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Editing RUNX2 KO in B16 melanoma cells with Crispr-Cas9 as a potential strategy to enhance *in vivo* tumor response to therapeutic approaches

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Background: Cutaneous melanoma (CM) is a heterogeneous and highly metastatic disease with variable clinical behavior, for which the most effective pharmacological strategies are still being sought.
RUNX2 is a transcription factor involved in many pathways, such as apoptosis, Epithelial-Mesenchymal Transition (EMT), and stem cell function, affecting WNT, NOTCH, BMP, and RAS signaling.
The present study presents the workflow for RUNX2 knockout (KO) generation in B16 melanoma cells, an approach that may contribute to defusing CM resistance to targeted and immune therapy.
Material and methods: Two gRNAs targeting exon 4 of RUNX2 were designed and cloned into pX459v2.0 plasmid. The B16 melanoma cells were transfected with 2.5 µg of plasmid DNA by Lipofectamine 3000 in DMEM (w/o antibiotics). After 24 hours, cells were selected with complete DMEM containing 1µg/mL puromycin for six days. Cells were further subjected to monoclonal cell isolation by seeding 0.3 cells/well into 96-well plates. Genomic DNA was extracted from isolated clones, PCR-amplified with primers spanning the gRNA-targeting region and subjected to Sanger sequencing. RUNX2 KO was confirmed by Western blot.
Results: Clones with a 108 bp-deletion showed by PCR were selected for Sanger sequencing and western blot analysis. Seven B16 clones showed editing events by sequencing. In particular, four clones showed in-frame deletion within the Runt domain of the RUNX2 gene. Three clones displayed heterozygous editing. These seven clones showed no RUNX2 protein expression by western blot.
Conclusions: Our study presents the workflow for obtaining genetically engineered melanoma cells that may be further exploited to dissect the biological roles of RUNX2 in B16 melanoma syngeneic mouse models.