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TERCEL 2019

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SANTIAGO DE COMPOSTELA
28-29 NOVIEMBRE 2019

CUADERNO DE SESIONES
Y POSTERS

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REUNIÓN ANUAL
TERCEL 2019

RED DE TERAPIA CELULAR

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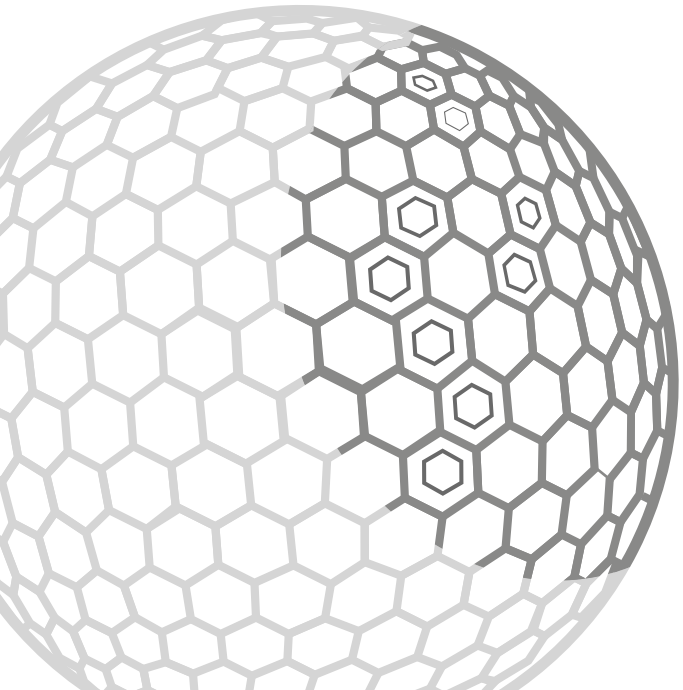

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NODO: RD16/0011/0019

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NODO: RD16/0011/0005

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NODO: RD16/0011/0005

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NODO: RD16/0011/0011

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GLIOBLASTOMA ABLATES PERICYTES ANTITUMOR IMMUNE FUNCTION THROUGH ABERRANT UP-REGULATION OF CHAPERONE-MEDIATED AUTOPHAGY. NODOS RD16/0011/0001 Y RD16/0011/0010

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The contractile perivascular cells, pericytes (PC), are hijacked by glioblastoma (GB) to facilitate tumor progression. PC's protumorigenic function requires direct interaction with tumor cells and contributes to the establishment of immunotolerance to tumor growth. Cancer cells up-regulate their own chaperone-mediated autophagy (CMA), a process that delivers selective cytosolic proteins to lysosomes for degradation, with pro-oncogenic effects. However, the possible impact that cancer cells may have on CMA of surrounding host cells has not been explored. We analyzed the contribution of CMA to the GB-induced changes in PC biology. We have found that CMA is markedly up-regulated in PC in response to the oxidative burst that follows PC–GB cell interaction. Genetic manipulations to block the GB-induced up-regulation of CMA in PC allows them to maintain their proinflammatory function and to support the induction of effective antitumor T cell responses required for GB clearance. GB-induced up-regulation of CMA activity in PC is essential for their effective interaction with GB cells that help tumor growth. We show that CMA inhibition in PC promotes GB cell death and the release of high immunogenic levels of granulocyte-macrophage colony stimulating factor (GM-CSF), through deregulation of the expression of cell-to-cell interaction proteins and protein secretion. A GB mouse model grafted in vivo with CMA-defective PC shows reduced GB proliferation and effective immune response compared to mice grafted with control PC. Our findings identify abnormal upregulation of CMA as a mechanism by which GB cells elicit the immunosuppressive function of PC and stabilize GB–PC interactions necessary for tumor cell survival.

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ESTUDIO PRECLÍNICO EN LESIÓN MEDULAR TRAUMÁTICA CRÓNICA: TRASPLANTE DE CÉLULAS HUMANAS MESENQUIMALES (HMSC) DE GELATINA DE WHARTON EN UN MODELO DE RATA. NODOS RD16/0011/0014, RD16/0011/0028 Y RD16/0011/0036.

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La terapia celular representa una de las opciones de interés para el tratamiento de lesiones traumáticas que afectan a la médula espinal (lesiones medulares, LM). En colaboración entre el grupo de referencia en el desarrollo de medicamentos de terapias avanzadas basados en terapia celular (Banco de Sangre y Tejidos, BST) y el grupo de la UAB se ha realizado un estudio pre-clínico de un ensayo clínico efectuado en colaboración entre el BST y el grupo clínico del Institut Guttmann. El objetivo del estudio clínico es demostrar la seguridad de un tratamiento alogénico de terapia celular de células humanas mesenquimales de gelatina de Wharton (WJ-MSC) en pacientes con una LM crónica, como primer paso que permita desarrollar, en el futuro, un tratamiento para la LM en fase aguda. Las células WJ-MSC, cultivadas en sala blanca y caracterizadas en el BST, siguen la normativa vigente de la Agencia Española del Medicamento (AEMPS). El estudio pre-clínico en un modelo de LM en ratas se realizó con el fin de comprobar el comportamiento de las WJ-MSC, siguiendo un protocolo similar al estudio clínico. La LM se realizó a nivel torácico, mediante laminectomía entre los segmentos T8 y T9, y contusión de 200Kdyn controlada por un dispositivo específico (Infinite Horizon Impactor device). El tratamiento consistió en administrar mediante punción lumbar intratecal entre L3 y L4 una dosis de aproximadamente 10 × 106 WJ-MSC o sólo vehículo. Un grupo de animales fue tratado con vehículo (medio) a los 7 días post lesión (VHC), un segundo grupo recibió una única dosis celular 7 días después de la LM (WJ-MSC 7), mientras que un tercer grupo experimental recibió dos dosis celulares, a los 7 y 14 días post lesión (WJ-MSC 7+14). Con el fin de reducir el rechazo de las células trasplantadas, los animales fueron administrados diariamente con un inmunosupresor (FK-506) hasta la finalización del estudio. Durante 70 días los animales fueron evaluados mediante un test específico de valoración de la locomoción (escala Basso, Beattie, Bresnahan, *BBB*). Al final del seguimiento se realizó un estudio electrofisiológico y finalmente se procesaron las médulas espinales para su estudio histológico.

La primera conclusión es que la administración intratecal a los 7 y 14 días después de la lesión no interfirió en la recuperación funcional después de la LM. El grupo experimental con dos dosis de WJ-MSC mostró una tendencia, no significativa, a una mejor locomoción con respecto a los otros dos grupos en la escala BBB. De los estudios electrofisiológicos podemos concluir que los animales WJ-hMSC 7+14 mostraron una disminución significativa frente a los animales VHC en el reflejo espinal H / M del músculo plantar. Estos datos indican una disminución de la espasticidad o hiperreflexia en el grupo con dos dosis.

En el estudio histológico e inmunohistoquímico no se identificaron células WJ-hMSC en la médula espinal de los animales administrados, a los tiempos en

que se obtuvieron las muestras, 21 y 70 días post lesión. El grupo experimental administrado con dos dosis de WJ-hMSC tuvo una reducción de la cavidad quística o lo que es lo mismo, un aumento del tejido medular preservado. No se observó evidencia de regeneración axonal ni diferencias en la activación microglial entre los tres grupos experimentales.
En conjunto, los datos preclínicos sugieren que la administración intratecal de WJ-hMSC tras una LM es segura, sin reacciones adversas, y produce una ligera mejoría en los resultados funcionales y en la preservación del tejido. Esta observación sugiere una vía indirecta de protección a través de la acción paracrina de las WJ-hMSC en la médula espinal lesionada.

