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Y
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Red de Terapia Celular 2020

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I. BASIC AND TRANSLATIONAL SCIENCE

PRIMERA MESA DE PONENCIAS

1. LA SOBREEXPRESIÓN DE HIF-1 EN CÉLULAS MESENQUIMALES (MSC) AUMENTA LA CAPACIDAD CLONOGÉNICA IN VITRO DE LAS CÉLULAS CD34+ HUMANAS Y SU CAPACIDAD DE INJERTO EN UN MODELO DE XENOTRASPLANTE.

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Antecedentes.

El fallo de injerto o injerto pobre se considera una complicación importante en el contexto del trasplante alogénico de progenitores hematopoyéticos. Se ha observado que la administración de células estromales mesenquimales (MSC) mejora la capacidad de injerto y la función hematopoyética en modelos pre-clínicos de xenotrasplante. Entre las diferentes fuentes de obtención de MSC, la pulpa dental se considera actualmente una de las fuentes más atractivas debido a su fácil accesibilidad, su baja inmunogenicidad y su capacidad de diferenciación a tejidos mesodérmicos. Debido a que se ha comprobado que la hipoxia y, más en concreto, la sobre-expresión mediante ingeniería genética del factor inducible por hipoxia (HIF-1) potencia los efectos terapéuticos de las MSC, en este estudio nos planteamos analizar si la co-administración de MSC humanas que sobre-expresan HIF-1 podría aumentar la capacidad de injerto de células CD34+ humanas en un modelo de xenotrasplante murino en comparación con las MSC control.

Métodos.

Se emplearon MSC de pulpa dental humana. La sobre-expresión de HIF-1 se produjo mediante transducción con lentivirus de los vectores pWPI-green fluorescent protein (GFP)(MSC) ó pWPI-HIF-1α-GFP (HIF-MSC). Las células CD34+ se aislaron de cordones umbilicales humanos mediante la separación de sus células mononucleadas por gradiente de densidad con Ficoll y purificación inmunomagnética con AutoMacs. 2x105 células CD34+ se co-cultivaron con 5x104 MSC o HIF-MSC (ratio 4:1) durante 72 horas en RPMI a 37ºC, 5% CO2 y 95% de humedad, tras las cuales se recogieron las células CD34+ y se realizaron los siguientes experimentos. Se analizó la expresión de algunas moléculas implicadas en el injerto hematopoyético (CD44, CXCR4, CD34, ITGA4, cKIT), viabilidad (Anexina V y 7AAD) y expresión de ROS mediante citometría de flujo. Además, se evaluó la capacidad clonogénica de las células CD34+ co-cultivadas con las distintas MSC. Para ello, 2.500 células CD34+ de cada condición se sembraron en medio semi-sólido de metilcelulosa MACSMedia Stem MACS HSC-CFU complete without Epo human durante 14 días tras los cuales se cuantificaron las CFU. Por último, se estudiaron los cambios funcionales en un modelo animal de xenotrasplante hematopoyético. Para ello, se inyectaron por vía intra-femoral 5x105 de cada tipo de MSC en el fémur derecho de ratones NOD SCID previamente irradiados con 3,5Gy. Cuatro horas después se administraron 1x105 células CD34+ por vía intravenosa estableciendo 3 grupos: 1) células CD34+ solas, 2) células CD34+ +MSC, 3) células CD34+ + HIF-MSC. A las 4 semanas post-trasplante, se analizó el injerto humano mediante CMF en los dos fémures y en el bazo.

Resultados.
La eficiencia de transducción fue siempre superior al 95%. Observamos un aumento significativo en la expresión de CD34, CXCR4 e ITGA4 (p=0,027; p=0,003; p=0,039) en las células CD34+ que habían sido co-cultivadas con HIF-MSC frente a las que habían sido co-cultivadas con MSC. No encontramos diferencias significativas en cuanto a su viabilidad o la expresión de ROS. La capacidad formadora de colonias CFU-GM fue significativamente superior en las células CD34+ que habían sido co-cultivadas con HIF-MSC (p=0,048). Finalmente, en cuanto a su capacidad de injerto en ratones NOD-SCID observamos un aumento significativo en la capacidad de injerto de las CD34+ en los ratones a los que se les había co-administrado HIF-MSC con respecto a los ratones con MSC o sin MSC tanto en fémur derecho (p=0,016; p=0,015) como en fémur izquierdo (p=0,024; p=0,008). En la hematopoyesis extramedular no se observaron diferencias.

Conclusiones.
La sobre-expresión de HIF-1 en MSC de pulpa dental mejora sus efectos terapéuticos, aumentando la expresión de CD34, CXCR4 e ITGA4 en las células CD34+, aumentando su capacidad clonogénica y su capacidad de injerto en un modelo de xenotrasplante murino.

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2. CONDICIONAMIENTO DE ASCS A CES COMO TERAPIA CELULAR EN EL TRATAMIENTO DE LA ISQUEMIA.

NODOS: RD16/0011/0018 - RD16/0011/0005

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La enfermedad arterial periférica (EAP) presenta una elevada prevalencia. Su forma más severa es la isquemia crítica, que se asocia con altas tasas de pérdida de la extremidad, morbilidad y mortalidad. La neovascularización es la piedra angular de la preservación de la extremidad. La formación de nuevos vasos mediante células endoteliales (CEs) obtenidas a partir de células mesenquimales autólogas derivadas del tejido adiposo (ASCs) se ha presentado como una gran promesa en el tratamiento de la enfermedad isquémica. Sin embargo, debido a la baja capacidad de auto-renovación y pluripotencialidad de las ASCs, su eficacia terapéutica es relativamente baja.

El condicionamiento de las ASCs mediante pre-diferenciación a células endoteliales incrementaría la capacidad angiogénica de las ASCs y por consiguiente su utilización en terapia celular. La diferenciación de las ASCs a otras estirpes celulares puede ser mediada a través de la regulación de miRNAs y sus dianas. Recientemente, nuestro grupo ha observado que el FGF-2 secretado por las CEs mediante la interacción con su receptor en las ASCs induce la señalización de AKT1 / FOXO1 que regula la expresión del miRNA-145 y la expresión de ETS1, factor de transcricón implicado en la diferenciación de las ASCs a CEs, aumentando la proliferación, migración, e inducción de la expresión de marcadores de CE (VE-cadherina, VEGFR2 o VWF), que finalmente lleva a la formación de estructuras similares a los capilares. En estudios in vivo, la implantación subcutánea o la inyección en un modelo murino de isquemia en las extremidades posteriores, de ASCs precondicionadas a CEs mediante regulación del miRNA-145 o ETS1, indujo la formación de neovasos y el incremento del flujo sanguíneo en la zona afectada. Este enfoque abre una nueva perspectiva en el tratamiento de la EAP al aumentar la disponibilidad de ASCs autólogas transformadas en CEs para ser usadas en terapia angiogénica.

3. CARDIAC PROGENITOR/STEM CELLS SHOW SUPERIOR CELL ENGRAFTMENT TO CARDIAC TISSUE AFTER MYOCARDIAL INFARCTION.

NODOS: RD16/0011/0004 - RD16/0011/0037

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Cardiovascular diseases are the leading cause of mortality in the world (>30%, WHO). Acute myocardial infarction (AMI) and arrhytmias are the most common diseases in clinical practice. At present, several therapies are being developed in order to regenerate the necrotic areas appearing in the heart after a heart attack. In this sense it is important to consider the cardiac progenitor/stem cells (CPCs) because of their therapeutic potential. Although cell retention after intramyocardial transplantation is important to improve cell therapy, few studies have addressed this topic.
In this work we evaluate the engraftment, biodistribution and capacity of cardiac repair of CPCs in a model of myocardial infarction (MI) nude rats. Cardiac function was measured by echocardiographic studies at basal conditions and one month after cellular injection in control, bone marrow mesenchymal stem cells (BM-MSCs) and CPC groups and the spreading of the cells was analyzed by biodistribution experiments. The plasmatic membrane of CPCs was fully characterized by proteomic analysis of biotin labelling surface proteins and the expression was compared against membrane fractions of BM-MSCs.

Our results show that CPCs have high capacity to improve the cardiac function in rats after MI and also to reduce the infarct size comparing to BM-MSCs when sub-optimal doses of cells were used (2.5x105 cells/animal). Most of the CPCs were localized in the heart after 2, 10 and 21 days after administration comparing with other organs analysed. Also, the analysis of the receptome of CPCs showed that gene ontology biological processes and KEGG pathways associated with adhesion mechanisms were up-regulated in CPCs compared with BM-MSCs.
Taken together, these results show the potential of CPCs to engraft in the infarcted area due probably to a more pronounced cell adhesion expression program.

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4. EXTRACELLULAR VESICLES DERIVED FROM CYTOKINE PRIMED MESENCHYMAL STEM CELLS OVEREXPRESSING HIF-1A MODULATE THE PRO-INFLAMMATORY IMMUNE RESPONSE OF MONOCYTE-DERIVED POPULATIONS.

NODOS: RD16/0011/0004 - RD16/0011/0002

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Introduction.

Mesenchymal stem cell (MSC) are a promising therapy for immunological disorders due to their regenerative and immunomodulatory capacities, carry out mostly through their paracrine activity. Increasing evidences show that the release of extracellular vesicles (EVs) can reproduce the effects of their parental cells and offer advantages being a cell-free therapy. It is known than MSC-EVs can target monocytes, macrophages and DCs, which play an essential role in innate immunity, adaptive immunity, and homeostasis. We have developed a no-senescing boosted MSC cell line that previously we demonstrated that represent a source of EVs with a higher immunoregulatory potential. The aim is to study the effect of these EVs on monocyte-derived populations.

Methods.

Monocytes were isolated by CD14 positive magnetic separation. To generate monocyte-derived macrophages (MDMs) M1 and M2, 5 ng/ml rhGM-CSF or 10 ng/ml rhM-CSF, were added respectively. Under M1 conditions, 15µg/ml of EVs were added in two different points of culture: on day 0, before the monocytes differentiation; or on day 5 along LPS stimulation. To generate immature monocyte-derived dendritic cells (iDCs), 20 ng/ml rhGM-CSF and 20 ng/ml rhIL-4 were added. MDMs and iDCs were stimulated with 10 ng/ml of LPS in the last 16 h. To characterize the phenotype of macrophages and DCs, the expression of surface markers and the cytokines released were analyzed were by flow cytometry, RT-qPCR and ELISA assay.

Results.

First, we observed that EVs derived from our improved MSCs decreased TNF-α/IL-10 ratio on undifferentiated monocytes. When we added EVs during differentiation to M1 macrophages, we saw that they were capable of repolarize monocytes towards M2-like phenotype. EVs increased by 60% CD14+CD163+ macrophages (M2 phenotype) and strongly reduced TNF-α/IL-10 ratio compared to control M1. EVs also increased the expression of other anti-inflammatory molecules like IDO, COX2, CXCL-10, CCL-2 or MMP-9. Despite EVs added in differentiated M1 macrophages showed a slight effect on surface markers, the pattern of molecules released was repolarized towards a more immunosuppressive phenotype. EVs also affected the differentiation of dendritic cells and redirected them towards a tolerogenic phenotype, increasing the CD14+/CD1a- population. Finally, we observed that our EVs reduced M1 macrophage infiltration in a Delayed-Type Hypersensitivity mouse model.

Conclusion.

We have demonstrated that our boosted MSC-EVs are able to immunomodulate macrophage population by reducing M1 polarization and promote M2 polarization. In addition, EVs bends DCs toward tolerance induction. MSC-EVs have effective anti-inflammatory properties, making them potential therapeutic agents handier and safer than MSCs for the treatment of diseases in which inflammation and immunity play a critical role.

5. DYNAMIC OF CARDIAC FIBROBLASTS ACTIVATION AFTER A MYOCARDIAL INFARCTION.

NODOS: RD16/0011/0005 - RD16/0011/0030

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Introduction.

