neuronal differentiation (hsa-miR-9-3p/9-5p/124-3p/125a-5p/125b-5p and 128-3p). From the list of differentially expressed miRNAs it caught our attention the hsa-miR-216a,b/217 cluster, which was only expressed in NSC and NEU. Moreover, this expression pattern was also validated by RT-qPCR. Furthermore, expression of this cluster was reported in published RNA-Seq bioinformatic datasets of other neural cell types but not along mesoderm and endoderm differentiations or in other species or types of pluripotent stem cells datasets. Besides, there is no bibliographic data of this family associated to neural differentiation or regulation of stemness. In a future, we aim to knock out the whole cluster using the CRISPR/Cas9 technology as a loss-of-function strategy. This knowledge will enable us to characterize the function and targets of this cluster in a neurogenic context.

301. (409) **3D CELL CULTURES IN HYDROGELS FOR BONE TISSUE ENFINEERING**

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Cells in living beings are disposed in three dimensions and, consequently, growing cells in 3D cultures are a more realistic, physiologically relevant than in a 2D environment, the last being the more extended due to their ease of use. For tissue engineering, 3D cultures can provide more representative information of the cell proliferation and differentiation capacity as well as a measure of the need of vascularization of the artificial tissue. Here, we explore the use of two hydrogels, alginate (ALG) and silk fibroin (SF) as scaffolds for 3D cultures of cells, making focus on soft gelation processes that avoid compromising the cell viability. Alginate gelation was performed by Ca2+ crosslinking, which was tailored by the addition of Ca2+ containing particles and pH control, while SF gelation was induced by sonication. Then, the rheological properties of the hydrogels containing ALG and/or SF was evaluated. Finally, the proliferation and differentiation capacity of MC3T3-E1 Subclone 4 cells were analyzed in both 2D and 3D environments. SF exhibited a more simple and customizable gelation procedure than ALG, although the latter showed a more stable gel than the former. 3D cultures, although more complicated to obtain, significantly changed the behavior of cells. Consequently, this type of culture is the more appropriate to determine the capacity of these hydrogels to perform as scaffolds for bone tissue engineering.

302. (411) **MESENCHYMAL STEM CELL-DERIVED EXOSOMES LOSE THEIR REGENERATIVE POTENTIAL UPON UV-C IRRADIATION**

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Exosomes are extracellular vesicles ranging from 30 nm to 150 nm that originate from the endosomal pathway. Their content will depend on the cell of origin and its physiological state, thus the message they convey might change in response to changes in cellular conditions. In particular, the DNA damage response (DDR) has been reported to modulate exosome secretion. This work aims to elucidate the effect that genomic damage induced by UV-C may have on the exosomal secretion of Mesenchymal Stem Cells derived from induced Pluripotent Stem cells (iPS-MSC).
To induce genomic stress, iPS-MSC were irradiated with three different UV-C intensities (0,001 J/cm2, 0,01 J/cm2 and 0,1 J/cm2) and it was assessed by immunofluorescence evidencing the expression of damage sensors such as S-15 phosphorylated p53 and H2AX-γ. Our group has previously demonstrated that although both the expression of genes involved in the exosomal pathway and the number of exosomes secreted by irradiated and non-irradiated iPS-MSC did not show a significant difference between conditions, a loss in pro-migratory properties was observed in irradiated iPS-MSC derived exosomes. For this reason, we hypothesized that a change in exosomal cargo could be responsible for such effects. To evaluate this, exosomes secreted by irradiated and not irradiated IPS-MSC were isolated from conditioned media using a Size Exclusion Chromatography column and proteomic analysis was performed by Tandem Mass Spectrometry. The results showed that a subset of cytoskeleton proteins and migration-inhibiting molecules were over-represented in exosomes from irradiated cells, such as Filamin A and Talin-1, known for negatively regulating cell motility. Altogether these results suggest that iPS-MSC irradiated exosomes carry a distinct and particular cargo that can explain the reverting of the pro-migratory capabilities of non-irradiated iPS-MSC exosomes.