EMBRYONIC NEURODEVELOPMENT IS AFFECTED IN HUNTINGTON'S DISEASE. NODO RD16/0011/0012

Vila, C.[1,2,3,4], Esteve-Codina, A.[5], Estirado, A.[6], Iacono, G.[5], Míguez, A.[1,2,3,4], Martínez, S.[6], Tornero, D.[1,2,3,4], Canals, J.M.[1,2,3,4].

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Huntington's Disease (HD) is a fatal neurodegenerative disorder that manifests itself through motor and cognitive symptoms due to a predominant loss of Medium Spiny Neurons (MSNs) in the striatum. HD research has classically focused on the adulthood. However, growing evidences show that developmental alterations could be a key factor in the disease, determining the future vulnerability of certain cell types, such as striatal MSNs. This work aims to study the striatal developmental alterations in HD mice (zQ175). We have isolated both mantle and germinal striatal zones of E16.5 WT and HD embryos by laser micro-capture and we then compared its gene expression by bulk RNA-seq. The results show significant differences in gene expression pattern in the mantle zone of HD embryos compared to controls. Genetic alterations suggest defects in neuronal generation, growth and maturation, as well as alterations in HD-related intracellular pathways such as mitochondrial metabolism, vesicular transport and calcium homeostasis. Analysis of specific subpopulations of striatal neurons (e.g. D1 and D2 MSNs) should help identifying the primary mechanisms behind the developmental defects and augmented sensitivity of HD MSNs. Indeed, our preliminary results show differences between genotypes. All together, these results indicate that HD pathogenesis starts early in neurodevelopment, thus opening a new promising therapeutic target for preventing HD.

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ADULT NEUROGENESIS AND DISEASE: EFFECTS OF **α**-SYNUCLEIN DOMINANT MUTATIONS IN THE REGULATION OF ADULT NEURAL STEM CELLS BY DOPAMINE. NODO RD16/0011/0025

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We have previously shown that **α-**Synuclein (**α**-SYN) in dopaminergic terminals originating in the substantia nigra (SN) is required for the maintenance of neural stem cells (NSCs) in the adult mouse subependymal zone (SEZ) and, therefore, for sustaining a normal rate of olfactory bulb (OB) neurogenesis. *Snca* homozygous mutant mice that lack **α-SYN** exhibited reduced production of OB newly-born neurons and impaired olfactory behavior. Furthermore, our results indicated that dopamine (DA) is important to keep NSCs undifferentiated and cycling and suggested that **α-SYN** could be regulating DA bioavailability in the SEZ niche (Pérez-Villalba et al., 2018, *J. Neuroscience* 38:814-825). Here, we decided to perform gain-of-function experiments by analyzing tyrosine hydroxylase (TH) promoter-based transgenic mice that express endogenous **α-SYN** plus either normal human **α-SYN** or a doubly mutated human **α-SYN** carrying the A30P and A53T mutations detected in some patients. Birth dating analysis with BrdU revealed reduced rate of neurogenesis in the OB of TH-SNCA*A30P*A53T double mutant mice that it is not observed in TH-SNCA mice. These data were in agreement with lower proportions of BrdU-label retaining NSCs in the SEZs of the same animals. Treatment with the drug 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) which selectively kills DAergic neurons results in reduced proportions of BrdU-label retaining NSCs in the SEZ and of newly-born neurons in the OB of wild-type mice but did not exacerbate the loss resulting from the presence of the doubly mutated **α-SYN**. In order to investigate whether the effects of altered **α-SYN** on NSC neurogenic activity were related to DA bioavailability we have studied DA release by amperometric detection in the SEZ and adjacent striatum using organotypic cultures of coronal slices obtained from *Snca* wild-type and mutant mice as well as from TH-SNCA and TH-SNCA*A30P*A53T transgenic mice. This analysis corroborates the physiological relevance of α-SYN for the appropriate DA release that is required to sustain normal adult neurogenesis.

NEUROCEL
POSTERS

CULTURING CELLS IN A BIOREACTOR FOR CELL-DERIVED PRODUCTS. NODO RD16/0011/0012

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Current interest in advanced therapy medicinal products (ATMPs) for clinical use is not only a scientific but also a technological challenge for the centers involved in the ATMPs manufacturing. In this sense, the translation of conventional scientific protocols to the GMP environment must rely on current technology with the main objective of generating homogeneous and high quality products. In the present work, we aim to establish a robust production system composed by the fixed-bed iCellis nano bioreactor and a concentration equipment based on the principle of tangential flow filtration (TFF). Based on the protocols previously established in the laboratory for the generation of lentiviral particles, various tests have been carried out.

Present results show how the technology is perfectly adaptable to lentiviral production protocols. In addition, the use of these systems have some benefits compared to conventional methods: 1) Reduction of handling time in the stages of cell line expansion, transfection and supernatant collection; 2) Continuous monitoring and control of critical cell culture parameters such as pH, temperature or gas levels; 3) Reduction of the risk of contamination due to the continuously working closed system; 4) Scalability and adaptability to different work volumes; and 5) Concentration of large volumes of supernatant in a short period of time. Altogether, it is intended to introduce these systems in future production projects, not only of lentiviral particles but also in obtaining other products such as other viruses, proteins and/or exosomes.

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DESIGN AND PRODUCTION OF AN INNOVATIVE AND SCALABLE CELL-BASED THERAPY TO TREAT NEURODEGENERATIVE DISEASES. NODO RD16/0011/0012

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Huntington's disease (HD) is a neurodegenerative disease caused by the toxic mutant huntingtin (mhtt) protein. HD primarily affects the medium spiny neurons (MSNs) of the striatum. Brain-derived neurotrophic factor (BDNF) can protect those neurons but its expression is compromised in the presence of mhtt. We are developing a cell-based therapy to repair or replace the damaged MSNs by applying striatal neuron precursors differentiated from pluripotent stem cells, and human bone marrow mesenchymal stem cells (hBM-MSCs) engineered to over-express BDNF using lentivirus. This approach is based on the encouraging outcomes obtained from *in vivo* applications of BDNF and the known immunomodulatory capability of hBM-MSCs. After designing the tools for the production of BDNF lentiviral particles, we optimized large-scale lentivirus (LV) production in a good manufacturing practices (GMP) environment, using calcium-phosphate mediated transfection followed by tangential flow filtration and diafiltration to concentrate the final product. We have transduced hBM-MSCs with BDNF-LVs and quantified BDNF release by ELISA. Furthermore, we have designed a bioassay to assess the effect of BDNF-expressing hBM-MSCs in a brain-on-chip system based on analysis of their effect on neuronal sprouting. In addition, we have also transplanted neuronal precursors in combination with BDNF-expressing hBM-MSCs in mouse model of HD, to study if damaged MSNs in HD are restored by this strategy. We anticipate that such an approach could be used to treat other neurodegenerative diseases that also display loss of neurons in specific brain areas. For this reason, we are developing a novel and scalable cell therapy medicinal product that fulfils the manufacturing needs for new advanced therapy medicinal products.

This work has been supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Innovative Training Networks (Training 4CRM, grant agreement N. 722779; and ASCTN Training Network, grant agreement N. 813851); the Ministerio de Ciencia, Innovación y Universidades, Spain; ISCIII-Subdirección General de Evaluación and European Regional Development Fund (ERDF) [RETICS and CIBERNED]; and Catalonia Trade and Investment, Generalitat de Catalunya and ERDF [ADVANCE(CAT)], Spain. We are very grateful to Dr. Pedro Marin (Hemotherapy and Hemostasis Department, Hospital Clínic de Barcelona, Barcelona, Spain) for his help for obtaining bone marrow samples.

