with unpredictable clinical behaviour, for which the most effective pharmacological strategies are still being sought. In the present study, we describe the workflow for RUNX2 KO generation in B16 melanoma cells, an approach that according to preliminary data from international databases may contribute to defusing CM resistance to targeted and immunotherapies.
Methods: The role of the RUNX2 gene in CM was investigated using the TIMER2.0 (http://timer.cistrome.org/) and TISIDB (http://cis.hku.hk/TISIDB/) databases. Two gRNAs targeting exon 4 of RUNX2 were designed and cloned into the px459v2.0 plasmid, as previously described[1]. The B16 melanoma cells were transfected with 2.5 µg of plasmid DNA using Lipofectamine 3000 in DMEM (without antibiotics). After one day, cells were selected with complete DMEM containing 1 µg/ml puromycin for six days. Cells were further subjected to monoclonal cell isolation by seeding 0.3 cells/well in 96-well plates. Genomic DNA was extracted from the transfected bulk cell population and isolated clones and PCR-amplified with primers spanning the gRNA-target region. RUNX2 KO was confirmed by Western blot.
Results: According to the TIMER2.0 and TISIDB databases, RUNX2 shows positive correlations with the most notorious immune inhibitors within the CM tumour microenvironment and is overexpressed in CM patients unresponsive to immune and targeted therapies. Therefore, we hypothesize that orchestrating RUNX2 KO in melanoma may be a promising strategy to improve tumour response to therapeutic approaches. Five B16 clones were subjected to Sanger sequencing and all showed editing events. In particular, they showed an in-frame deletion within the Runt domain of the RUNX2 gene. In addition, these five clones showed no RUNX2 protein expression by Western blot.
Conclusions: Our study presents the workflow for obtaining genetically engineered melanoma cells that can be further exploited to dissect the biological roles of RUNX2 in syngeneic B16 melanoma mouse models.
References:
[1]Deiana M. et al., 2018. New insights into the Runt Domain of RUNX2 in melanoma cell proliferation and migration. *Cells*. 7(11):220.