Ventricular remodeling is the natural process that happens after a myocardial infarction (MI), and it is characterized by the replacement of the necrotic, ischemic tissue by a fibrotic scar. We have described a new subpopulation of cardiac fibroblasts (CF) responsible for the generation of the healing scar within the first week after MI (Reparative Cardiac Fibroblasts, RCF). For this reason, the knowledge of the cellular dynamic and the molecular mechanisms that underlies its activation is extremely relevant in terms of potential cell therapies toimprove MI.

Methods & Results.

Using bulk RNA-seq analysis on CF isolated from Col1α1-GFP infarcted mice each day during the first week after MI, we identified the peak of overexpression of the top RCF defining marker genes between 3 and 5 days post-infarction (dpi). Then, we performed droplet-based single cell RNA-seq to define the transcriptomic profile of 27,995 CF isolated from healthy hearts, together with 3, 5 and 7 dpi. This unbiased approach helped us to identify 17 clusters of CF. Interestingly, only four of them revealed transcriptomics signatures similar to that of RCF, indicating they are at a transitional stage of activation. These data were validated in silico through a trajectory approach using Velocity. Finally, we identified a set of genes potentially responsible of the transition between homeostasis and damage heart.

Conclusion.

We have identified that RCF appear between 3 and 5 dpi. This subpopulation of cells is the result of a transition of 4 different subpopulation of CF (clusters 8, 9, 10, 11) into a new one in charge of the main response to MI. This transition is mediated by a set of specific genes that are potentially responsible of this process. The identification of the window of activation and the target genes that are responsible for the transition between a basal and a disease-activated CF should help to improve and personalize the current cell therapies against MI.

6. GENERATION OF IPSCS-DERIVED HEART AND VASCULAR SYSTEM IN VIVO.

NODOS: RD16/0011/0005 - RD16/0011/0019

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Organ transplantation is the ultimate approach to treat end-stage organ failure. However, the gap between the demand and supply of organs for transplantation is a major problem worldwide. To cover this clinical demand, one of the latest goals of regenerative medicine is the generation of humanized organs in pigs from pluripotent stem cells (PSCs), by blastocyst complementation.

Here we report the generation of iPSCs-derived heart and vascular system in mice. Mouse iPSCs have been injected in preimplantational embryos unable to form the heart or the vascular system, as they express the difteria toxin subunit A (DTA) suicide gene in the cardiac or endothelial precursors, respectively, during development. In this environment, microinjected iPSCs have been able to colonize the empty developmental niche, rescuing the lethal phenotype and generating chimera embryos, whose cardiomyocytes or endothelial cells were derived exclusively from exogenous cells.

The results obtained with these tissue-specific cell ablation models pave the way for the generation of organs with both parenchymal and vascular tissues derived from donor cells. We believe that the production of an exogenous “vascularized organ” in vivo would be a great step forward in the field of regenerative medicine, as endothelial cells play a key role in the host versus-graft rejection of every organ after a xenotransplantation. Future application of this strategy to form humanized organs in large animals could provide an unlimited source of compatible organs for transplantation.

7. RENIN-ANGIOTENSIN SYSTEM ON THE CAROTID BODY: RELEVANCE IN ANTIPARKINSONIAN CELL THERAPY AND COVID-19 DISEASE.

NODOS: RD16/0011/0025 - RD16/0011/0016

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The Renin-Angiotensin system (RAS) is a well-established humoral circulating system where Angiotensin II is the principal effector, mainly acting through type 1 and 2 receptors. In addition to the systemic RAS, local RAS has been described in different tissues, including central and peripheral nervous system. A local RAS system with its principal components (angiotensinogen, angiotensin converting enzyme, and angiotensin receptors) has been described in the carotid body (CB), a highly dopaminergic neural crest-derived organ which is the principal arterial chemoreceptor inducing the cardiorespiratory reflexes necessary for the adaptation to hypoxia. Our group has demonstrated, in different animal models, that intrastriatal CB transplantation exerts a trophic protection, by the release of GDNF, of the damaged nigrostriatal pathway. Here, we studied the effects of the local RAS in the CB neurogenic niche and the GDNF expression. Moreover, we identified the angiotensin-converting enzyme 2 (ACE2) as a new element of the local CB RAS, being highly expressed both in murine and human CB tissue. Since ACE2 has been identified as the functional receptor by which SARS-CoV-2 enters in human cells, we propose that a potential SARS-CoV-2 infection of the CB tissue could alter its ability to sense the arterial oxygen tension and trigger the cardiorespiratory reflexes as occur in the cases of silent hypoxemia observed in Covid-19 patients. This hypothesis should to be further studied by analysis of CBs obtained from humanized ACE2-transgenic mice infected with SARS-CoV-2.

II. METHODOLOGICAL ADVANCES AND ENGINEERING

SEGUNDA MESA DE PONENCIAS

1. DESIGN OF CONDUCTIVE ELECTROSPUN NANOFIBROUS POLY (CAPROLACTONE)/GELATIN/ POLY-ANILINE SCAFFOLDS FOR CARDIAC REGENERATION.

NODOS: RD16/0011/0004 - RD16/0011/0005

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In 2016, approximately 17.6 million deaths worldwide were attributed to cardiovascular disease (CVD), representing 40% of total disease deaths. CVD is, in absolute terms, the leading cause of death in the world and is expected to rise to more than 23.6 million deaths in 2030. One of the main risk factors for CVD is closely related to the poor conductivity of the damaged heart muscle and the appearance of arrhythmias. Most of the strategies used to correct these defects try to use matrices and/or supports in combination with different additives (cells, growth factors, conductive particles, etc) to improve cardiovascular repair and restore cardiac function.

In this work electrospun scaffolds based on polycaprolactone (PCL), gelatin (Ge) and polyaniline (PAni) were prepared in different PCL/Ge/PAni compositions. PCL and Ge varied between 60/40, 50/50 and 40/60, and PAni was added as conductive cue in different percentages of 0.25, 0.50 and 1.00 %wt. Scaffolds were fully characterized by physico-chemical assessment and biocompatibility with cardiomyocytes in culture was evaluated.

Our results show that the presence of PAni on the scaffolds does not significantly influence the structural and morphological features of the scaffolds, but offers a controlled increase of conductivity. In biocompatibility experiments, cardiomyocytes showed similar proliferation rates on all the scaffolds tested and beat spontaneously with similar beating frequencies. Taken together, we have designed a potential PAni/Ge/PCL based scaffold that could be used to improve cardiac repair. In vivo experiments should be performed to validate its potential.

Funding:

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2. NANOPARTÍCULAS LIBERADORAS DE SECRETOMA DE CÉLULAS MADRE MESENQUIMALES PARA EL TRATAMIENTO DE HERIDAS CRÓNICAS.

NODOS: RD16/0011/0022 - RD16/0011/0008

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Las células madre mesenquimales (MSC) regulan procesos celulares secretando factores de crecimiento, citokinas, hormonas y vesículas extracelulares. Este conjunto de factores constituye el secretoma y se ha demostrado tener propiedades terapéuticas. Si bien el secretoma se puede administrar directamente, su rápida eliminación en el organismo sigue siendo un desafío. Por lo tanto, se han realizado nuevos esfuerzos para desarrollar plataformas que mantengan la liberación de secretoma y aumenten su tiempo de retención.

El objetivo de este trabajo es encapsular el secretoma en nanopartículas (NP) para lograr una liberación sostenida y evaluar su potencial en la cicatrización de heridas. El secretoma se obtuvo cultivando MSC de cordón umbilical humano en condiciones de hipoxia. El contenido de proteínas se determinó utilizando un kit de análisis de proteínas. El secretoma se encapsuló en NP de Poli-láctico-co-glicólico (PLGA) mediante doble emulsión. El tamaño de las NP y el potencial zeta se midieron utilizando un Zetasizer. El contenido de carga y la liberación se evaluaron utilizando un microBCA. También se evaluó la integridad del secretoma. Se estudió la compatibilidad celular de las NP utilizando fibroblastos dérmicos y queratinocitos humanos. Los resultados indican que las moléculas bioactivas más expresadas en el secretoma son TIMP-2, TIMP-1, IL-6, IL-8, RANTES. Las NP encapsulan entre 7,2-13,5 µg/mg NP, con tamaños de 300-400 nm y potenciales zeta por debajo de -20 mV. Los NP son biocompatibles y mantiene la liberación durante 7 días.

La composición del secretoma evidencia su potencial en la cicatrización de heridas. El secretoma se encapsuló con éxito en las NP, mostrando una liberación sostenida. Nuestros resultados indican que estas nanoplataformas pueden ser utilizados como terapias para la cicatrización de heridas.

3. CRISPR/CAS9-MEDIATED GENERATION OF A TYROSINE HYDROXYLASE REPORTER IPSC LINE FOR LIVE IMAGING AND ISOLATION OF DOPAMINERGIC NEURONS.

NODOS: RD16/0011/0024 - RD16/0011/0003

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Patient-specific induced pluripotent stem cells (iPSCs) are a powerful tool to investigate the molecular mechanisms underlying Parkinson's disease (PD), and might provide novel platforms for systematic drug screening. Several strategies have been developed to generate iPSC-derived tyrosine hydroxylase (TH)-positive dopaminergic neurons (DAn), the clinically relevant cell type in PD; however, they often result in mixed neuronal cultures containing only a small proportion of TH-positive DAn. To overcome this limitation, we used CRISPR/Cas9-based editing to generate a human iPSC line expressing a fluorescent protein (mOrange) knocked-in at the last exon of the TH locus. After differentiation of the TH-mOrange reporter iPSC line, we confirmed that mOrange expression faithfully mimicked endogenous TH expression in iPSC-derived DAn. We also employed calcium imaging techniques to determine the intrinsic functional differences between dopaminergic and non-dopaminergic ventral midbrain neurons. Crucially, the brightness of mOrange allowed direct visualization of TH-expressing cells in heterogeneous cultures, and enabled us to isolate live mOrange-positive cells through fluorescence-activated cell sorting, for further differentiation. This technique, coupled to refined imaging and data processing tools, could advance the investigation of PD pathogenesis and might offer a platform to test potential new therapeutics for PD and other neurodegenerative diseases.

4. MESENCHYMAL STEM CELLS AS POSSIBLE MEDIATORS OF SUBLINGUAL VACCINE MV130 EFFECTS.

NODOS: RD16/0011/0002 - RD16/0011/0011

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Recent clinical observations indicate that bacterial vaccines induce cross-protection against infections produced by different microorganisms. In the case of MV130, a polyvalent bacterial preparation designed to prevent recurrent respiratory tract infections, the vaccine treatment significantly reduces the patient infection rate. On the other hand, mesenchymal stem cells (MSC) are key components that contribute to the maintenance of tissue homeostasis and exert both immunostimulatory and immunosuppressive functions. Herein, we study the effects of MV130 in human mesenchymal stem cell functionality as a potential mechanism that contributes to the above mentioned clinical benefits. We provide evidence that during murine sublingual MV130 vaccination, oral mucosa resident MSC can take up MV130 components and their numbers remain unchanged after vaccination, in contrast to granulocytes that are recruited from extramucosal tissues. On the other hand, MSC treated with MV130 show an increased viability without affecting their differentiation potential. In the short term, MSC treatment with the bacterial preparation induces higher leukocyt recruitment and T cell expansion. In contrast, once T-cell activation is initiated, MV130 stimulation induces an up-regulated expression of immunosuppresor factors in MSC. Accordingly, MV130-primed MSC reduce T lymphocyte proliferation, induce the differentiation of dendritic cells (DCs) with immunosuppressive features and favor M2-like macrophage polarization, thus counterbalancing the immune response. In line with this, MSC treated with MV130 induce a clearly reduced leukocyte infiltration in a in vivo model of acute inflammation. Finally, MSC trained with MV130 vaccine undergo functional changes, enhancing their immunomodulatory response to secondary stimulus. In addition, we show that MSC are able to uptake, process and retain a reservoir of the TLR ligands derived from MV130 digestion which can be subsequently transfer to DCs, an additional feature that also may be associated to trained immunity.