LA INTERACCIÓN RECÍPROCA DE LOS RECEPTORES AT1 Y AT2 DE ANGIOTENSINA REGULA LA NEUROGÉNESIS ADULTA EN ROEDORES. NODO RD16/0011/0016

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La zona subventricular de los ventrículos laterales (V-SVZ) es un nicho neurogénico bien establecido. Sin embargo, los factores que controlan el microambiente y el comportamiento de las poblaciones celulares que lo componen no son del todo conocidos. La angiotensina II es el principal efector del sistema renina-angiotensina (SRA), y actúa a través de los receptores AT1 y AT2, con efectos contrarios. Diversos trabajos sugieren la existencia de una regulación recíproca entre los receptores AT1 y AT2 en distitntos tipos celulares y tejidos, incluidos los ganglios basales cerebrales. Sin embargo, se desconoce si esta interacción también se produce en la V-SVZ. Por ello, nos propusimos estudiar las posibles interacciones de los receptores AT1 y AT2 en la V-SVZ y el efecto de dicha interacción en la neurogénesis de la zona en animales jóvenes y envejecidos.

Nuestros resultados muestran que en la V-SVZ de ratones jóvenes deficientes (KO) para los receptores AT2 y en cultivos de neuroferas de ratones salvajes tratados con el antagonista de los receptores AT2 PD123319, se observa un aumento en la expresión de AT1, así como una disminución de la proliferación y la generación de neuroblastos. Además, en la V-SVZ de ratones jóvenes KO para AT1 y en cultivos de neurosferas tratadas con el antagonista de AT1 candesartán, se observa una disminución en la expresión de AT2, aunque el tratamiento no afecta la proliferación ni la generación de neuroblastos. Sin embargo, y al contrario que en ratones, nuestros resultados muestran un incremento marcado en la expresión de los receptores AT2 en ratas tratadas con candesartán, en las que también se observa un aumento de la proliferación y la generación de neuroblastos. A la vista de estos resultados, estudiamos las posibles diferencias en los niveles de expresión de los receptores AT1 y AT2 entre ratas y ratones. Nuestros resultados muestran que los niveles de expresión de ambos receptores son mayores en ratas que en ratones, aunque la ratio AT1/AT2 es de alrededor de 1 en ratas y mientras que es 3 veces mayor en ratones. Además, estudiamos los niveles de expresión de AT1 y AT2 en animales envejecidos y observamos que los niveles de AT1 aumentan significativamente en la V-SVZ de animales envejecideos respecto a jóvenes, lo que es consistente con la disminución en la proliferación y la generación de neuroblastos observada en animales envejecidos. Sin embargo, esta disminución puede ser contrarrestada modulando RAS, lo que sugiere que las células madre/progenitoras de animales envejecidos muestran capacidad de respuesta tras los estímulos adecuados.

Estos resultados sugieren la existencia de interacciones mútuas y complejas entre los receptores AT1 y AT2 de angiotensina en la V-SVZ, que podrían explicar las diferencias observadas entre especies y la respuesta asociada a la edad que la modulación de RAS ejerce en la neurogénesis de este nicho. Trabajo financiado por RD16/0011/0016, RD16/0011/0017, BFU2015-70523, SAF2017-86690-R, ED431C2018/10, ED431G/05, FEDER, Prometeo 2017-030 y Fundación Emilio Botín-Banco Santander.

EFECTO DE LA MANIPULACIÓN DEL SISTEMA RENINA ANGIOTENSINA EN LA NEUROGÉNESIS DE LA ZONA SUBVENTRICULAR DEL CEREBRO ADULTO. NODO RD16/0011/0016

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La zona subventricular de los ventrículos laterales (V-SVZ) es un nicho neurogénico adyacente al estriado, que contiene células madre/progenitoras que podrían contribuir a la reparación de los circuitos afectados en la enfermedad de Parkinson. De ahí la importancia de conocer los mecanismos y factores responsables del control del nicho, así como las posibles alteraciones que se producen con la edad. La angiotensina II, a través de los receptores AT1 y AT2, es el principal efector del sistema renina-angiotensina (RAS). Estudios en distintos tejidos, incluido el cerebro, demuestran que la activación del receptor AT1 da lugar a un ambiente prooxidativo y proinflamatorio, mientras que los receptores AT2 contrarrestan los efectos deletéreos de los receptores AT1. Además, existen datos que sugieren que los receptores AT2 están implicados en la proliferación celular, maduración, apoptosis y regeneración. Sin embargo, el papel de RAS en la regulación de la neurogénesis de la V-SVZ y las posibles alteraciones relacionadas con el envejecimiento no han sido determinadas.

En este trabajo estudiamos el papel de los principales receptores de RAS en la neurogénesis en la V-SVZ en roedores jóvenes y envejecidos. *In vitro*, observamos una reducción en el número de neurosferas derivadas de la V-SVZ de ratones jóvenes deficientes (KO) para AT2, así como en cultivos derivados de ratones salvajes tratados con antagonistas de los receptores AT2. Por otra parte, el tratamiento con agonistas de AT2 induce un incremento en el número de esferas. *In vivo*, observamos una reducción en la proliferación y la generación de neuroblastos en animales KO para AT2, mientras que no se observaron diferencias respecto al control en ratones KO para el receptor AT1 o en ratones salvajes tratados con antagonistas de AT1. De nuevo, el tratamiento con agonistas de AT2 induce un aumento en ambos parámetros. Sin embargo, en ratas tratadas con antagonistas de AT1 se observó un aumento en la proliferación y generación de neuroblastos en la V-SVZ. Por otra parte, en animales envejecidos se observó una reducción en la proliferación y generación de neuroblastos, así como una disminución en el número de neurosferas obtenidas en cultivo respecto a los animales jóvenes . Sin embargo, estos valores se revirtieron a niveles de animales jóvenes control tras el tratamiento con agonistas de los receptores AT2.

En conclusión, nuestros resultados muestran que RAS juega un papel importante en la regulación de la neurogénesis en la V-SVZ de animales jóvenes y envejecidos, y sugieren que los moduladores de RAS podrían tener efectos potencialmente beneficiosos en el control de la neurogénesis adulta. Trabajo colaborativo financiado por RD16/0011/0016, RD16/0011/0017, BFU2015-70523, SAF2017-86690-R, ED431C2018/10, ED431G/05, FEDER, Prometeo 2017-030 y Fundación Emilio Botín-Banco Santander.

INTERACCIÓN DE LOS SISTEMAS DOPAMINÉRGICO Y RENINA-ANGIOTENSINA EN LA ZONA SUBVENTRICULAR. IMPLICACIONES EN EL CONTROL DE LA NEUROGÉNESIS ADULTA. NODO RD16/0011/0016