303. (414) **EFFICIENCY OF POINT MUTATION BY CRISPR/CAS9 IN IPSCS DETERMINED BY NEXT GENERATION SEQUENCING**

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The arrhythmogenic cardiomyopathy (ACM) is a genetic disease characterized by the replacement of contractile myocardium by adipose tissue, causing ventricular arrhythmias and eventually sudden death in patients. One of the most commonly mutated genes is the Plakophilin-2 (PKP2) that codifies for a desmosomal protein. The aim of this work was to generate an induced pluripotent stem cell (iPSCs) line with a reported point mutation in PKP2 gene (C>T that generates a missense mutation p.S140F) by CRISPR/Cas9 for modeling the ACM in vitro. In order to generate this edition we designed two RNA guides (gRNA 1 and 2) targeting the Cas9 to the desired region of the PKP2 gene and a template DNA for each gRNA (ssODN 1 and 2) complementary to the sequence containing the point edition. We co-transfected the plasmid containing the CRISPR system with gRNA1 or
gRNA2 together with the ssODN1 or ssODN2, respectively, to $2x10^5$ iPSCs in two different
concentrations (1 ug or 2.5 ug of each DNA construction): gRNA1-1, gRNA1-2.5, gRNA2-1, gRNA2-2.5 groups. After puromycin selection, genomic samples from the 4 groups were taken for amplicon sequencing analysis. PCR amplicons from the pool were sequenced using Miseq in CD-Genomics. These results were analysed with CRIS.py, a python-based program for multiple sequence analysis. The analysis revealed 24.7%, 27.6%, 0.2% and 5.5% of C>T edition, 9%, 10.9%, 3.2%, 25.7% of indel, and 67.8%, 62.9%, 91.2% and 47% of wild type sequences for gRNA1-1, gRNA1-2.5, gRNA2-1, gRNA2-2.5, respectively.
With these results, cells from gRNA1-1 were clonally expanded and 15 clonal cell lines were Sanger sequenced, obtaining 3 clonal cell lines with the desired edition (20%).
In conclusion, gRNA1 was more efficient than gRNA2 independently of the DNA concentration used. Our next steps are to characterize the phenotype of the C>T PKP2 clonal cell lines and to determine whether we can model the ACM in-vitro after differentiating these cell lines into cardiomyocytes.

304. (436) **WALKING BACK OSTEOSARCOMA STEPS: PROTEOMIC PROFILING OF BONE MARROW MESENCHYMAL STEM CELLS AND PRIMARY AND LUNG COLONIZING OSTEOSARCOMA HUMAN CELL LINES**

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Osteosarcoma (OS), the most common malignant bone tumor, has a 20% five-year survival rate for metastatic disease and treatment-resistant patients. Rapid lung dissemination and acquired chemotherapy resistance remain as major clinical challenges. Mesenchymal stem cells (MSC) may contribute directly or indirectly to OS origin and progression. To identify potential metastasis biomarkers, we made a proteomic screening of non-metastatic SAOS2, metastatic LM7 OS cells and BM-MSC using a shotgun approach by a tandem nanocapillary liquid chromatography-mass spectrometry system. We identified 1049 proteins for BM-MSC, 1567 for SAOS2, and 1424 for LM7. To obtain gene ontology terms of the identified proteins, an enrichment analysis of the gene groups was carried out. The three cell populations shared 661 proteins corresponding to protein metabolism, metabolism, and energy-related pathways (25.72%, 22.37%, and 22.37% respectively). Individually, SAOS2 and LM7 cells showed the same number of shared proteins with BM-MSC, but the 64-shared proteins were not the same. Most relevant differences were that VEGF and PDGF signaling pathways were 2.25 fold-increased in LM7-MSC vs. SAOS2-MSC shared proteins. Further, citric acid and electron transport pathways were upregulated in SAOS2-MSC shared proteins. A comparison between SAOS2 and LM7 also shows upregulation of VEGF/PDGF signaling and other metastatic-related pathways in LM7 cells. Our results on the comparison of both OS cells to MSC, suggest that MSC may have a relevant role in OS progression, dictating not only tumor initiation but also metastatic dissemination. Further, LM7 cells had higher expression levels of proteins related to a mesenchymal phenotype and stem-related genes, suggesting a closer relation with MSC. Lung disease remains a major mortality factor in OS. Identification of mechanisms and differentially expressed genes associated with metastasis would help in discovering promising markers and therapeutic targets.