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5. MODELLING COMPLEX INHERITANCE PATTERNS OF LVNC IN THE MOUSE.

NODOS: RD16/0011/0021 - RD16/0011/0030 - RD16/0011/0024

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Left Ventricular Non-compaction (LVNC) is a cardiomyopathy characterized by persistent trabeculae, thin ventricular walls and impaired heart function, whose etiology and genetics are poorly understood. We have previously identified two inactivating mutations in the NOTCH pathway regulator MINDBOMB-1 (MIB1R530X and MIB1V943F), inherited in an autosomal dominant pattern in two different LVNC pedigrees. We have introduced both Mib1mutations in the mouse genome using CRISPR-Cas9 gene editing. Phenotypic analysis reveals that the Mib1R530X mutation causes a NOTCH lethal phenotype in homozygosity, while Mib1R530X heterozygous mice are viable and show LVNC in trans with the Mib1flox allele. In contrast, Mib1V943F mice do not show LVNC in hetero or homozygosis, but do show NOTCH-related valve phenotypes in trans heterozygous combination with NOTCH mutations. These data suggested that the expressivity of the LVNC phenotype may be influenced by genetic modifiers present in our LVNC families. We have carried out an exome analysis in the expanded pedigrees of the MIB1R530X and MIB1V943F families, sequencing 15 V943F proband's relatives' exomes from three generations, and 17 exomes of the MIB1R530X proband's kin from two generations. We have identified three candidate mutations in the MIB1V943F family (TMX3, CEP192 and BCL7A), and two variants in the MIB1R530X family (ASXL3 and APCDD1). These mutations co-segregate with LVNC and with MIB1. We have generated the corresponding murine models harboring those mutations, all in chrs. 18 together with MIB1 with the exception of BCL7A, located in chrs. 12. Mib1 Apcdd1 Asxl3 mutant mice show a thinned ventricular wall and a decreased compact/trabecular area ratio, suggestive of LVNC, while preliminary analysis of Mib1 Cep192 Tmx3 mutants show a high penetrance of defective valvular phenotype, suggesting a genetic interaction in cardiovascular development. We are currently characterizing the chamber and valve phenotypes of all the required mutant combinations, and the biochemical interaction of the molecules involved.

6. PRECLINICAL EVALUATION OF THE SAFETY AND IMMUNOLOGICAL ACTION OF ALLOGENEIC ADSC COLLAGEN PATCHES FOR THE TREATMENT OF CHRONIC ISCHEMIC CARDIOMYOPATHY.

NODOS: RD16/0011/0005 - RD16/0011/0029

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The long-term benefit of epicardial collagen scaffolds (CS) seeded with autologous adipose derived-mesenchymal stem cells (ADSC-CS) has been previously shown in rat and pig models of chronic myocardial infarction (MI). In contrast to direct intramyocardial administration of ADSC, epicardial delivery using collagen scaffolds enhanced stem cell engraftment and improved cardiac function, which was mediated through a decrease on myocardial remodeling and an increased vasculogenesis. In order to get into the clinical scenario, collagen scaffolds were seeded with allogeneic ADSC and manufactured under GMP standards. Their safety was confirmed under GLP conditions in different rodent models of tumorogenicity, biodistribution and toxicity and their putative immunogeneic action analyzed in a pig chronic MI model induced by coil. All the animal studies were performed according to the principles of laboratory animal care (NIH Publication no.85-23 revised 1985). Thus, tumorigenicity was evaluated in Rag2-/-gc-/- immunodeficient mice subcutaneously implanted with the cellularized patch. No tumoral or differentiated cells were detected after 3 and 8 months of implantation (n=10/group). Also, ADSC distribution was shown to be confined to the cardiac tissue when implanted in the infarcted rat hearts. Cells were no detected in reproductive organs or brain (among other organs) 7 or 30 days post-implant (n=5/group). Importantly also, toxicity studies showed no adverse effects 2, 10, 30 and 90 days post-implantation in infarcted and healthy Nude rats (n=6/group). Clinical biochemistry, hematological and coagulation blood parameters together with urine analysis did not show significant changes. Necropsy and anatomo-pathological analysis did not reveal significant alterations due to the patch, neither.

Next, the inflammatory and immune response towards the allogeneic patch was analyzed in chronically infarcted pigs implanted with 50 million ADSC injected or implanted in combination with the CS. Untreated infarcted animals were included as control group (n=8/group). The immune response was analyzed before implantation and 15, 30 and 90 days post-implant, determining the evolution of lymphocyte populations, monocytes, granulocytes and leukocytes in blood as well as levels of immunoglobulins and serum proteins. No significant changes were globally detected in any of the parameters analyzed. Moreover, blood biochemical parameters indicated a well preserved hepatic and renal function in all groups thorough the study.

Finally, a co-culture of the human patch with human-allogeneic inflammatory cells was set to deeper analyze the allollogeneic ADSC-CS immunogenic and immunomodulatory action. An alloreactive response towards the cellularized scaffold was not found. Moreover, allogeneic ADSC-CS significantly inhibited lymphocyte´s response, confirming the patch immunomodulatory action in the human context.

In conclusion, treatment of the heart with an allogeneic Cg-ADSC patch is a safe treatment and do not induce an adverse inflammatory reaction, which could be a promising therapy for the treatment of ischemic patients.

7. GENERATION OF HUMAN AND PIG KIDNEY DECELLULARIZED EXTRACELLULAR MATRIX FOR THE FABRICATION OF KIDNEY-SPECIFIC HYDROGELS: TOWARDS THE DERIVATION OF KIDNEY ORGANOIDS FOR APPLICATIONS IN DISEASE MODELING.

NODOS: RD16/0011/0027 - RD16/0011/0005

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Decellularized extracellular matrix (dECM) from tissues and organs constitutes a type of biomaterial that contains tissue-specific biochemical cues and the right proportion of ECM proteins capturing the composition of that found in the original native tissue. These propierties are being exploited in the field of bioengineering to further recapitulate organ-specific microenvironments for cell growth and function allowing the generation of strategies facilitating the derivation of tissue grafts for applications in regenerative medicine. We have relayed in this knowledge to derive for the first time, cardiac dECMs for the generation of cardiac grafts from human pluripotent stem cells (hPSCs). This approach allowed us to mature hPSCs into cardiac cells and develop a platform for cardiac disease modeling.

Nowadays different laboratories, including us, are trying to design new strategies allowing for the generation of organoid systems from hPSCs. In this line, our work has recently proved on the possibility to generate kidney organoids from hPSCs and their utility, for the first time, to study first steps of SARS-CoV-2 infection identifying a clinical grade compound blocking virus infection in a phase IIb clinical trial. One of the major challenges in the field of organoid differentiation stands in the maturation of the generated cell cultures which inherently exhibit incomplete maturation and vascularization compared to that found in the original native tissue.

Since dECMs offer a suitable biomaterial to generate biocompatible hydrogels we hypothesized that the combination of hPSCs derived renal progenitors with kidney derived dECM bioinks would better recapitulate the kidney specific ECM microenvironment allowing for the derivation of organoids with enhanced cellular complexity and function. Here, we have defined the suitable decellularization conditions for fabricating pig and human kidney dECMs using an immersion decellularization protocol. After extensive characterization (i.e., immunohistochemistry, quantification of major ECM proteins, rheology, among others) dECMs have been also assayed for their bioactivity and capability to induce blood vessel growth using the chick chorioallantoic membrane (CAM) assay. Next, through the enzymatic digestion of the dECMs, we have established a procedure to fabricate pig and human kidney dECM hydrogels using different proportions of well-known natural biomaterials (i.e., gelatin, fibrinogen). We have further repurposed our procedure for the generation of kidney progenitor cells from hPSCs which upon extensive characterization (including immunohistochemistry, qPCR and single cell RNA seq) have been further assembled in three dimensional culture conditions upon aggregation with different proportions of human kidney dECM hydrogels. This approach has allowed us to generate hPSCs-kidney derived organoids exhibiting important features of enhanced differentiation including the acquisition of different ECM markers and endogenous vascularization. We have further exploit this novel culture platform for the assessment of nephrotoxicity assays establishing different read outs as metabolic profiling and viability.

III. CLINICAL RESEARCH

TERCERA MESA DE PONENCIAS

1. ENSAYO CLÍNICO FASE I PARA EVALUAR LA SEGURIDAD DE LA INFUSIÓN DE CÉLULAS ESTROMALES MESENQUIMALES DE MÉDULA ÓSEA AUTÓLOGAS FUCOSILADAS EN EL TRATAMIENTO DE LA OSTEOPOROSIS: RESULTADOS CLÍNICOS Y BIOLÓGICOS PRELIMINARES TRAS FINALIZAR EL PERÍODO DE SEGUIMIENTO.

NODOS: RD16/0011/0001 - RD16/0011/0022

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Introducción.
La osteoporosis es una enfermedad ósea sistémica caracterizada por disminución de la masa ósea y deterioro de la microarquitectura del hueso que causa un alto riesgo de fractura. Nuestro grupo demostró que las células mesenquimales de médula ósea que dan origen a las células osteoprogenitoras e influyen en la homeostasis ósea, migran de forma más eficaz al hueso cuando se fucosilan y se infunden por vía intravenosa en modelo murino, potenciando la osteoblastogénesis en el hueso del ratón. En base a esos datos preclínicos, planteamos este ensayo clínico en pacientes con osteoporosis.

Objetivo.
Analizar la seguridad y factibilidad de la infusión de células estromales mesenquimales de médula ósea autólogas fucosiladas (CSM-MOfuc) en pacientes con osteoporosis. Analizar la eficacia potencial, en términos clínicos, biológicos, radiológicos e histológicos.

Métodos.
La población a estudio fueron pacientes de entre 50-75 años con criterios densitométricos de osteoporosis (T< 2,5 DE en cuello femoral o columna lumbar) y al menos una fractura de bajo impacto. El medicamento se procesó bajo condiciones GMP para obtener la cantidad requerida de CSM-MOfuc que se administraron por vía venosa periférica. Se realizó un seguimiento de 24 meses con 10 visitas presenciales y 2 telefónicas en las que se evaluaron acontecimientos adversos, y variables secundarias de eficacia como la evolución clínica (aparición de nuevas fracturas, dolor mediante escala EVA, función mediante cuestionario de Incapacidad por dolor lumbar ODI, y calidad de vida evaluada con Test EuroQoL-5D). También se estudió como variable de eficacia el estado de mineralización y la microarquitectura del hueso mediante biopsia ósea basal y a los 4 meses del tratamiento. Las biopsias de cresta ilíaca posterosuperior se realizaron guiadas por radioscopia para mejor rendimiento y se enviaron fijadas en formol a LABRET-UMA (BIONAD). Se realizaron TAC de las biopsias recibidas con el sistema multimodal de imagen pre-clínica ALBIRA CT (Bruker) y tras incluirlas en metacrilato, realizar secciones de 5 micras por abrasión (EXAKT) y teñir con von Kossa se procedió a su análisis histomorfométrico.