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La neurogénesis en la zona subventricular de los ventrículos laterales (V-SVZ) se mantiene gracias a la existencia de un nicho neurogénico finamente regulado por factores específicos, entre los que destacan los neurotransmisores. Existen datos que sugieren que la dopamina tiene un papel importante en el control de la neurogénesis de la V-SVZ a través de receptores de la familia D1 (D1 y D5) y D2 (D2, D3 y D4). De hecho, en modelos experimentales de enfermedad de Parkinson (EP), en los que se produce denervación dopaminérgica de la V-SVZ, se observa un descenso en la proliferación de la zona. Además, estudios postmortem de pacientes afectados por la EP muestran igualmente una disminución en el número de células proliferativas de la V-SVZ. Sin embargo, los mecanismos subyacentes continúan siendo poco conocidos. Por otra parte, estudios recientes de nuestro grupo muestran la existencia de los principales receptores del sistema renina-angiotensina (RAS), AT1 y AT2, en la V-SVZ, y que la modulación RAS tiene efectos en la proliferación y generación de neuroblastos en la zona. Además, datos previos indican que dopamina y angiotensina II, el principal efector de RAS, interactúan y se contrarregulan en distintos tejidos, incluido el estriado y la sustancia negra. Con estos antecedentes, nos proponemos conocer si RAS media los efectos de la dopamina sobre la neurogénesis de la V-SVZ.
Nuestros resultados muestran que la activación en cultivo de los receptores de dopamina tipo D2 aumenta el número de neurosferas de células madre/progenitoras derivadas de la V-SVZ de ratones. Estos efectos estarían mediados por los receptores D3 de dopamina. Sin embargo, el tratamiento con antagonistas de los receptores AT2 de angiotensina bloquea el aumento en la generación de neurosferas inducido por los agonistas de D2. La activación de los receptores tipo D1 no induce cambios en el número de esferas, en presencia o ausencia de antagonistas de los receptores AT1 y AT2. Sin embargo, el tratamiento con agonistas de los receptores D1 bloquea el aumento en la generación de esferas inducido por el tratamiento con agonistas de los receptores AT2 de angiotensina. Estudiando los niveles de expresión de ARNm observamos que la activación de los receptores D2 de dopamina da lugar a un aumento en la expresión de los receptores AT2 de angiotensina, y el bloqueo de los receptores AT1 induce una reducción en la expresión de los receptores D1. Por otra parte, experimentos *in vivo* confirman una reducción en la proliferación y generación de neuroblastos en la V-SVZ de animales con denervación dopaminérgica, que es revertida a niveles similares al control tras el tratamiento con antagonistas de los receptores AT1 o agonistas de los receptores AT2.
Estos resultados muestran que el sistema dopaminérgico y RAS se contrarregulan en la V-SVZ y sugieren que manipulaciones de RAS podrían ser potencialmente útiles en la modulación de la neurogénesis en condiciones patológicas en las que se produce un declive de la neurogénesis como en la EP. Trabajo financiado por RD16/0011/0016, BFU2015-70523, SAF2017-86690-R, ED431C2018/10 y ED431G/05.

LAS CÉLULAS MADRE MESENQUIMALES FAVORECEN LA SUPERVIVENCIA DE LAS NEURONAS DOPAMINÉRGICAS TRASPLANTADAS EN MODELOS DE ENFERMEDAD DE PARKINSON. ENSAYO DE TERAPIA CELULAR COMBINADA NEUROPROTECTORA Y RESTAURADORA. NODO RD16/0011/0016

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En la enfermedad de Parkinson (EP) se produce la degeneración de las neuronas dopaminérgicas de la sustancia negra del mesencéfalo y la consecuente denervación del estriado, dando lugar a las alteraciones motoras típicas de la enfermedad El tratamiento clásico consiste en la administración de L-DOPA, con una eficacia limitada. De ahí la importancia de la búsqueda de nuevas estrategias terapéuticas. Una alternativa prometedora la representa la terapia celular, cuyo éxito depende en buena medida de la supervivencia e integración de las células trasplantadas. Por otra parte, las células madre mesenquimales (MSCs) poseen propiedades inmunomoduladoras y antiinflamatorias, además de propiedades antiapoptóticas, antioxidantes y tróficas, que las hacen especialmente interesantes en estrategias de terapia celular combinada neuroprotectora/restauradora. De hecho, estudios previos de nuestro grupo, en colaboración con otros nodos de la RED TERCEL, han mostrado que el medio condicionado derivado de las MSCs favorece la supervivencia de neuronas dopaminérgicas en cultivo. Estos efectos, al menos en parte, estarían mediados por factores de naturaleza lipídica como la prostaglandina, que actuaría a través de los receptores EP2. Por ello, nos planteamos conocer si combinaciones de MSCs o sus efectores con progenitores mesencefálicos favorecerían la supervivencia de células dopaminérgicas trasplantadas en modelos de EP.

Nuestros estudios mostraron que cuando se realizan cotrasplantes de precursores dopaminérgicos combinados en la misma suspensión con un número elevado de MSCs se produce una reducción en la supervivencia dopaminérgica. Sin embargo, el número de precursores dopaminérgicos que sobreviven tras el implante aumenta significativamente cuando se introduce en la suspensión un número menor de MSCs. Estos resultados sugieren la importancia de ajustar la proporción de células de ambas poblaciones que contiene la suspensión, y que los posibles efectos beneficiosos de las MSCs podrían depender del tipo y cantidad de factores que producen. En otra serie de experimentos, realizamos trasplantes de precursores dopaminérgicos a los que añadimos en la suspensión agonistas de los receptores EP2 de prostaglandina. Nuestros resultados mostraron que el tratamiento no inducía cambios en la supervivencia de las células dopaminérgicas trasplantadas. Sin embargo, se observó un aumento en la densidad de fibras dopaminérgicas en el área de reinervación del trasplante. Por tanto, el cotrasplante con MSCs o factores derivados de estas células conjuntamente con progenitores dopaminérgicos podría ser una estrategia terapéutica útil para favorecer la supervivencia de las neuronas dopaminérgicas utilizadas en terapia celular en la EP. Trabajo financiado por RD16/0011/0016, BFU2015-70523, SAF2017-86690-R, ED431C2018/10 y ED431G/05.

TNFR2 AS A NEGATIVE REGULATOR OF CELL REPROGRAMMING. NODO RD16/0011/0017

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Tissue damage is followed by an inflammatory response that is key to promote repair. Regeneration usually involves cell reprogramming, and this process is known to be boosted by proinflammatory cytokines such as IL-6. Moreover, *in vivo* cell reprogramming also triggers tissue damage. Therefore, a positive forward loop is established to facilitate reprogramming. However, negative forward loops halt every non-pathological activation of the innate immune system, and therefore inflammation and its related processes, after its goal has been achieved. Then, as IL-6 promotes reprogramming, we hypothesized that other inflammation-related cytokines should be doing the opposite.

We had a characterized model of reprogramming of neural stem cells (NSCs) to iPSCs by the viral transduction of Sox2, Oct4 and Klf4. For that reason, we first did RNA-Seq of NSCs and NSC-derived iPSCs to look for differentially expressed cytokine receptors. *Tnfrsf1b* gene, which encodes for TNFR2, emerged as a good candidate, as it was much more expressed in iPSCs than in NSCs. To explore its role we used the same system to reprogram NSCs from TNFR2 *wild-type* and *knock-out* mice and compared iPSC formation. Interestingly, we were able to generate more alkaline phosphatase-positive colonies, more SSEA-1-positive cells, and more Nanog gene expression in absence of TNFR2. This suggests that this receptor could be part of a negative forward loop that stops reprogramming after a damaged tissue has been restored.

IN SEARCH OF VASCULAR SENESCENT CELLS. NODO RD16/0011/0017

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Cell senescence is an alternative cellular process to apoptosis in which the cell cycle is in permanent arrest. Senescent cells have an active secretome in which proinflammatory factors are released with consequently deleterious effects for the neighbouring cells. These cells accumulate with age, causing a reduction in tissue function that leads to the development of age-associated diseases, such as neurodegenerative disorders [1].

In the adult mammalian brain, the subependymal zone (SEZ), lining the wall of the lateral ventricle, represents the major reservoir of neural stem cells (NSCs) and continually generates new neurons [2]. Several signals are implicated in the regulation of NSC activity, for instance, vasculature plays an important role by providing a number of factors to the neurogenic niche [3].

