nografts in nude mice. In vitro we found that PTHrP induces Ser552 phosphorylation of β-catenin and its subsequent nuclear translocation. Once in the nucleus, β-catenin can activate the expression of molecular markers associated with other events of CRC progression such as cancer stem cell (CSC) phenotype and epithelial to mesenchymal transition (EMT). In both experimental models we observed that PTHrP regulates protein levels of two CSC markers, CD44 and CD24 and also modulates protein expression of the EMT markers, CK-18, E-cadherin and ZEB-1. Met is a receptor tyrosine kinase (RTK) with aberrant expression and signaling in advanced CRC. We found that PTHrP decreases Met protein levels being this effect reverted by ERK1/2 and p38 MAPK specific inhibitors, suggesting that in PTHrP-treated HCT116 cells this RTK is degraded after its activation via MAPK signaling. According with our hypothesis, PTHrP increases Met protein levels in the murine model. Finally, the specific Met inhibitor reverted β-catenin phosphorylation and EMT markers expression induced by PTHrP suggesting that Met signaling is involved in these molecular events. Advances in the knowledge of the characteristics of aggressive CRC, such as the induction of CSC or EMT, will provide valuable information in understanding this disease and will facilitate the development of new therapeutic approaches

GENÉTICA / GENETICS ORAL SESSION

603. (231) **DESIGN OF A LENTIVIRAL VECTOR TRANSCRIPTIONALLY REGULATED FOR SPECIFIC NEURON THERAPEUTIC GENE DELIVERY**

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Alzheimer's disease is a neurodegenerative disorder characterized by a progressive loss of cognitive functions. One hallmark is the formation of amyloid plaques, composed mainly by Aß peptide oligomers (AβOs). Neprilysin (NEP) is the main endopeptidase for the degradation and clearance of Aß in the brain. Strategies aiming to increase NEP levels should contribute to decrease the amount of AβOs and could have a therapeutic effect. In this work we developed a LV coding the NEP cDNA under SYN promoter (LV-SYN-NEP) for the delivery of transgenic NEP. The objective is to evaluate its performance in neuron-like cells at different days of differentiation and correlate vector-encoded NEP expression with Synaptophysin, which is a marker of neuronal differentiation.

Neuronal progenitor cell line SH-SY5Y was transduced with LV-SYN-NEP and LV-CMV-NEP. 72 hours post transduction SH-SY5Y cells were put under neuron differentiation conditions (Retinoic Acid 10 uM). Expression of NEP and Synaptophysin was measured at day 0, 5 and 7 of differentiation by flow cytometry and immunofluorescence, respectively.

We observed in SH-SY5Y transduced with LV-SYN-NEP 0%, 16% and 33% of NEP+ cells at days 0, 5 and 7 of differentiation, respectively, while SH-SY5Y transduced with LV-CMV-NEP resulted in more than 90% of NEP+ cells at all time points assessed. NEP was not expressed in mock-transduced cells. We also found that LV-delivered NEP expression correlates with Synaphophysin, demonstrating that LV-SYN-NEP is able to express in differentiated neurons. We also demonstrated that SYN promoter activity increases with the degree of neuronal differentiation.

In conclusion, we developed a neuron-specific lentiviral vector to deliver NEP transgene to neuronal cells which is expressed as neurons become differentiated. In the future, we will test its protective potential against toxic aβ peptides.

604. (235) **EFFICIENT GENOME EDITING AND GENE ADDITION USING BABOON ENVELOPE GP PSEUDOTYPED VIRAL DERIVED "NANOBLADES" LOADED WITH CAS9/SGRNA RIBONUCLEOPROTEINS AND AAV6 FOR DONOR DNA CASSETTE DELIVERY.**

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Programmable nucleases have enabled rapid and accessible genome engineering in cells and living organisms. However, their delivery into target cells can be challenging into primary cells. Here, we have designed "Nanoblades", a new technology to deliver a genomic cleaving agent into cells. These are murine leukemia virus-derived virus like particle (VLP) loaded with Cas9 protein through fusion with the gag viral protein and guide RNAs. Cas9 together with gRNAs introduces site specific double strand break (DSBs) in target genes which can be repaired by non-homologous end-joining (NHEJ) or by homology-directed repair (HDR) introducing a new sequence from an exogenous template DNA bearing homology to the sequences flanking the DSBs (donor-DNA).

Previously, we demonstrated that Nanoblades were extremely efficient in delivery of Cas9/sgRNA cargo into K562 cell line and human T, B , HSCs and HSC-derived progenitors T-cells (pro-T cells), thanks to their surface co-pseudotyping with baboon retroviral and VSV-G envelopes.

The objective of this work was to edit Wiscott Aldrich Syndrome (WAS) gene locus by HDR using Nanoblades and AAV6 carrying a donor-DNA, consisting in GFP reporter gene flanked by homologous arms of the WAS gene.

AAV6 was added to K562 cells at different time points with respect to Nanoblades addition, in order to find the optimal addition dynamic that maximizes HDR. Different multiplicities of infection (MOIs) of AAV were tested. HDR-mediated gene editing was determined by PCR and GFP expression by flow cytometry 7 days after Nanoblades addition.

We show that HDR-mediated edition of WAS gene occurred in 50% of the cells adding nanoblades and AAV6 (MOI 100000 vg/cell) simultaneously. We are currently testing this protocol in HSCs and pro-T cells.

In summary, Nanoblades in combination with AAV6 carrying donor-DNA are efficient tools for gene editing and have important prospects for basic and clinical translation for gene therapy.

605. (259) **IDENTIFICATION OF HYPOPITUITARISM RELATED VARIANTS IN ARGENTINEAN PATIENTS BY MOLECULAR INVERSION PROBE SEQUENCING (MIPS), A NOVEL MOLECULAR APPROACH FOR LOW COST SEQUENCING**

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Pituitary hormone deficiency occurs ~1:4,000 live births. Over 30 genes have been implicated in isolated and/or combined pituitary hormone deficiency (IGHD/CPHD). Mutations are estimated to account for ~16% of patient cases, thus the majority of familial and sporadic cases have no known genetic origin. We recently implemented a novel and cost-effective approach based on Molecular