Resultados.
se incluyeron 13 mujeres con una mediana de edad de 60,9 años (rango 52-72) con una o más fracturas vertebrales (n=9) o periféricas (n=4). 3 lotes de CSM-MOfuc autólogas no se administraron al no cumplir especificaciones (2 por baja proliferación y 1 por inestabilidad genética) por lo que las pacientes salieron del estudio. 10 pacientes recibieron 1 infusión intravenosa de CSM-MOfuc autólogas de 2x106 células/kg (n=4) o 5x106 células/kg (n=6). El seguimiento de la última paciente finalizó en junio 2020. No se observaron diferencias en cuanto a seguridad entre las pacientes que recibieron la dosis alta o baja de CSM-MOfuc. Dos pacientes presentaron acontecimientos adversos graves que precisaron ingreso hospitalario. La paciente nº 4 (dosis baja de CSM-MOfuc), presentó un dolor torácico atípico 20 meses después de la infusión. La paciente nº12 (dosis alta) presentó una sub-oclusión intestinal y cólico reno-ureteral 6 meses tras la infusión de CSM-MOfuc. Ninguno de los dos eventos adversos se consideró relacionado con el tratamiento. Los acontecimientos adversos no graves más frecuentes estuvieron relacionados con la biopsia y el harvest de médula ósea (dolor y hematomas). Sólo 1/10 pacientes presentó una nueva fractura en los 24 meses de seguimiento. 8/10 pacientes presentaron mejoría de al menos 1 punto en la escala de dolor EVA durante el seguimiento, alcanzándose los niveles más bajos en el primer mes. La media de puntuación en la escala EVA fue de 6,9±1,6 en la visita basal frente a 4,7±2,8 en la visita fin de estudio ($p<0,05$) donde se observa una estabilización del dolor. Se observó una mejoría de la capacidad funcional que pasó de limitación intensa (45%) a limitación moderada en el grupo completo, y mejora o estabilización de la calidad de vida percibida a los 24 meses respecto a la situación basal en 7/10 pacientes.
El análisis global de los resultados mediante esta histomorfometría evidencio un incremento de los valores medios de área de tejido óseo en la biopsia realizada a los 120 días postratamiento en 7/10 pacientes sin que este resultado se viese influenciado por la situación de partida.

Conclusión.
El uso de CSM-MOfuc autólogas para la osteoporosis establecida con fractura de bajo impacto es factible, seguro y potencialmente eficaz.

2. TREATMENT OF DEGENERATIVE DISC DISEASE WITH ALLOGENIC MESENCHYMAL STEM CELLS: LONG-TERM FOLLOW-UP RESULTS.

NODOS: RD16/0011/0003 - RD16/0011/0001

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Degenerative disc disease with low back pain is a public health problem with great economic and quality of life impact. Mesenchymal stromal cell (MSC) treatments have shown feasibility, safety, and strong indications of clinical efficacy 1 year after cell transplantation. Here, we report the results of the patient follow-up at (mean ±SE; n = 23) 3.5 ±0.1 years. No serious adverse effects were recorded during this extension period for either treatment or control group. The early pain and the Oswestry disability index improvements seen during the first year persisted 2.5 years later. The therapeutic efficiency of the MSC treatment was estimated from the pain relief and the disability improvement in the Huskisson plot, and was 0.28 at 1 year after the intervention. By 3.5 years, the therapeutic efficiencies increased to 0.60 (pain relief) and 0.71 (disability). The control patients did not show any significant healing at 3.5 years after intervention. Regarding the structural changes of the affected discs, we reported significant improvements with decreased Pfirrmann grade at 1 year which was maintained at 3.5.
Overall, these long-term data reaffirms MSCs as a valid alternative for treatment of degenerative disc disease because they can provide effective and durable pain relief together with objective improvements to the disc degeneration. Future studies are now needed to confirm these durable results in a large series of patients (e.g., the pan-European RESPINE trial), and to investigate upgrades to the MSC production protocol to make the generalization of this MSC therapy possible.

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Noriega DC, Ardura F, Hernandez-Ramajo R, et al. Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial. Transplantation. 2017; 101: 1945-1951.

3. ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF PATIENTS WITH SEVERE SARS-COV-2 PNEUMONIA REQUIRING MECHANICAL VENTILATION.

A PROOF OF CONCEPT STUDY BY TERCEL GROUPS.
NODOS: RD16/0011/0015 - RD16/0011/0005-RD16/0011/0013 - RD16/0011/0001 - RD16/0011/0002 - RD16/0011/0029-RD16/0011/0010

López-Parra M, García-Arranz M, Sánchez-Guijo F, Monedero P, Mata-Martínez C, Santos A Sagredo V, Álvarez-Avello JM, Guerrero JE, Pérez-Calvo C, Sánchez-Hernández MV, Del Pozo JL Andreu EJ; Fernández-Santos ME, Villarón EM, Soria-Juan B, Martínez S, Zapata A, Moraleda JM, Soria B, Fernández-Avilés F, García-Olmo D, Prósper F.

TerCel nodes involved:
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.

Background.
Identification of effective treatments in severe cases of COVID-19 requiring mechanical ventilation represents an unmet medical need. Our aim was to determine whether the administration of adipose-tissue derived mesenchymal stromal cells (AT-MSC) is safe and potentially useful in these patients.

Methods.
Thirteen COVID-19 adult patients under invasive mechanical ventilation who had received previous antiviral and/or anti-inflammatory treatments (including steroids, lopinavir/ritonavir, hydroxychloroquine and/or tocilizumab, among others) were treated with allogeneic AT-MSC. Ten patients received two doses, with the second dose administered a median of 3 days (interquartile range-IQR- 1 day) after the first one. Two patients received a single dose and another patient received 3 doses. Median number of cells per dose was 0.98 x 106 (IQR 0.50 x 106) AT-MSC/kg of recipient's body weight. Potential adverse effects related to cell infusion and clinical outcome were assessed. Additional parameters analyzed included changes in imaging, analytical and inflammatory parameters.

Findings.
First dose of AT-MSC was administered at a median of 7 days (IQR 12 days) after mechanical ventilation. No adverse events were related to cell therapy. With a median follow-up of 16 days (IQR 9 days) after the first dose, clinical improvement was observed in nine patients (70%). Seven patients were extubated and discharged from ICU while four patients remained intubated (two with an improvement in their ventilatory and radiological parameters and two in stable condition). Two patients died (one due to massive gastrointestinal bleeding unrelated to MSC therapy). Treatment with AT-MSC was followed by a decrease in inflammatory parameters (reduction in C-reactive protein, IL-6, ferritin, LDH and D-dimer) as well as an increase in lymphocytes, particularly in those patients with clinical improvement.

4. TWO PHASE I/II CLINICAL TRIALS FOR THE TREATMENT OF URINARY INCONTINENCE WITH AUTOLOGOUS MESENCHYMAL STEM CELLS.

NODOS: RD16/0011/0013-RD16/0011/0029

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Con el ensayo realizado nos propusimos evaluar la seguridad y viabilidad de células troncales mesenquimales autólogas (ASC) de origen adiposo para tratar la incontinencia urinaria endoscópicamente tras prostatectomía radical en varones o estrés urinario en mujeres. Diseñamos dos ensayos clínicos independientes fases I-IIa prospectivos, no aleatorizadas en los que participaron 9 hombres (8 tratados) y 10 mujeres para evaluar la viabilidad y seguridad de las ASC para este uso. Las ASC fueron obtenidas mediante liposucción (150 a 200 g de grasa) realizadas a cada paciente.

Después de 4 a 6 semanas y bajo sedación, las ASC se inyectaron mediante endoscópica intrauretral bajo sedación y posteriormente los pacientes fueron dados de alta. En cada visita (basal, 1, 3, 6 y 12 meses) se recogieron parámetros clínicos, muestras de sangre, urocultivo y se realizó una uroflujometría.En todas las visitas se realizó una medida de peso de compresa (pad-test) y se rellenaron test de calidad de vida (SF-12) y de incontinencia. Además, a cada paciente se le realizó una uretrocistoscopia y estudios urodinámicos en la visita pre-inclusión y en la última visita. El análisis estadístico se realizó mediante prueba Wilcoxon. Los resultados obtenidos a nivel seguridad fueron óptimos ya que no se han recopilado ningún efecto adverso. A nivel eficacia, aunque son pocos casos los resultados fueron alentadores. Tres hombres (37,5%) y cinco mujeres (50%) mostraron una mejora objetiva de> 50% ($p < 0,05$) y una mejora subjetiva del 70% al 80% desde el inicio.

En conclusión, la aplicación intrauretral de ASC es un procedimiento seguro y factible para tratar la incontinencia urinaria tras prostatectomía radical y la incontinencia urinaria de esfuerzo femenina. Estadísticamente se obtuvo una diferencia significativa para la mejora del pad-test en 3/8 hombres y en 5/10 mujeres. Nuestros resultados animan a realizar estudios para confirmar la seguridad y analizar la eficacia con un número de pacientes más alto.

Resultados publicados en: STEM CELLS Transl Med. 2020;1–9.

5. TRATAMIENTO DE LA ARTROSIS DE RODILLA MEDIANTE LA INYECCIÓN INTRAARTICULAR DE CÉLULAS ESTROMALES MESENQUIMALES: SITUACIÓN DEL ENSAYO ARTROCELL.

NODOS: RD16/0011/0015 - RD16/0011/0005 - RD16/0011/0003 - RD16/0011/0001- RD16/0011/0022 - RD16/0011/0012 - RD16/0011/0013 - RD16/0011/0029

Lamo-Espinosa JM, Blanco JF, Vega A, Crespo I, Combalia A, Medina-Quirós M, Montánez E, Fernández Gutiérrez B, Vaquero J, Calvo E, Andreu E, López-Parra M, Villarón EM, Fernández ME, García-Hernández AM, Perpiñá U, Canals J, Becerra J, Soler R, Orozco L, García-Arranz M, Moraleda JM, Prósper F, García-Sancho J, Sánchez-Guijo F.

EudraCT: 2019-002446-21

Nodos TerCel participantes:
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RD16/0011/0005 (Pamplona)
RD16/0011/0003 (Valladolid)
RD16/0011/0001 (Murcia)
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Introducción.

En grados avanzados de artrosis, el tratamiento definitivo es la prótesis total de rodilla. En las últimas dos décadas han sido numerosos los avances realizados por el conjunto de la red TerCel en el tratamiento de estos pacientes con el uso de células estromales mesenquimales derivadas de médula ósea (BM-MSC), con mucha experimentación básica y preclínica y varios ensayos fase 2 publicados con resultados favorables. Por ello, el siguiente paso por parte de TerCel ha sido el desarrollo de un ensayo clínico fase III, financiado en la convocatoria de investigación clínica independiente del ISCIII del año 2018 (PIC18/00001; inicio en 2019), cuyo diseño se describe a continuación.

Método/Diseño.

ARTROCELL es un ensayo clínico Fase III, multicéntrico, aleatorizado en pacientes afectos de artrosis de rodilla, cuyo objetivo principal es evaluar comparativamente la eficacia de las BM-MSC alogénicas y de las BM-MSC autólogas frente a un control activo con ácido hialurónico. El ensayo clínico incluirá a 120 pacientes entre 18 a 80 años, con grados de artrosis 2-4 de Kellgren-Lawrence, distribuidos en 3 grupos experimentales: Grupo 1 (n=40): Control activo con una inyección intraarticular de ácido hialurónico. Grupo 2 (n=40): Inyección intraarticular de 40 millones de BMMSC alogénicas. Grupo 3 (n=40): Inyección intraarticular de 40 millones de BMMSC autólogas. El seguimiento se realizará durante 12 meses, evaluando la evolución clínica mediante escalas de dolor (EVA); funcionales (WOMAC y Lequesne) y de calidad de vida (SF-12). De la misma forma se realizará seguimiento mediante pruebas de imagen para la valoración tanto del cartílago articular como de las estructuras de la rodilla que se encuentran implicadas en la fisiopatología de la artrosis mediante radiografía y secuencias de resonancia magnética específicas para cada una de ellas.

Situación actual.

El ensayo fue aprobado por el CEIm de referencia (Salamanca) el 17/02/2020 y por la AEMPS el 20/04/2020. Las validaciones de la producción celular se han retrasado por la pandemia pero ya se han retomado, y el master cell bank de las células alogénicas (Salamanca y Pamplona) ya tiene casi la mitad de las dosis necesarias para cubrir las necesidades del ensayo. La fecha estimada de apertura de centros es noviembre de 2020, la de inicio del reclutamiento es diciembre de 2020.