With age, the capacity for self-renewal and proliferation of NSCs declines, resulting in a loss of neurogenic potential in the SEZ [4]. However, it is still unknown whether this phenomenon is influenced by cell senescence.

Given the important role of the vasculature in sustaining the neurogenic niche, vascular changes associated with age could account, at least in part, for the reduced neurogenic function [3]. Altogether, we suggest that a process of vascular senescence could be involved in the age-related NSCs alterations.

To explore this relationship, an on-off two photon probe (AHGa) has been design by our group and utilised as a tool for senescence detection. AHGa consists of a compound that binds a galactose. This bond is susceptible of being hydrolysed by lysosomal senescence associated-β-galactosidase activity (SA β-gal), converting the compound into fluorescent mainly in senescent cells [5]. This device has the advantage of detecting senescent cells by fluorescence, which is interesting for obtaining quantitative data from assays, such as flow cytometry.

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KEYDWORDS

- Cellular senescence
- GalNP
- NSCs
- SEZ
- β-gal

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ANALYSIS OF THE EFFECTS OF THE RENIN-ANGIOTENSIN SYSTEM ON THE CAROTID BODY MORPHOLOGY AND GDNF EXPRESSION: IMPLICATIONS FOR ANTIPARKINSONIAN CELL THERAPY. NODOS RD16/0011/0025 Y 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 that mainly acts through type 1 and 2 receptors (AT1 and AT2). In addition to the circulating RAS, local RAS has been described in different tissues, including central and peripheral nervous system. The carotid body (CB) is a highly dopaminergic neural crest-derived organ, which after intrastriatal transplantation exerts a trophic protection, by the release of GDNF, of the nigrostriatal pathway on experimental models of parkinsonism. Different studies have shown the presence of the principal elements of the local RAS system, including AT1 and AT2 receptors, in the CB. Moreover, regulation of the expression of different RAS elements during the hypoxic CB growth, which is mediated by neurogenic niche activation, has been described. Here, we studied the effects of the local RAS on the CB morphology and its GDNF expression. Furthermore, we also analysed the putative role of the RAS in the CB growth after chronic hypoxia. The results obtained from this study could open the possibility to increase the CB GDNF expression and/or to improve the "in vitro" expansion of CB cells by pharmacological modulation of the local RAS, with the aim to increase the clinical outcome of anti-parkinsonian CB cell therapy.

COMBINATORY TREATMENT OF NEURAL PRECURSOR CELLS AND A NEW NANOCONJUGATE OF FASUDIL FOR THE CLINICAL APPLICATION IN ACUTE SPINAL CORD INJURY. NODO RD16/0011/0028

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Banc de Sang i Teixits (BST) is a public agency of the Catalan Department of Health whose mission is to guarantee the supply and proper use of human blood, tissue and advanced therapies in Catalonia. In the context of advanced therapy development, our group at BST (RD16/0011/0028) establishes collaborations with leading clinical researchers to contribute in the translation of novel therapies. Herein, in collaboration with the researchers in charge of the preclinical development of the candidate medicine, BST performs a Good Manufacturing Practice (GMP)-ization of bioprocesses, the definition and validation of suitable quality control panels and the design of clinical studies. Of note, this kind of approach has been previously successfully proved in a number of collaborations (i.e. Dr. Joan Vidal, Institut Guttmann, RD16/0011/0036, EudraCT No. 2015-005786-23; Dr. Antoni Bayès-Genís, RD16/0011/0028, EudraCT No. 2018-001964-49).

While significant improvements in rescuing neuronal activity after SCI have been reported at the preclinical stage, their translation to the clinic remains inefficient. The complexity of SCI, involving cascades of concatenated extrinsic and intrinsic events, requires rapid intervention with a combination of therapeutic strategies. We believe that neural precursor cell transplantation can create a permissive environment for neuronal survival. However, limited spontaneous regeneration of axonal tracts requires additional support. Solid preclinical data from Dr. Moreno's group has demonstrated the efficacy of combining epSPCs (ependymal progenitor/stem cells) transplantation with poly-L-glutamic acid-Fasudil (PGA-SS-FAS), which allows the controlled and sustained release of a Rock kinase inhibitor rescuing voluntary motor tasks, promoting neuronal neuroprotection, and enhancing regeneration by reducing glial scar formation. The main objective here is the clinical translation of an already established methodology from experimental models for the isolation and expansion of epSPC-derived from human foetal tissue. Human foetal neural precursor cells will be isolated from spinal cords dissected from legally induced abortions during the first trimester in compliance with current GMP and other regulatory requirements. The final medicinal product will be activated by preconditioning of the neural precursor cells with PGA-SS-FAS. The final goal of this project is to present a dossier (Producto en Investigación, PEI) to AEMPS and move towards a pilot Phase I clinical trial for the treatment of chronic SCI patients.

NEURAL STEM CELLS OPTOGENETICALLY MODIFIED FOR EXPERIMENTAL PARKINSON'S DISEASE TREATMENT. NODO RD16/0011/0032

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Parkinson's disease is the most common neurodegenerative movement disorder and it is characterized by the selective loss of nigrostriatal dopaminergic neurons and therefore the depletion of striatal dopamine levels. Therapies available only transiently ameliorate the symptoms but none of them can cure it, nor block disease progression. In the last decades several clinical trials have shown that neural stem cell therapy can be a feasible approach, although, so far, it lacks the possibility to control the biology and function of the transplanted cells. Having this in account a new cell line was developed with the potential to produce dopaminergic neurons with the possibility to be functionally controlled and regulated by blue light.

In the present work, *v*-myc immortalized human neural stem cells from ventral mesencephalon overexpressing $BclX_L$ (Hi-$BclX_L$-hVM1 cell line, Courtois et al, JBC 2010) were genetically modified to express channelrhodopsin-2. Several clones were selected to identify a cell line expressing the transduced vector while retaining a good neurogenic potential. After 2-3 weeks of differentiation induced by GDNF and cAMP, the cells were studied by immunocytochemistry and western blot, revealing the expression of markers of different neuronal maturation stages (synapsin-1) and dopaminergic neurogenesis (TH, β-III-Tubulin). Electrophysiology studies confirmed the presence of immature neurons with a resting membrane potential of -70±3mV, as well as some in an early stage of maturation with a resting membrane potential of -70±6mV, moreover, the depolarization upon blue light stimulation (440-500nm) has been recorded.

In conclusion the present optogenetic human cell line has the potential to be used for future studies in vitro and in vivo and also to help to understand the fundaments of dopaminergic neurogenesis and grafting in PD.

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EFFECT OF ELECTRICAL STIMULATION DURING EARLY DEVELOPMENT OF HUMAN BRAIN ORGANOIDS. NODO RD16/0011/0032

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Cerebral organoids are a model of human brain development that allows scientists to study brain development from human stem cells, overcoming some of the limitations of using animal models. Nevertheless, this model has limitations as well; there is a high variability between batches of organoids and among organoids within the same batch, and of course the connexion with the whole organism is missing. In order to improve brain organoids quality, we explored the effect of electrical stimulation during development. Electrical activity is known to modulate brain development together with gene modulation. For this reason, we modulated the electrical activity through electrical stimulation and checked if there was an improvement in neural development of brain organoids. In previous preliminary studies from our laboratory, we observed that electrical stimulation has a stronger effect during early developmental stages. Therefore, in the present study we explore the effect of electrical stimulation during the neural expansion phase of organoid generation. The stimulation was done using our *in house* developed system and all organoids were analysed after 30 days of maturation, when neural markers (B-III tubulin, DCX, MAP2, GFAP) are already detectable. We studied the expression of neural markers both using *in toto* immunohistochemistry and western blot analysis. Here we show or preliminary results that seem to indicate that electrical stimulation is affecting neuronal maturation mainly after stimulation at early time-points during development.