305. (482) **STUDY OF NON-CO-LINEAR EVENTS IN HUMAN PLURIPOTENT STEM CELL**

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RNA sequences topologically inconsistent with the correspondent DNA sequence in the reference genome are known as “non-co-linear events” (NCLe). These events can be linear (trans-splicing) or circular (circRNA) and both are post-transcriptional events. In human pluripotent stem cells (iPSC), NCLe were described to contribute to the regulation of early lineage differentiation; in particular circRNAs formed by quaking protein (QKI) 5 were described as necessary for cardiac differentiation. Trans-splicing events are formed by separate pre-mRNA with inverted and repeated sequences (Alu) while circRNA originate from a backspliced junction in a pre-mRNA. The aim of this work was to characterize NCLe and the role of QKI 5/6/7 in circRNA formation in iPSC. RNAseq data from an iPSC line was analyzed with NCLscan pipeline, revealing 1109 NCLe, among which 3 occurred between different genes. To validate these intergenic junctional events, we amplified them by RTq-PCR with specific primers and sequenced the product, corroborating that these alternative junctional organizations were not informatic artifacts. PCR on purified DNA showed they are not genomic rearrangements. Furthermore, using magnetic oligo dT beads we also demonstrated that the 3 events are polyadenylated and they are sensitive to degradation with RNase R, thus linearly conformed. In parallel, we designed RNA guides to knock out QKI in FN2.1 and H9 using CRISPR/Cas9. PCR and immunofluorescence analysis revealed the absence of the target, indicating that the strategy was successful. In conclusion, we identified NCLe in an iPSC line and characterized 3 different trans-splicing. We were also successful in preparing knockout lines for QKI to assess its role in differentiation as well as the circRNAs dependent on its function. In the future we plan to assess these knockout lines by RNAseq and functionality of the trans-splicing with CRISPR/Cas13 and characterize using northern blot.

METABOLISMO Y NUTRICIÓN

306. (001) **EFFECTS OF METFORMIN AND LOSARTAN ON NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH MESENTERIC ADIPOSITY IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME IN THE RAT**

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Non-alcoholic fatty liver disease (NAFLD) has been described as a histological manifestation of metabolic syndrome (MS). Mesenteric fat that drains into the portal circulation is the largest contributor to visceral adiposity. There is great interest in the pleiotropic effects of metformin (M) and losartan (L) in the treatment of risk factors for MS. We studied the effects of M (500 mg/kg/day) and L (30 mg/kg/day) on NAFLD and its relationship with mesenteric vascular bed (MVB) adiposity, insulin resistance (IR) and systolic blood pressure (SBP) in an experimental model of MS for 9 weeks. Six groups of Sprague-Dawley rats were used: control (C, standard diet), high-fat plus fructose-overload (HFF, 50% w/w bovine fat plus 10% w/v fructose solution), M-treated (CM), L-treated (CL), M-treated HFF diet (HFFM) and L-treated HFF diet (HFFL). Adiposity index was calculated as MVB adipose tissue weight/body weight x 100. Homeostasis model of assessment of IR (HOMA-IR), SBP, hepatic steatosis and perivascular fibrosis (hematoxylin-eosin and Sirius Red techniques) were measured.
HFF diet produced significant ($p<0.001$) increments on MVB adiposity index (%, 1.75±0.07 vs C: 0.81±0.04), HOMA-IR (0.50±0.06 vs C: 0.11±0.003), SBP (mmHg, 154±2 vs C: 120±2), hepatic steatosis (%, 81.5±2.5 vs C: 1.3±0.3) and perivascular fibrosis (%, 52.0±3.3 vs C: 12.3±1.1). Compared with HFF rats, M and L treatments (HFFM and HFFL respectively), significantly ($p<0.001$) ameliorated MVB adiposity index (%, 1.23±0.02 and 1.18±0.08), HOMA-IR (0.13±0.01 and 0.20±0.03), SBP (mmHg, 127±1 and 116±3), hepatic steatosis (%, 51.6±3.2 and 56.5±5.2) and perivascular fibrosis (%, 33.4±3.4 and 31.0±2.8). Moreover, we found that both steatosis and perivascular fibrosis positively correlated with MVB adiposity index, HOMA-IR and SBP.
Both M and L prevented MVB adiposity increase and consequently exhibited beneficial effects on the stages of NAFLD in a context of IR and hypertension.

307. (016) **TOTAL AND UNDERCARBOXYLATED OSTEOCALCIN (OCN) IN NON-DIABETIC WOMEN HAVING OR NOT METABOLIC SYNDROME (MS)**

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Bone controls glucose homeostasis and insulin resistance through OCN. We wanted to know if body mass index (BMI) and the presence of MS could influence total and/or undercarboxylated OCN (tOCN and ucOCN, respectively) levels.
We compared ucOCN and total tOCN levels in 95 non-diabetic normoglucemic women (52.7±13.2 years) having or not metabolic syndrome (MS and nMS, respecively) and different degree of obesity. ELISA was used except for 25OHD where an immune-competitive