6. TRANSPOCART PROJECT: SLEEPING BEAUTY CD19 CART CELLS FOR R/R ALL

NODOS: RD16/0011/0001 - RD16/0011/0005 - RD16/0011/0011 - RD16/0011/0015

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In the last decade, CART therapies have shown very impressive results, especially in CD19+ malignances like relapsed/refractory (R/R) acute lymphoid leukemia (ALL) and Non-Hodgkin lymphoma (NHL). Of note, most CART therapies tested nowadays rely on genetic T cell engineering with integrating viral vectors, that although effective, have several limitations which become in some cases insurmountable, such are risk of malignant transformation and prohibitive costs.

The TranspoCART project has been conceived to bring a highly innovative cell product to the clinic by including truly cutting-edge technology into the CART cell approach. In particular through i) novel modular multifunctional CAR designs for enhanced safety and efficacy, ii) revolutionary virus-free gene-transfer strategy of the CAR using Sleeping Beauty transposition, and iii) highly innovative methods through next-generation sequencing and flow cytometry to evaluate the antitumoral response at unprecedented level of resolution. Altogether these innovations will enable TranpoCART to achieve its ambitious goals: i) to obtain clinical proof-of-concept of the efficacy of transposon-based CD19-specific CART cells (TranspoCART19 cells) in R/R ALL, as well as ii) to substantially reduce production costs and manufacturing time compared to conventional CART products.

To implement the innovative Sleeping Beauty technology for TranspoCART19 cell production we have generated a 4-1BB CAR construct targeting CD19 with a truncated EGFR as a safety switch. During this first year of TranspoCART project we have optimized the transduction of T cells from healthy donors by using plasmid minicircles and mRNA for the delivery of the transposon vector containing the CAR and the transposase (SB100X) respectively. Our optimized protocol reached transfection efficacies of up to 45-55% of CAR+cells, compatible with clinical applications. Moreover, TranspoCART19 cells showed high expansion rates (rounding 30-50x), and were extensively characterize in vitro at the end of the production, showing an enriched stem-cell memory/central memory phenotype, with no signs of cell exhaustion, and high level of specific cytotoxicity activity and cytokine production (IFNg, IL2 and TNFa) against CD19+ cells. Finally, in vivo antitumoral efficacy of TranspoCART19 cells was evaluated in NALM6 xenograft models using immunodeficient NSG mice. Treated animals showed a statistically improved survival indicating proper antitumoral efficacy of TranspoCART19 cells. Currently, we are optimizing T cell transfection from ALL patient samples and large-scale cell expansion in order to move forward and validate TranspoCART19 cell production to GMP conditions.

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7. ENSAYO CLÍNICO DE LA ADMINISTRACIÓN INTRATECAL DE CÉLULAS MADRE MESENQUIMALES DE GELATINA DE WHARTON EXPANDIDAS EN PACIENTES CON LESIÓN MEDULAR COMPLETA DE MÁS DE UN AÑO DE EVOLUCIÓN.

NODOS: RD16/0011/0036 - RD16/0011/0014 - RD16/0011/0028

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La lesión de la médula espinal (LM), ya sea de origen médico como traumático causa, en la mayoría de los casos, una parálisis severa y permanente de las extremidades, ausencia total o parcial de la sensibilidad, falta de control de esfínteres, trastornos en la esfera sexual, alteraciones del sistema nervioso autónomo y el riesgo de graves complicaciones de por vida, siendo causa de una discapacidad grave, permanente e irreversible.

Hoy en día no existe un tratamiento eficaz para la LM, pero se han diseñado varias estrategias terapéuticas dirigidas a minimizar las graves secuelas que se producen, incluyendo terapias celulares, farmacológicas y neurorehabilitadoras. Si bien muchas de los tratamientos farmacológicos con potencial neuroprotector y agentes pro-regenerativos han sido ensayados en modelos animales, hoy en día, el uso clínico de cualquiera de estos fármacos no ha mostrado efectos favorables. Los trasplantes de células madre se consideran posibles terapias emergentes para estimular los procesos neuroplásticos y regenerativos en este tipo de lesiones. Presentamos los resultados de un ensayo clínico que investigó los efectos de seguridad y recuperación clínica de la infusión intratecal de células madre mesenquimales de gelatina de Wharton expandidas (WJ-MSC) en pacientes con LM traumática completa de más de un año de evolución.

Se diseñó un ensayo clínico de fase I / IIa, aleatorizado, doble ciego, cruzado, controlado con placebo (NCT03003364). Se reclutaron 10 pacientes (7 hombres, 3 mujeres, rango de edad: (25-47 años) con una LM crónica completa (AIS-A) a nivel dorsal (T3-T11). Todos los pacientes fueron asignados al azar para recibir MSC en infusión intratecal de cordón umbilical humano expandido ex vivo) o placebo y cambiaron al otro brazo a los 6 meses. (figura 1)

Evaluación clínica (puntuación AIS motor y sensorial, espasticidad, dolor neuropático y percepción eléctrica y umbrales de dolor) (figura 2), potenciales evocados sensoriales y motores de miembros inferiores (MEP y SSEP), medida de independencia de la médula espinal (SCIM) y calidad de vida (WHOQOL-BREF) fueron evaluados al inicio del estudio, 1, 3 y 6 meses después de cada intervención. Se realizaron estudios urodinámicos y calidad de vida específica urinaria (cuestionario Qualiveen), así como manometría anorrectal, evaluación funcional de la disfunción intestinal (cuestionario diagnóstico Roma III) y gravedad de la incontinencia fecal (puntuación de Wexner) al inicio del estudio y a los 6 meses después de cada intervención.

Resultado.
El trasplante intratecal de WJ-MSC se consideró seguro, sin efectos secundarios significativos. Después de la infusión de MSC, encontramos una mejora significativa de la sensibilidad táctil superficial, y una reducción significativa de la intensidad del dolor neuropático, en comparación con el placebo. Otros efectos clínicamente relevantes, como un aumento de la capacidad máxima y elasticidad de la vejiga y una disminución de la hiperactividad neurogénica de la vejiga y la disinergia del esfínter externo, solo se observaron a nivel individual. No se observaron cambios en la función motora, espasticidad, MEP, SSEP, función intestinal, calidad de vida o medidas de independencia.

Conclusión.
El trasplante intratecal de WJ-MSC derivadas del cordón umbilical humano es una intervención segura. La infusión intratecal de una dosis única de WJ-MSC en pacientes con una LM completa de origen traumático en fase crónica puede inducir algún efecto neuroregenerativo, limitado a ligeras mejoras sensoriales, que podrían estar determinadas por la gravedad y cronicidad de la lesión espinal, infusión tardía y baja concentración de células WJ-MSC. Este proyecto de colaboración entre tres grupos de la Red TERCEL: Banc de Sang i Teixits (BST), Universitat Autònoma de Barcelona (UAB) e Institut Guttmann (IG) se encuadra en los objetivos iniciales de la Red, en que se priorizaba la realización de un ensayo clínico con humanos en el modelo de LM y ha recibido financiación de la Fundació Marató de TV3, del ISCiii y fondos propios del BST. El proyecto global incluye la realización de un estudio in vitro (BST), un estudio preclínico en modelo animal (UAB) y, finalmente, un ensayo clínico en pacientes con LM traumática crónica (IG). La fuente celular utilizada fueron las células mesenquimales expandidas de gelatina de Wharton (MSC,WJ) aprovechando la plataforma de criopreservación de células madre generada a partir del programa CONCORDIA de donación de sangre de cordón umbilical. El estudio pre-clínico en modelo de LM en rata se realizó con el fin de comprobar el comportamiento de las células madre, siguiendo un protocolo similar al del estudio clínico y demostrando la seguridad de la administración intratecal de las células, sin que se observaran reacciones adversas y resultando en cierta mejoría funcional y mayor preservación del tejido medular comparado con el grupo control.

IV. TERCEL ABSTRACTS

Compilation 2020

1. ENGINEERED SMALL EXTRACELLULAR VESICLES FROM MESENCHYMAL STROMAL CELLS PRESENT AN ENHANCED ANTIFIBROTIC EFFECT INVENTRICULAR CARDIAC FIBROBLASTS IN VITRO.

NODO: RD16/0011/0004

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Mesenchymal stromal cells (MSC) are multipotent cells that raised the scientific community interest to use them for cell therapy and tissue regeneration applications. Nevertheless, increasing scientific evidence has shown that beneficial effects initially attributed to MSC are an effect of their secretome. Among the paracrine factors that MSC secrete, extracellular vesicles (EV), and more specifically exosomes, have gained special attention during the last years. Exosomes are a type of small EV (sEV) formed by a lipid bilayer ranging between 30 and 150 nm whose composition is rich growth factors, lipids, cytoplasmic and membrane proteins and different types of RNA. The use of SEV for clinic purposes instead of cells would be of especial interest in terms of biosecurity, since they are not able to replicate and have a minimal risk to enhance tumorigenesis in vivo. In addition to this, engineering tools are being developed to improve native characteristics of sEV and enhance their therapeutic potential. In this work, we have focused on the specific anchoring of a protein with anti-fibrotic potential (from now on called ProtX) to the surface of MSC-derived sEV to test if the specific incorporation of this protein to sEV is able to enhance MSC-derived sEV native potential against pathological fibrotic processes.

With this objective, MSC were genetically modified with two lentiviral vectors to obtain new stable cell lines, one expressing the candidate sEV anchoring protein (XStamp) and GFP as a reporter and the second one expressing the ProtX fused to XStamp and GFP. The resultant cell lines were called MSC-XStamp and MSC-ProtX-XStamp, and native MSC were used as a control. The obtained MSC-based cell lines were expanded and sEV from the three different cell lines were isolated by serial steps of ultrafiltration and ultracentrifugation and characterized by nanoparticle tracking analysis (NTA), electronic microscopy and Western Blot (WB). NTA and electronic microscopy results showed that our samples ranged the expected size for sEV (between 50 and 180 nm). Additionally, the presence of sEV markers (such as CD81, CD63, TSG101 and Alix) along with the absence of markers of cell contamination (Calnexin) was corroborated by WB analysis. In addition, the presence of the desired fusion protein (ProtX-XStamp) in MSC-ProtX-XStamp cells and SEV samples derived from those cells was verified by WB. Functional assays were performed with the engineered sEV to test their biological effect and compared with native MSC-derived sEV samples. In this context, tube formation assays in human coronary microvascular endothelial cells and proliferation test in ventricular cardiac fibroblasts showed an anti-fibrotic effect of sEV derived from MSC-ProtX-XStamp. Future in vivo studies using a model of aortic constriction in mice will reveal if engineered sEV can enhance native sEV potential in vivo and be a useful tool in the field of cardiac repair.

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2. C-MYC, NUEVO CANDIDATO PARA MODULAR LA REPARACIÓN MUSCULAR.

NODOS: RD16/0011/0005 - RD16/0011/0019

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Las células satélite, células madre adultas de músculo esquelético, presentan una expresión heterogénea en los niveles endógenos de c-Myc. Células satélite con niveles bajos de c-Myc poseen una capacidad regenerativa a corto plazo, mientras que aquellas que expresan niveles altos de c-Myc presentan un alto potencial regenerativo a largo plazo. c-Myc participa en las divisiones simétricas/asimétricas de las células satélite que determinan su diferenciación miogénica, produciendo nuevas fibras musculares, o su regreso a un estado quiescente, renovando el pool de células satélite. La eliminación específica de una o dos copias de c-Myc en las células satélite disminuye notablemente su función tras inducir daños musculares consecutivos. Si niveles endógenos de c-Myc determinan el potencial de las células madre musculares, estableceremos una conexión entre inducción de quiescencia, stemness y reparación muscular. Esto permitirá desbloquear la terapia celular regenerativa en músculo esquelético, mejorando el potencial regenerativo a largo plazo de las células donantes terapéuticas.