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DIRECT NEURONAL REPROGRAMMING OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS. NODO RD16/0011/0010

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Since many years, cell fate and diversity field has been governed under the non-reversible paradigm represented by the Waddington's landscape. But the hypothesis in which cell fate acquisition is an irreversible state has changed dramatically since the Induced Pluripotency Stem Cell (iPS) discovery.

Nowadays, the iPS vision has been expanded. Many laboratories are starting to see different cell types which could be transformed into others directly, which implies bypassing the intermediate immature state and avoiding some problems of the iPS technique. This phenomenon is called direct reprogramming, and the technology has many interesting applications, such as the production of neurons from other cell types *in vitro*, which could be useful to analyze patient specific neuronal diseases or test different drugs.

In our case, our interest is the production of neurons from human adipose-derived mesenchymal stem cells (hADSC), which is an easily accessible source of cells and a less differentiated cell (which could have less restrictions for reprogramming).

In order to test the possible neuronal differentiation capacity of hADSC *in vitro*, two different approaches have been developed: a non-genetic manipulation approach (based on the use of different small molecules to induce different pathways), and a genetic-manipulated approach (based on the use of lentiviral vectors with important transcription factors for neuronal development). Both strategies have been analyzed by the observation by immunocytochemistry of Neurofilament-M+ and Tuj1+ neuronal markers.

Our initial results are pointing towards the potential of hADSC to produce neurons. But further assays using electrophysiology tools should be done in order to test if these induced neurons (iN) could have electrical activity, functionality and the ability to form synapses.

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CARDIOCEL
SEGUNDA MESA DE PONENCIAS

SESIONES

CHARACTERIZATION OF MURINE AND HUMAN EPICARDIAL FAT INTERSTITIAL FRACTION. NODOS RD16/0011/0006, RD16/0011/0021 Y RD16/0011/0030

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Adult mesenchymal cells with a progenitor profile (MSC) are present in the interstitium of different tissues. MSC have been widely used as substrates for experimental cell-based therapies to treat a great variety of diseases. These cells display a restricted differentiation potential and are therefore not suitable to support substitutive cell therapies, but their immunomodulatory properties have made them relevant for the treatment of specific conditions.
Remarkably, MSC could also be involved in the activation of pathologic responses of multiple organs. In the case of the heart, fibroblast mesenchymal progenitors are key to the development of post-myocardial infarction (MI) fibrotic ventricular remodeling, and might also be involved in fibrous or fibro-fatty myocardial substitutions in dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ACM), respectively. In the case of ACM, fibro-fatty myocardial substitution starts in the epicardial surface and progresses towards the myocardium until reaching the endocardial surface. Our working hypothesis is that cells of the embryonic epicardial lineage at the heart surface are the source of fibroblasts and adipocytes involved in this pathological transformation. We have therefore focused in the analysis of the interstitial/stromal mesenchymal fraction of the epicardial fat (EF-IC), a poorly known cardiac mesenchymal fraction, aiming at:

1. Characterizing the diversity of EF-IC cells as related to specific developmental lineages using genetic-tracing methods (mouse).
2. Establishing parallels between murine and human EF-IC subpopulations.
3. Defining the differentiation potential of EF-IC cells (mouse, human).

This research should allow us to further understand the properties of epicardial-derived mesenchyme as both a possible source of cardiovascular cell types and as a pathologic cell substrate for various heart conditions.

This is a collaboration between TERCEL groups RD16/0011/0006, RD16/0011/0021 and RD16/0011/0030. Suggested for oral presentation.

H2020-SC1-2019-SINGLE-STAGE-RTD. REGENERATIVE MEDICINE: FROM NEW INSIGHTS TO NEW APPLICATIONS. PROYECTOS REANIMA Y BRAVE. NODOS RD16/0011/0019, RD16/0011/0005, RD16/0011/0027 Y RD16/0011/0029

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REANIMA

Heart failure is a major epidemic world-wide, resulting in a large burden for society in deaths, morbidity and economic sustainability. The inability of the human heart to regenerate the myocardium lost due to acute myocardial infarction underlies a large part of chronic heart failure cases. To tackle this problem, REANIMA aims to deliver new therapies for heart regeneration, thereby reverting the conditions that lead to heart failure. Thus far, clinical trials based on introducing stem cells into the heart have not shown regenerative ability. In contrast, studies of spontaneous and induced heart regeneration in animal models strongly suggest a change in paradigm towards the reactivation of endogenous regenerative mechanisms. To develop this new path, REANIMA presents the first European basic-translational integrated effort in the cardiac regenerative field, with activities that span from the discovery of new targets in animal models to clinical trial design. REANIMA brings together knowledge on species that can regenerate their hearts -fish and amphibians-, animals that cannot regenerate their hearts -adult mammals- and human engineered heart tissues. Strong synergy between partners will allow REANIMA to identify new pathways in regenerative animals and design strategies for their reactivation in non-regenerative animals and human tissue. REANIMA incorporates industrial and academic partners specialised in translational and pre-clinical research, who will develop advanced therapeutic medicinal products and new translational platforms. REANIMA will thus establish a translational pipeline using cross-species gene/protein therapy products with high translational potential, thereby boosting the rapid transition from basic research to the design of clinical trials. We expect that REANIMA will change current paradigms underlying clinical efforts in regenerative cardiology by transforming basic knowledge on endogenous pathways into effective new therapies.

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BRAVE

Ischemic heart disease is the main cause of death in the EU, straining patients and economies. Regenerative Medicine has failed at delivering a definitive solution, and even the breakthrough of cell reprogramming, biomaterials or 3D printing, have not been able to find a curative solution. Generating a muscle with efficient pumping requires a careful recapitulation of the
myocardial architecture. BRAVƎ is born with the ambition of shaping this quantum leap in the field. The overall concept is to provide a lasting functional support to injured hearts through the fabrication of regenerative personalized advanced tissue engineering-based biological ventricular assist devices (Bio-VADs). To do so, we will apply multimodal deep cardiac phenotyping, coupled to advanced Computational Modelling and biomechanical analysis in a large animal model of disease, to create a personalised 3D printable design. We will for the first time create a fibre-reinforced human heart-sized cardiac tissue able to recapitulate the low Young´s Modulus of the myocardium while withstanding pressures generated during the
cardiac circle. Using the latest human induced pluripotent stem cell (hiPSC) technology and industrial-scale growth and differentiation, we will cellularize this novel human heart-sized constructs, creating a highly efficiently aligned cardiac tissue (including vasculature). BioVADs will be matured in in-Consortium built electromechanical stimulation bioreactors before
transplantation in a porcine model of disease. We anticipate our BioVADs will constitute a one-shot regenerative treatment of IHD, decreasing the burden on healthcare providers and improving the quality of life of patients. Crucially, we will for the first time generate a wealth of information on heart development at a human scale. Delivering this novel application whilst
developing the technological environment (bioreactor, chamber, pacemaker) will boost the capacity of the EU to grow economically and lead the field.

CARDIOPROTECTIVE EFFECT OF MIRNAS DERIVED FROM MESENCHYMAL STEM CELLS EXTRACELLULAR VESICLES IN DOXORUBICIN-INDUCED DAMAGE. NODO RD16/0011/0004

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Doxorubicin is an anthracycline effective against several types of cancer but its use is limited due to cardiotoxocity. Nowadays there is no effective treatment to avoid the mentioned cardiac damage. Mesenchymal stromal cells (MSCs) derived extracellular vesicles (EVs) are an excellent candidate to be used as next generation therapy since they showed excellent results in different preclinical models of heart diseases. In this regard, EVs contain cytokines, signalling molecules and different miRNAs, small chains of nucleotides able to regulate the expression of a wide variety of protein in the cells.

In this work, we isolated EVs from MSCs and observed that they reduce oxidative stress and senescence trigger by doxorubicin in cardiac cells. In addition, MSC derived EVs partially reverted fibrosis induced by doxorubcin and recovered angiogenesis of coronary microvasculature. As mentioned, EVs deliver miRNAs to target cells. In this regard we identified miRNAs related to cardiatoxicity in MSC derived EVs. Moreover, transfecting these sequences individually or in combination in cardiac cells increased their viability and decreased their oxidative stress when treated with doxorubicin.

In conclusion, we showed that EVs secreted by MSCs have beneficial effect on doxorubicin treated cardiomyocytes and that the miRNAs carried by these vesicles plays a key role in this effect. This piece of work indicates that EVs enriched in miRNAs could be an effective treatment for doxorubicin damaged heart and opens the door to design synthetic EVs loaded with a combination of therapeutic miRNAs.

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ROLE OF AUTOPHAGY DURING CELL COMPETITION IN HEART DEVELOPMENT AND REGENERATION. NODO RD16/0011/0019

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Cell Competition (CC) is the process by which viable cells are eliminated from tissues by comparison with neighboring cells. Myc overexpression in a mosaic fashion induces CC in heart, a mechanism by which Myc-high cardiomyocytes actively eliminate neighboring cardiomyocytes with lower Myc levels. Our current interest focuses on understanding the relationship between metabolism and autophagy during Myc-dependent CC at both embryonic development and postnatal maduration of cardiomyocytes (CMs) in mice. Our results show that autophagy is reduced in wildtype embryonic CMs when Myc is overexpressed in neighboring CMs (iMOS-Myc). More interestingly, hypoxia, which induces autophagy in wildtype hearts, completely abolishes this lysosomal degradative process and CC in Myc-overexpressed hearts. In addition, blocking the monocarboxylate transporters (MCTs) prevents the reduction of wildtype CMs in iMOS-Myc hearts. Together, these results could suggest that some metabolites that are released through MCTs and whose levels can vary upon hypoxic conditions are implicated in CC.

On the other hand, we are studying the autophagy pathway during the neonatal maduration of the heart by analyzing its role in the binucleation and polyploidization, both processes associated with cell cycle arrest in cardiomyocytes. We have observed that autophagy is inhibited at postnatal stages, specially from P7, when the majority of CMs are binucleated. More importantly, this process increases in both antioxidant and hypoxic conditions, two stimuli that promote cardiomyocyte proliferation in the postnatal heart. In summary, our main goal is to determine if autophagy flux depends on metabolism and is regulated by Myc during CC as well as it could be relevant for heart regeneration.

CARDIOCEL
POSTERS

LAS VESÍCULAS EXTRACELULARES DE MSCS MEJORADAS MEDIANTE CONDICIONES DE PRECONDICIONAMIENTO ***IN VITRO*** Y MODIFICACIONES GENÉTICAS MUESTRAN PROPIEDADES INMUNOMODULADORAS. NODOS RD16/0011/0004 Y RD16/0011/0002

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A pesar del aumento en los últimos años de ensayos preclínicos y clínicos basados en MSCs, los resultados terapéuticos obtenidos son inconsistentes y muestran una eficacia modesta hasta ahora. Únicamente 2 ensayos han reportado resultados positivos tras su finalización. En los últimos años, varios autores han descrito que las Vesículas Extracelulares (EVs) secretadas por las MSCs son capaces de recapitular las propiedades regenerativas e inmunoreguladoras inducidas por las MSCs y ofrecen ventajas en términos de bioseguridad y producción en condiciones de grado clínico. Para mejorar la producción de EVs hemos generamos una línea de células MSC no senescentes. Además, hemos aumentados su potencial inmunosupresor modificándolas genéticamente mediante la sobreexpresión del Factor Inducible por Hipoxia HIF-1α y desarrollando un medio de cultivo específico basado en citoquinas proinflamatorias. Una vez caracterizados los EVs mediante NTA, microcopia electrónica y marcadores de membrana, observamos que ésta eran incorporados por distintas células inmunes como monocitos, linfocitos T y B y células NK. Las EVs derivadas de células modificadas genéticamente (mg-EVs) reducían la proliferación de linfocitos T activados de forma más efectiva que los EVs normales. Por otra parte, las EVs redujeron la actividad citolítica de las células NK, aunque las mg-EVs no mostraron mejores resultados. Sobre la población de monocitos las EVs son capaces de redirigir su diferenciación hacia un fenotipo de macrófagos M2 de manera similar a lo observado en presencia de MSCs. Tras analizar el cargo de los EVs hemos observado diferencias en citoquinas y miRNAs que podrían explicar su potencial inmunosupresor. Finalmente, se utilizó un modelo de hipersensibilidad retardada en ratón para validar los resultados obtenidos in vitro. Las mg-EVs redujeron la inflamación de la oreja del ratón, disminuyeron la infiltración de células inmunitarias y mejoraron la integridad de los tejidos de manera más efectiva. En resumen, hemos generado una fuente de EVs de larga duración que secreta no sólo más EVs, sino también con una mayor capacidad inmunosupresora, lo que facilitará la obtención de un producto terapéutico más estándar y de menor coste. Estas EVs pueden proporcionar una herramienta nueva y segura para el tratamiento de enfermedades autoinmunes y otras enfermedades inflamatorias.

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EL N1ICD INCORPORADO EN LOS EXOSOMAS DE LA LÍNEA DE CÁNCER DE MAMA MDA-MB-231 CONTRIBUYE A SU INDUCCIÓN SOBRE LA PROLIFERACIÓN Y LA MIGRACIÓN A TRAVÉS DE LA ACTIVACIÓN DE LA TRANSICIÓN EPITELIO MESÉNQUIMA (TEM). NODOS RD16/0011/0004 Y RD16/0011/0005

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Colaboración con grupo Felipe Prosper.

Las células de los mamíferos secretan una gran variedad de vesículas extracelulares (EV) que participan activamente en la comunicación intercelular. Entre ellas, los exosomas son un tipo de EV cuyo tamaño está comprendido entre los 20 y 150 nanómetros que juegan un papel importante en la comunicación intercelular. De este modo, se ha descrito el papel de los exosomas en distintos procesos como la regeneración de tejidos, la respuesta inmunitaria o en la progresión de diferentes tipos de cánceres.

Por otro lado, se ha observado que la actividad de la vía de señalización de Notch juega un papel muy importante en procesos de diferenciación celular, la angiogénesis o la proliferación, entre otros. Además, se ha relacionado con la aparición y progresión de un gran número de patologías, como el cáncer. Dos trabajos recientes, entre ellos uno publicado por nuestro grupo de trabajo, han advertido que la actividad de la vía de Notch puede regularse mediante un nuevo mecanismo de señalización mediado por exosomas. En este trabajo se profundizó en este nuevo de mecanismo de señalización de la vía de Notch en dos contextos diferentes como son las terapias regenerativas y el cáncer.