3. BMP2 OVEREXPRESSION EFFECTS OVER APPENDICULAR SKELETON DEVELOPMENT.

NODOS: RD16/0011/0005 - RD16/0011/0021

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Background.
The development of the vertebrate skeleton is a complex biological event where diverse highly coordinated processes take place. Bone morphogenetic proteins (BMPs), multifunctional growth factors which belong to the TGF-b superfamily of proteins, have been reported to play pivotal roles in the skeletal morphogenesis signalling network. Among the BMP family, BMP-2 has important roles in joint development, endochondral bone formation and bone maintenance and repair. Bmp2 expression during early stages of fracture healing is compulsory for starting the reparative process.

Objective.
Here we evaluate the effects of the overexpression of Bmp2 over osteochondral development.

Methods.
Transgenic mice conditionally overexpressing Bmp2 (Rosa26-Bmp2GOF) were crossed with transgenic mice expressing Cre recombinase under the control of Prrx1 promoter (Prrx1-Cre). With this strategy, mice offspring (Bmp2GOF) would overexpress Bmp2 in their limb buds, calvaria and sternum. Appendicular skeleton structure was analyzed employing micro CT, histology and immunofluorescence.

Results.
Bmp2 overexpression deeply affect appendicular skeleton development, generating severe deformity in the appendicular skeleton of mutant mice. Bmp2GOF mice suffer the fusion of radius and ulna, as well as tibia and fibula, the last is also bended and results in hind limb major deformity. Bmp2 overexpression also modifies cartilage differentiation, delaying chondrocyte hypertrophy and endochondral ossification of long bones.

4. TOWARDS BIOLOGICALLY-INSPIRED AND COMPUTATIONALLY-DESIGNED HUMAN CARDIAC ENGINEERED TISSUES USING MELT ELECTROWRITING AND HIPSCS.

NODOS: RD16/0011/0005 - RD16/0011/0029

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Introduction.
Successful biomimetism requires exquisite design of engineered tissues. Fibre-reinforcement of hydrogels is able to overcome their inherent mechanical weakness, but introduces cues, affecting the functional outcome, especially in high-anisotropy tissues as the myocardium. Here, we generate human cardiac tissues, and apply computational modelling to optimize fibre reinforcement for the desired functionality.

Methods.
Uniaxial and biaxial mechanical tests were employed. hiPSC-cardiomyocytes (CMs) were embedded in matrigel, casted on MEW-PCL fibres and cultured for 28 days. Gene-expression, structure (IF) and functionality (optical mapping) were assessed and compared with conventionally-cultured hiPSC-CMs in 2D. This information was used to generate computational electrophysiological models to investigate the impact of the MEW-PCL fibres on the activation pattern of hiPSC-CMs.

Results.
Fibre mechanics dominated the properties of the composites. hiPSC-CMs formed microtissues and were able to survive and contract for 4 weeks. Gene expression highlighted an increased maturity, with fibres aligning CMs, which redounded in faster conduction velocities. In silico simulations revealed a high impact of the composite's pore size on the velocity of electrical propagation. Maximum local activation time (LAT) was 51 ms and increased up to 71 ms for pore sizes of 100 μm and 700 μm. Simulation of 2D-cultured hiPSC-CMs led to maximum LAT values of 350 ms due to the loose coupling of the cardiomyocytes.

Conclusion.
Composite systems matured, at structural, gene-expression and functional levels. Simulations provided insight on the impact of the MEW-fibres on the activation pattern of hiPSC-CMs, and open the way for the fabrication of tailored human cardiac tissues.

5. REPROGRAMMING CARDIAC MYOFIBROBLASTS INTO CARDIOMYOCYTE-LIKE CELLS BY BAF60C OVEREXPRESSION AFTER MYOCARDIAL INFARCTION.

NODO: RD16/0011/0006

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Background.
The alleviation of cardiac muscle after myocardial infarction (MI) is an outstanding clinical concern. In this context, reprogramming of cardiac myofibroblasts directly into newly functional cardiomyocytes could be a valuable therapeutic strategy. Baf60c is a protein subunit implicated in heart development and its overexpression in cultured embryonic stem cells can mediate interactions between cardiac transcription factors.

Hypothesis.
The overexpression of Baf60c gene in cardiac myofibroblasts could promote its differentiation into cardiomyocyte-like cells.

Methods and Results.
We first cultured explants from mouse MI scars and characterized the cells. Primary culture analysis by flow cytometry revealed a high percentage of myofibroblasts (90% CD90+CD44+CD31-CD144- cells) and poor levels of smooth muscle cells (3% CD144+ cells) and endothelial cells (0.3% CD31+ cells). This was confirmed by elevated levels of two myofibroblasts markers, SMA and vimentin by qPCR and immunofluorescence. Moreover, gene and protein evaluation showed expression of MEF2, desmin, GATA4, collagen III, MyoD and Serca2 in cardiac myofibroblasts. We then selected from our primary cultures the CD90+CD44+CD31-CD144- cells by cell sorting, and we analysed if cell passaging and manipulation altered their integrity in terms of SMA expression and structure. Comparison of sorted and non-sorted cells demonstrated that 96% of sorted cells were positive for SMA, but only 8% was functional. In non-sorted cells, 93% were SMA+ and 46% of them presented a well-structured protein. We finally transduced non-sorted myofibroblasts with retrovirus bearing Baf60c gene. Gene expression analysis revealed that Baf60c induced and increased expression of cardiac genes such as GATA4, Nkx2.5, Tbx5, TnI, Cx43 and Serca2.

Conclusions.
Our results demonstrate that 1) increasing passages and cell manipulation altered myofibroblasts culture integrity and 2) Baf60c is able to potentiate a cardiovascular lineage-specific gene expression programme in mice explanted myofibroblasts. Further experimentation is required to reach fully functional cardiomyocytes after in vivo Baf60c-mediated reprogramming.

6. MODELING LEFT VENTRICULAR NON COMPACTION (LVNC) IN VITRO.

NODOS: RD16/0011/0021 - RD16/0011/0024

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Left Ventricular non Compaction (LVNC) is a cardiomyopathy characterized by prominent trabeculae into the lumen of the ventricles and reduced ventricular walls thickness. We previously identified two-point mutations in the MIND BOMB1 gene (MIB1), encoding a type 3-ubiquitin ligase of the NOTCH pathway. The MIB1V943F and MIB1R530X mutations cause LVNC with autosomal dominant inheritance. Conditional inactivation of Mib1 in the mouse produces LVNC. In order to understand the mechanisms underlying ventricular development and maturation, in vitro we generated human induced pluripotent stem cells (hiPSC) from skin fibroblast of MIB1V943F and MIB1R530X LVNC patients. Using CRISPR/Cas9 genomic edition we have reverted these mutations obtaining hiPSC isogenic controls. These hiPSC lines have been differentiated in vitro into cardiomyocytes. MIB1V943F and MIB1R530X hiPSC-derived cardiomyocytes (hiPSC-CM) showed higher proliferation, lower percentage of multinucleation, and predominant nuclear versus cytosolic cardiac Troponin I (cTNI) compared to wild type hiPSC-CM, indicating that mutant and edited hiPSC-CM remain immature. RNA from MIB1V943F and MIB1R530X hiPSC lines has been collected at different time points of cardiac differentiation to assess a global gene expression analysis that would help us to understand the mechanism by which MIB1 affects the maturation of these cardiomyocytes.

7. TRABECULATED MYOCARDIUM IN HYPERTROPHIC CARDIOMYOPATHY: CLINICAL CONSEQUENCES.

NODOS: RD16/0011/0021 - RD16/0011/0024

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Aims: Hypertrophic cardiomyopathy (HCM) is often accompanied by increased trabeculated myocardium (TM)—which clinical relevance is unknown. We aim to measure the left ventricular (LV) mass and proportion of trabeculation in an HCM population and to analyze its clinical implication. Methods and Results: We evaluated 211 patients with HCM (mean age 47.8 ± 16.3 years, 73.0% males) with cardiac magnetic resonance (CMR) studies. LV trabecular and compacted mass were measured using dedicated software for automatic delineation of borders. Mean compacted myocardium (CM) was 160.0 ± 62.0 g and trabecular myocardium (TM) 55.5 ± 18.7 g. The percentage of trabeculated myocardium (TM%) was 26.7% ± 6.4%. Females had significantly increased TM% compared to males (29.7 ± 7.2 vs. 25.6 ± 5.8, $p< 0.0001$). Patients with LVEF < 50% had significantly higher values of TM% (30.2% ± 6.0% vs. 26.6% ± 6.4%, $p = 0.02$). Multivariable analysis showed that female gender and neutral pattern of hypertrophy were directly associated with TM%, while dynamic obstruction, maximal wall thickness and LVEF% were inversely associated with TM%. There was no association between TM% with arterial hypertension, physical activity, or symptoms. Atrial fibrillation and severity of hypertrophy were the only variables associated with cardiovascular death. Multivariable analysis failed to demonstrate any correlation between TM% and arrhythmias. Conclusions: Approximately 25% of myocardium appears non-compacted and can automatically be measured in HCM series. Proportion of non-compacted myocardium is increased in female, non-obstructives, and in those with lower contractility. The amount of trabeculation might help to identify HCM patients prone to systolic heart failure.

8. ASSESSING THE LANDSCAPE OF LNCRNAS DYNAMICS IN CARDIAC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS.

NODOS: RD16/0011/0027 - RD16/0011/0029 - RD16/0011/0005

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In recent years, it has been revealed that majority of the genome is transcribed into RNAs that do not encode proteins. Increasing evidence suggests that non-coding RNAs, especially long non-coding RNAs (lncRNAs), are emerging as important regulators of gene expression and other cellular processes, such as developmental pathways. Advances in developmental cardiology have increased our understanding of the early aspects of heart differentiation, but the role of lncRNAs in human cardiac differentiation remains elusive. One of the most important goals is to understand if they can play a main role in the processes that give rise to mature and functional cardiomyocytes. To assess this, hPSCs were differentiated into cardiomyocytes using a previously established protocol based on a stage specific activation and suppression of the canonical Wnt signaling pathway. We collected total RNA from five important developmental stages, ranging from early embryonic to cardiomyocyte differentiating cells, to construct transcriptomes for both coding and noncoding genes based on high-throughput sequencing datasets.

We identified 5,726 differentially expressed RNAs that significantlymchange during differentiation: 4,881 corresponed to protein coding RNAs and 845 are non-coding RNAs. Among the non-coding RNAs, we identified 519 long non-coding (lncRNAs), 177 pseudogenes, 17 small nuclear RNAs (snRNAs), 19 miRNAs, 9 RNAs that cannot be defined by other RNA keys (miscRNAs), 55 processed transcripts and 49 RNAs to be experimentally confirmed (TEC). Using the lncRNA classification based on their location in the genome, we identified 21 sense RNAs (overlapping a protein-coding gene), 172 antisense, 1 bidirectional promoter (transcribed within 1Kb of promoters antisense to the protein coding transcript), 283 intergenic (between two protein coding genes) and 42 intronic RNAs (transcribed from an enhancer region of a protein-coding gene). We further investigated the role of non-coding genes which expression constantly increases during differentiation or which expression is very high at the final time points. We identified 70 non-coding RNAs with these characteristics. We reasoned that most of them could sustain the late stage of cardiac differentiation and could be responsible for cardiac precursor maturation to give rise to functional cardiomyocytes. We started checking the expression of these 70 non-coding genes within 51 human tissues analysed by the GTEx consortium, and decided to focus on 15 non-coding RNAs with at least two-fold higher expression level in the heart as compared to the average expression value of all 51 analysed tissues. Ten out of these 15non-coding RNAs were lncRNAs (4 antisense, 4 linc, 2 sense intronic and 1 processed transcript) while five were pseudogenes.

9. STUDY ON THE OF THE INTERPLAY BETWEEN GLUCOSE METABOLISM AND SARS-COV-2 INFECTION IN KIDNEY ORGANOIDS.

NODOS: RD16/0011/0027 - RD16/0011/0005

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Severe acute respiratory syndrome 2 (SARS-CoV-2) infection leads to a high risk of hospitalization and mortality in diabetic patients. SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in key metabolic organs such as pancreas, muscle, heart, adipose tissue, the small intestine, and the kidneys. As a result, it is likely that SARS-CoV-2 may cause alterations of glucose metabolism that could complicate the pathophysiology of preexisting diabetes or lead to new mechanisms of disease.

We have previously showed that SARS-CoV-2 can directly infect engineered human blood vessel organoids and human kidney organoids. Here, we infected human kidney organoids cultured under hyperglycaemic condition. We found that kidney organoids under hyperglycaemic condition have higher expression of ACE2 by western blot, Immunofluorescence and RT-PCR. Furthermore, viral loads were determined by qRT-PCR showing higher expression in kidney organoids under hyperglycaemic condition. Together, our results provide first evidence that SARS-CoV-2 infection altered glucose metabolism and support the use of kidney organoids as a platform to investigate the cellular susceptibility, disease mechanisms, and treatment strategies for SARS-CoV-2 infection in hyperglycaemic condition.

10. LA DELECIÓN CONDICIONAL DEL GEN SUPRESOR DEL TUMOR DE WILMS EN EL LINAJE TROPONINA-T CARDIACA PROVOCA DEFECTOS ESTRUCTURALES EN EL MIOCARDIO ADULTO.

NODO: RD16/0011/0030

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El gen supresor del tumor de Wilms (Wt1) codifica un factor de transcripción del tipo "dedos de zinc" que además de su función transcripcional interviene también en procesamiento del mRNA y en interacciones proteína-proteína. Este factor desempeña una función esencial en el desarrollo de muchos órganos (riñones, gónadas, bazo, corazón, adrenales...), y recientemente se ha revelado su importante papel en la homeostasis y reparación de tejidos adultos. Durante el desarrollo cardiaco, la proteína WT1 se expresa fundamentalmente en el epicardio, y esta expresión es crucial para la formación del mesénquima derivado del epicardio y el correcto desarrollo de las arterias coronarias. La falta de función de WT1 en el epicardio murino provoca la muerte de los embriones hacia la mitad de la gestación.

Hemos obtenido evidencias de la expresión de WT1 en una parte del miocardio embrionario y adulto, y hemos realizado experimentos de deleción condicional de este gen en cardiomiocitos utilizando el sistema Cre-Lox en ratones WT1flox/flox. Realizamos la deleción sistémica de WT1 en cardiomiocitos usando el driver Tnnt2Cre (Troponina T cardiaca) y para la deleción adulta utilizamos el modelo aMHCMerCreMer.

Los embriones de ratón con falta de función de WT1 en cardiomiocitos mostraron importantes anomalías en el miocardio (discontinuidades de las paredes libres, defectos del septo interventricular, compactación ventricular deficiente), aunque estas anomalías no implicaron letalidad embrionaria significativa ni afectaron al sistema coronario. Sin embargo, la frecuencia de ratones adultos mutantes se redujo del 25% esperado al 14%. Estos ratones adultos presentaron defectos estructurales en las paredes cardiacas, incluyendo divertículos y aneurismas ventriculares, hipertrabeculación y áreas fibróticas.

La deleción adulta de WT1 en cardiomiocitos inducida por tamoxifeno en el modelo aMHCMerCreMer no reveló defectos significativos, aunque nuestro siguiente objetivo es comprobar si la falta de función inducida de WT1 puede afectar a procesos reparativos en el miocardio.

En resumen, el papel desempeñado por WT1 en el desarrollo cardiaco no parece estar restringido a su función epicárdica, y una parte de los defectos atribuidos hasta ahora a la falta de función de WT1 en el epicardio embrionario podría estar relacionada con funciones poco conocidas desempeñadas por este factor en cardiomiocitos.

11. PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM NORMOXIC AND HYPOXIC EPICARDIAL CELL LINE CULTURES.

NODO: RD16/0011/0030

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The epicardium has an essential role in the biology of the heart, from cardiac development to adulthood. Deficient epicardial transcriptional and signaling activity can compromise the development of coronary vasculature and the proliferation of cardiomyocytes. Besides its importance as a signaling center during cardiac embryogenesis, the epicardium, that becomes dormant during adult life, is reactivated after cardiac injury. Among the numerous agents that take part in relevant cell signaling routes, extracellular vesicles (EVs) hold critical roles for the cell to perceive and respond to surrounding stimuli. Indeed, EVs function as cell-to-cell message carriers. These vesicles, whose contents (DNA, miRNAs, mRNAs and diverse proteins) are thought to be an indicator of the physiological status of the cell they derive from, provide instructive information to the surrounding cells on relevant changes in their environment. Accordingly, EVs are regarded as potential markers of organ disease. It has been shown that cardiac fibroblasts involved in post-MI ventricular remodeling are derived from the embryonic epicardium, and that they are specifically activated (proliferation, migration, collagen synthesis) upon ischemic heart damage to compensate for cardiomyocyte loss. Our work focuses in isolation and characterization of EVs from EPIC, an epicardial cell line immortalized in our laboratory from embryonic epicardial cells. In this study, EPIC cells are cultured under normoxic and hypoxic conditions and exosomes isolated from the culture. First, we have characterized EPIC-derived EVs from normoxic and hypoxic cultures by TEM, Western Blot and NTA. EPIC EVs have a size range closely related to exosomes (30-150 nm) and contain what are currently considered as exosomal markers such as CD63, ALIX and TSG101 in both culture conditions. Then, we have compared their cargo using multiplex proteomics. EVs isolated from hypoxic conditions are enriched in proteins related to glycolysis. We have validated these results by assessing proliferation and metabolic assays in HUVECs. In conclusion, EPIC EVs seem to significantly induce HUVECs proliferation. Moreover, we show that EPIC EVs modify the metabolic activity in endothelial cells, independently of the experimental condition. These findings suggest that epicardial-derived promote vascular growth in conditions associated to tissue damage. These results may allow us to improve our knowledge on the role played by epicardial derived agents in the context of hypoxic stress-induced cardiac damage.

12. EVALUATION OF THE BMI1-CPC REGULATION MECHANISMS. THE CARDIAC PROGENITOR-ENDOTHELIUM NICHE RELATIONSHIP.

NODO: RD16/0011/0037

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Bmi1+ cardiac progenitor cells (Bmi1-CPC) is a population of mice cardiac progenitors which has been confirmed to actively participate in the renewal of all cardiac cell lineages, showing a preference for the endothelial lineage (Valiente-Alandi et al, 2015). This progenitor population seems to have a close relationship with cardiac endothelium that generates low-ROS areas in their proximity, where Bmi1-CPC are confined in a low proliferative state, during homeostasis (Herrero et al, 2019). Nonetheless, Bmi1-CPC actively respond to cardiac damage augmenting their proliferation and increasing their differentiation rate, participating directly in the recovery after acute myocardial infarct (AMI) (Herrero et al, 2018).

To elucidate the epigenetic regulation of the Bmi1-CPC population, RNA sequencing studies were performed in different homeostatic and AMI conditions (Valiente-Alandi et al, 2016). These results revealed genes that were highly upregulated in the Bmi1-CPC population, although significantly reduced after the cardiac damage. Two promising candidates were the T-box family member Tbx3, implicated in self-renewal and differentiation (Lu R et al, 2011 & Zhang W et al, 2019) and Mbd3, a NurD subunit involved in the maintenance of the cellular pluripotency and proliferation (Kaji et al. 2006 & Mor et al. 2018). Together, these genes could be epigenetically regulating the Bmi1-CPC population and their response to a severe cardiac damage.

Besides their intrinsic mechanisms, the Bmi1-CPC shows a niche-like interaction with cardiac endothelium which seems crucial for their regulation. For example, this interaction modulates the proliferation status of this progenitor population: the Bmi1-CPC are maintained in a low proliferative state close to the low-ROS areas generated by cardiac endothelium (Herrero et al, 2019). We also suggest this regulation seems to be mediated by direct contact between Bmi1-CPC and the endothelium. A similar niche-like relationship between specific tissue progenitor/stem cells and the surrounding endothelium has been described in other organs and is crucial for controlling their proliferation ratio and maintain their progenitor capacity (Verma et al, 2018). In this work, we propose several intercellular signaling molecules which could be involved in this cell-to-cell regulation of the Bmi1-CPC proliferation.

13. MANUFACTURING OF NKG2D CAR T CELLS FOR ALLOGENEIC APPLICATION USING CRISPR/CAS9 TECHNOLOGY.

NODO: RD16/0011/0003

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Cell therapy based in Chimeric Antigen Receptor (CAR) T cells represents a milestone in the treatment of cancer. However, the broad use of this therapy is limited mainly because it is an autologous cell product that requires to be individually manufactured for each patient and tumor type. Therefore, in order to allow scalability of the process, it is urgently needed to develop new strategies directed to manufacture CAR T cells useful for allogeneic application and that target different cancer types.

To that aim, we designed a procedure that combines CRISPR/Cas9-based gene-editing to simultaneously eliminate TCR and HLA-I expression (key players of graft-versus-host disease and therapy rejection, respectively) with the integration of NKG2D-CAR, which recognizes eight different ligands. NKG2D ligands are absent or rarely expressed in normal tissue but induced in different cancer types. Since several of these ligands are simultaneously expressed in many hematologic and solid tumors, NKG2D-CAR T cells are less prone to develop cell therapy resistance and allow the treatment of a broad range of cancers. For allogeneic NKG2D-CAR T cell production, we firstly isolated T cells from peripheral blood of healthy donors. Subsequently, we carried out the disruption of TRAC and B2M genes by using Cas9 ribonucleoprotein and specific guide RNAs in a multiplex manner. NKG2D-CAR expression was induced by lentiviral transduction. Finally, TCR, HLA-I and NKG2D-CAR expressions were determined by flow cytometry.

By using this CRISPR/Cas9-based procedure, we obtained a cell product containing over 60% NKG2D-CAR+ cells from which around 2/3 of the cells lack both TCR and HLA-I complexes, thus being potentially useful for allogeneic treatment of both hematologic and solid tumors. Further work is ongoing to study the potential utility of these cells as universal CAR T cell therapy.

14. CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MICROGLIA FROM A FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS PATIENT.

NODO: RD16/0011/0014

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by motor neuron degeneration, whose cause remains unknown in 95% of ALS patients. The most common ALS mouse model, which expresses mutant G93A superoxide dismutase 1 (SOD1G93A) gene, have provided important insights about the detrimental microglial role in its pathogenesis. However, rodent mouse models have strong limitations since they represent a small proportion of ALS cases. Moreover, mouse microglial transcriptome is divergent compared to the human, being an important obstacle for the study of microglia. The development of humanized mouse models with human microglia have become a promising tool to understand better the role of ALS-associated risk genes in microglial cells, since engrafted human microglia in the mouse CNS mimic more closely the primary human microglia transcriptome rather than cultured microglial cells.

In the present study, we characterized in vitro human induced pluripotent stem cells (iPSCs)-derived microglia from a familial ALS patient, carrier for the SOD1I114T mutation, as preliminary data to further develop a humanized ALS microglia mouse model. The obtained microglial cells expressed the main microglial markers (CD45low, CD11b, Iba1, P2RY12), exhibited a ramified morphology and responded to a proinflammatory stimulus. Additionally, human SOD1I114T iPSC-derived microglia released neurotoxic factors able to induce motor neuron degeneration in the spinal cord of C57Bl/6 mice. Finally, transplantation of human SOD1I114T iPSC-derived macrophage precursors differentiate into microglia in the mouse spinal cord, being a starting point to further optimize our chimeric mice with human ALS microglia.

15. COMBINED CELL THERAPY, INCLUDING INDUCED NEURAL STEM CELLS AND MESENCHYMAL STEM CELLS, WITH PA-CURCIMIN FOR THE TREATMENT OF TRAUMATIC SPINAL CORD INJURY.

NODO: RD16/0011/0014

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Spinal cord injuries (SCI) lead to a devastating loss of the neurological function that lasts throughout the patient's life, since there are no effective treatments available. Given the social, psychological and economic impact of SCI, the search for new therapies brought cell therapy into the spotlight, and it has become a promising field of translational research [1]. Current induced pluripotent stem cell (iPSC) technology allows the generation of genetically stable and high-quality human neural stem cells (iNSCs) providing the therapeutic potential of autologous transplants [2]. Furthermore, in preclinical studies human mesenchymal stem cells (MSCs) have shown anti-inflammatory, anti-apoptotic, immunomodulatory, angiogenic effects and substantial recovery of the motor function after SCI [3, 4]. On the other hand, Curcumin, a natural component of turmeric (Curcuma longa), has shown neuroprotective, antioxidant and anti-inflammatory effects in the treatment of SCI [5-7]. In the present work we have developed a combinatorial cell and pharmacological therapy for the treatment of SCI seeking the synergy between the anti-inflammatory effects of MSCs and Curcumin –in a conjugated form with a poly-acetyl polymeric chain (PA) for sustained release- and the regenerative potential of iNSCs. Briefly, animals were subjected to SCI by a moderate contusion (200 kdyn) and randomly divided into 6 groups: (1) Control, (2) MSCs, (3) iNSCs, (4) MSCs + iNSCs, (5) PA-Curcumin and (6) iNSCs + MSCs + PA-Curcumin. One week after, in the sub-acute stage, we performed the corresponding cell transplants by intramedullary injections (into the lesion, rostrally and caudally). The PA-Curcumin was delivered into the intrathecal space through a catheter connected to an osmotic pump. Locomotor function was evaluated by the BBB open field locomotor scale. Even though we did not find statistically significant improvements in the BBB scale, the MSCs, PA-Curcumin and the iNSCs + MSCs + PA-Curcumin treatments showed better locomotor performance compared to the control. The histological analysis of the injury size, measured as the GFAP negative area that delimits the inhibitory scar, showed smaller area for the PA-Curcumin and the combinatory cell treatments (iNSCs + MSCs). Furthermore, the axonal projecting filaments, measured as TUJ1 positive staining, showed a significant increase in the number of neural fibers at the injury area in the combinatory group including cell transplants and pharmacological treatment (iNSCs + MSCs + PA Curcumin). We further evaluated the microglia inflammatory profile and found a significant presence of anti-inflammatory microglia, identified by an increase in the Arginase/Iba1 ratio, in the PA-Curcumin treated group. Hence, combinatory therapies, including cell and pharmacological treatments confer a more extensive neuroprotective effect; however, further investigation is needed to identify whether synergistic mechanisms are involved.

16. DOPAMINE-ANGIOTENSIN INTERACTIONS IN THE VENTRICULAR-SUBVENTRICULAR ZONE AND THEIR RELEVANCE FOR ADULT NEUROGENESIS.

NODO: RD16/0011/0016

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Different works suggest the involvement of dopamine receptors in regulation of adult neurogenesis in the ventricular-subventricular zone (V-SVZ). Dopaminergic denervation results in a reduced proliferation in this niche in animal models of Parkinson's disease (PD). However, the underlying regulatory mechanisms are still poorly understood. We have recently demonstrated that interactions between RAS receptors play a major role in the regulation of proliferation and generation of neuroblasts in the V-SVZ. We have also described counterregulatory interactions between RAS receptors and dopamine in the substantia nigra and striatum. However, possible interactions between RAS and dopaminergic systems in the V-SVZ have not been investigated. Our results revealed a marked increase in the number of neurospheres derived from mice V-SVZ and upregulation of AT2R expression after treatment with D2-like receptor agonists. Previous results showed that stimulation of AT2 receptors increased the number of neurospheres. However, cotreatment with D1-like receptor agonists inhibited AT2-mediated proliferation. Interestingly, treatment with AT2R antagonists blocked the increase in the number of neurospheres obtained after treatment with D2-like agonists. In addition, we observed that dopaminergic grafts implanted into the striatum induced an increase in proliferation and generation of neuroblasts in the V-SVZ relative to dopamine-denervated animals. Moreover, treatments with AT1R antagonists or AT2R agonists are also capable of reversing neurogenesis to non-lesioned control levels. In conclusion, our results show dopamine-angiotensin interactions in the adult V-SVZ and suggest that manipulations of local RAS may counteract the decline in the V-SVZ neurogenesis observed after dopaminergic denervation in animal models of PD. Grant sponsor: Retic TerCel RD16/0011/0016, and Spanish Ministry of Economy and Competitiveness (RTI2018-098830-B100).

17. PARKINSON'S DISEASE PATIENT-SPECIFIC NEURONAL NETWORKS UNVEIL EARLY FUNCTIONAL ALTERATIONS THAT PREDATE NEURODEGENERATION.

NODOS: RD16/0011/0024 - RD16/0011/0025

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A deeper understanding of early disease mechanisms occurring in Parkinson's disease (PD) is needed to reveal novel restorative targets. Here we report that human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (DAn) obtained from healthy individuals or patients harboring LRRK2 PD-causing mutation can create highly complex networks with evident signs of functional maturation over time. Compared to control neuronal networks, LRRK2 PD patients' networks displayed an elevated bursting behavior, in the absence of neurodegeneration. By combining functional calcium imaging, biophysical modeling, and DAn-lineage tracing, we found a decrease in DAn neurite density that triggered overall functional alterations in PD neuronal networks. Our data implicate early dysfunction as a prime focus that may contribute to the initiation of downstream degenerative pathways preceding DAn loss in PD, highlighting a potential window of opportunity for pre-symptomatic assessment of chronic degenerative diseases.

18. LOW-FREQUENCY GENETIC VARIANTS CONFERRING PROTECTION AGAINST PARKINSON'S DISEASE.

NODOS: RD16/0011/0024 - RD16/0011/0012

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A deeper understanding of early disease mechanisms occurring in Parkinson's disease (PD) is needed to reveal novel restorative targets. Here we report that human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons (DAn) obtained from healthy individuals or patients harboring LRRK2 PD-causing mutation can create highly complex networks with evident signs of functional maturation over time. Compared to control neuronal networks, LRRK2 PD patients' networks displayed an elevated bursting behavior, in the absence of neurodegeneration. By combining functional calcium imaging, biophysical modeling, and DAn-lineage tracing, we found a decrease in DAn neurite density that triggered overall functional alterations in PD neuronal networks. Our data implicate early dysfunction as a prime focus that may contribute to the initiation of downstream degenerative pathways preceding DAn loss in PD, highlighting a potential window of opportunity for pre-symptomatic assessment of chronic degenerative diseases.

19. BORATE INDUCES PROLIFERATION OF BCAS1 EXPRESSING OLIGODENDROCYTE PROGENITORS IN VITRO.

NODOS: RD16/0011/0026

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BCAS1/NaBC1 is an electrogenic sodium-coupled borate transporter. The expression of this transporter has been previously described in different cell types such as osteoclasts, oocytes and neoplastic cells. After borate supplementation, BCAS1 expressing cells increase their proliferation. Recently, BCAS1 expression has been reported in a population of actively-myelinating immature oligodendrocytes in the human and rodent white matter as well as in multiple sclerosis lesions. On the other hand, animals lacking BCAS1 expression display abnormal behavior and hypomyelination, and the presence of BCAS1-positive oligodendrocytes has been related to different neurodegenerative diseases such as multiple sclerosis and multiple system atrophy. We have assessed the effect of borate supplementation in cultured oligodendrocyte precursor cells (OPCs) isolated from P4 mouse brains. Our results indicate that this intervention leads to a reduction in early progenitors, while increasing the proliferation of late OPCs expressing BCAS1. We conclude that borate supplementation might be an interesting way to facilitate robust remyelination through BCAS1-expressing OPC stimulation in demyelinating degenerative diseases.

20. NANOMETRIC 3D SUBSTRATES FOR NEURAL STEM CELLS BEHAVIOUR CONTROL.

NODO: RD16/0011/0032

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The use of stem cells in medicine is of great interest for their huge therapeutic potential. However, much work is still needed to understand their biology and to control cell behaviour and response to external stimulus. There are numerous evidences that mechanical stimuli such as matrix topography and stiffness have an impact over cellular fate and behaviour [1] and as such, there is a considerable interest in establishing the relationship between the mechanical cues and the mechano-transductive pathways involved in cell response [2]. It is widely accepted that micro/nano topographical cues can set off specific physiological processes that ultimately dictate the cell response or fate commitment [3]. As such topography could serve as a tool to regulate the stem cell function and lineage commitment to fulfil their potential therapeutic application. In this context, this work examines cellular behaviour of immortalized human Neural Stem Cells (hNS1) [4] when cultured on a pattern of dense high aspect ratio (HAR) polystyrene nanopillars with the final goal of guiding hNS1 fate commitment.

Towards this aim, topographical substrates were fabricated by nanoimprinting lithography. O2 plasma treatment and poly-L-lysine coating were applied on substrates to facilitate cell adhesion. Metabolic assays were carried out to assess cell viability on substrates. Immunofluorescence and scanning electron microscopy were used to characterise cell morphology. The expression of characteristic proteins of the neural lineages including Doublecortin (DCX) and microtubule-associated protein (MAP2) was evaluated to assess differentiation of the cells seeded on the substrates.

It was found that HAR nanopillars had an influence over the hNS1 in proliferation conditions, increasing population doubling time and reducing metabolic activity in comparison with controls. At the same time, the hNS1 morphology was found affected by the topographical features as cells presented more round shape as well as a smaller cell projected area while attached onto the nanopillars. In addition, the HAR nanopillars guided the orientation of hNS1 along the square pillar lattice where cytoplasmic projections along the perpendicular lattice directions were often seen. Preliminary differentiation tests of hNS1 on HAR nanopillars indicate the formation of a higher percentage of mature neurons compared to flat controls.

Our results suggest that HAR surface nanotopographies module neural stem cell behaviour in proliferation and differentiation conditions and may direct hNS1 towards neuron differentiation.

21. GENERATION OF MIDBRAIN FLOOR PLATE-DERIVED DOPAMINERGIC NEURONS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.

NODO: RD16/0011/0032

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Rationale.

Human induced pluripotent stem cells (hiPSCs) represent an unlimited source for generation of standardized, quality-controlled midbrain dopaminergic (mDA) neurons for cell replacement therapy in Parkinson's disease [1,2]. Authenticity and purity of ventral midbrain floor plate (mFP) progenitors and subsequent mDA neurons are critical for their clinical application [3–5]. Differentiation protocols of high efficiency and high reproducibility are therefore needed to yield correctly patterned DA neurons. Methods: Hereby, we used Gibco™ PSC Dopaminergic Neuron Differentiation Kit as a standardized and validated culture system for generation and characterization of hiPSC-derived dopaminergic cultures. The differentiation workflow consists of three steps, namely specification to mFP progenitors; expansion and cryopreservation of derived mFP cells; and maturation to mDA neurons.

Objectives.

The objective of the study was to test the differentiation efficiency in two conditions, namely FP progenitor expansion in suspension and monolayer culture.

Results.

First, a pure mFP progenitor population was obtained after 10 days of specification, as characterized by the expression of FP/mesencephalic markers FOXA2, LMX1A and OTX2. Following expansion and 10 days of maturation, the phenotype of the generated neurons was assessed for the presence of neuronal markers TUBB3 and MAP2, as well as mDA markers FOXA2, LMX1A and TH. While mature mDA cultures were obtained in both conditions, higher yields of mDA neurons were achieved in the suspension FP progenitor expansion setup.

Conclusions.

In summary, Gibco™ PSC Dopaminergic Neuron Differentiation Kit provided high yields of hiPSC-derived mDA neurons, with suspension culture being the preferred condition for FP progenitor expansion.

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