En la última década, se ha observado que los exosomas procedentes de células tumorales contribuyen a la progresión tumoral a través de mecanismos como la activación de la transición epitelio mesénquima (EMT), el establecimiento de nichos pre-metastáticos o la inmunomodulación. En este sentido, se sabe que la vía de señalización de Notch es capaz de activar la EMT durante la progresión de distintos tipos de cánceres. Así mismo, los cánceres de mama de peor prognosis han sido reiteradamente relacionados con una desregulación de la vía de señalización de Notch. Por estas razones, hipotetizamos que la señalización de la vía de Notch a través de exosomas estaba influyendo en la progresión del cáncer de mama. De este modo, estudiamos la presencia de componentes de la vía de señalización de Notch en los exosomas procedentes de dos líneas tumorales de cáncer de mama, una más agresiva (MDA-MB-231) y otra menos agresiva (MCF-7). Así, se observó que varios componentes de Notch se sobreexpresaron en los exosomas procedentes de MDA-MB-231 en comparación con los procedentes de MCF-7. Fue de particular interés la detección del dominio intracelular con actividad transcripcional de Notch1 (N1ICD) sobreexpresado en los exosomas procedentes de MDA-MB-231. Debido a que previamente había sido demostrado que una sobreexpresión de N1ICD en MCF-7 aumentaba su tumorigenicidad a través de una inducción de la EMT, se evaluó el efecto de los componentes de Notch incorporados en los exosomas procedentes de ambas líneas tumorales sobre cultivos de MCF-7. Nuestros resultados sugieren que los componentes de Notch incorporados en los exosomas procedentes de MDA-MB-231 son funcionales y contribuyen a la inducción de la EMT al ser agregados a cultivos de MCF-7 en parte debido a la actividad de N1ICD.

ANTHRACYCLINE-DERIVED CARDIOTOXICITY STUDY IN IPSC-CM FROM PAEDIATRIC ONCOLOGIC PATIENTS: MIRNA-BASED APPROACH. NODOS RD16/0011/0004 Y RD16/0011/0024

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Cardiac toxicity is a side effect which restricts anthracyclines (AC) treatment in cancer patients. Cardiotoxicity derived from this type of chemotherapy directly affects patient survival and life quality, independently of their oncologic prognostic. In this project, the development of a predictive miRNA signature for chronic or late cardiotoxicity development caused by AC in paediatric cancer patients is proposed.

human induced pluripontent stem cells (hiPSC) have been derived from paediatric patients blood samples with and without cardiotoxicity development after AC treatment. Two cell lines have been generated and characterized by karyotyping, finger print analysis, differentiation to the 3 lines germ layers, immunofluorescence for pluripotent markers and expression of pluripontent related-genes by qPCR. Then, hiPSC lines will be differentiated to cardiomyocytes (hiPSC-CM) and differentiation efficiency tested. Then, iPSC-CM will be treated with Doxorrubicin (Dox), the most common AC used in chemotherapy. After that, differences between iPSC-CM from controls and patients will be measured in terms of oxidative stress, DNA damage, apoptosis and spontaneous calcium activity will be measured. This way, we will be able to establish if iPSC-CM can recapitulate patient phenotype.

In the near future, additional cell lines from pediatric oncologic patients will be generated and miRNA expression patterns before AC treatment will be compared. The present experimental design will allow the validation of the predictive capability of our model to identify which patients are more susceptible to develop cardiotoxicity after chemotherapy. The development of a predictive model of cardiac toxicity will have an important clinical relevance in order to choose a correct treatment for each patient. Moreover, clinicians will be able to decide if it is necessary to administrate cardioprotective drugs during AC treatment. In conclusion, this project will allow give a step forward precision medicine.

ACKNOWLEDGEMENTS

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MYC AND MYCN-MEDIATED CELL COMPETITION IN HEART DEVELOPMENT AND REGENERATION. NODO RD16/0011/0019

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Cell competition ins a mechanism by which fitter cells in a given context eliminate neighbouring cells that are less fit. We have previously demonstrated that Myc is able to induce cell competition in the developing heart in homeostatic conditions by generating genetic mosaics with differences in Myc levels. Myc is considered an essential transcription factor for heart development, but cardiac defects have only been studied in global Myc loss of function models. We eliminated Myc and observed no anatomical, cellular or functional alterations in either fetuses or adult cardiac Myc-deficient mice. We re-examined Myc expression during development and found no expression in developing cardiomyocytes. In contrast, we confirmed that Mycn is essential for cardiomyocyte proliferation and cardiogenesis. Mosaic Myc overexpression in a Mycn-deficient background shows that Myc can replace Mycn function, recovering heart development. We further show that this recovery involves the elimination of Mycn-deficient cells by Cell Competition. Our results indicate that Myc is dispensable during cardiogenesis and adult heart homeostasis and Mycn is exclusively responsible for cardiomyocyte proliferation during heart development. Nonetheless, our results show that Myc can functionally replace Mycn and that cardiomyocytes compete according to their overall Myc+Mycn levels. In the developing heart, Cell Competition eliminates flawed cardiomyocytes, suggesting its relevance as a quality control mechanism in cardiac development. We now seek to explore the role of Cell Competition in neonatal heart regeneration, by LAD ligation in Myc mosaic hearts and testing the effect of Cell competition in promoting a regenerative response

MYC TRANSCRIPTIONAL REGULATION IN MOUSE EMBRYONIC STEM CELLS. NODO RD16/0011/0019

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The Transcription Factor MYC is a general activator of cell anabolism associated with almost every process of the cellular life such as proliferation, growth, differentiation, metabolism and apoptosis. Recently, MYC has been reported as a key factor implicated in cell competition observed in pluripotent stem cells in mammals. During cell competition, cells from the same tissue compare between them by its heterogeneous *Myc* expression in an endogenous manner. In this context, the loser cells with low MYC levels are eliminated in contact with the winner cells, which have high MYC levels. And the winner cell population proliferate in order to maintain tissue homeostasis. This suggest that *Myc* expression has to be precisely regulated during spontaneous cell competition and it is critical for the early development of the whole embryo.

The transcriptional regulation of *Myc* is still a mystery particularly in pluripotent stem cells as mouse Embryonic Stem Cells (mESCs). The major aim of our work is to find cis-regulatory elements involved in *Myc* transcriptional regulation in mESC. Based on previous studies, we focused on a *Myc´*s downstream surrounding region of around 300 kilobases. We established a collaboration with Álvaro Rada-Iglesias group and we have analyzed relevant epigenetic marks of enhancers in the surrounding non-coding regions. Using several published DNA modifications data from different pluripotency states ESCs, we identified 13 putative enhancers with H3K27 acetylations. Moreover, we have created a full picture of the epigenetic landscape of the region of the interest including others epigenetic marks such as H3K4me1, H3K4me3 and H3K27me3, also p300 and Foxd3 binding sites, and 5-Methylcytosine modifications. On the one hand, we have split the putative enhancers into 4 groups, and analyzing Myc expression by generating knock-out (KO) by CRISPR-Cas9 of each group in mESC. After that, we plan to generate KO of each putative enhancer by the same strategy. On the other hand, we are also exploring to perform CRISPR-Cas9-mediated genetic screening which is recently reported to efficiently identify enhancers.

CHARACTERIZATION OF NT3 EXPRESSION IN CORONARY VESSELS. NODO RD16/0011/0030

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Described for the first time in the nervous system, neurotrophins are secreted molecules that perform essential functions by contributing to axonal growth, neuronal survival or synaptic plasticity among. However, neutrophins are also expressed in non-nervous tissues as the bone, the immune or the cardiovascular system. The available information on the role of neurotrophins during heart development is scarce, although several studies have highlighted the importance of these molecules in early cardiomyocyte proliferation, sympathetic nerve growth and coronary vessel maturation. In particular, neurotrophins seem to be involved in the development of the characteristic neurovascular bands (spatial association of coronary blood vessels and sympathetic nerves) of the adult heart. Since neurovascular interactions are thought to be vital to the homeostasis of multiple organs, we suggest they play a similar role in the adult heart.

In this study, immunohistochemical (laser confocal microscopy) and FACS techniques have been applied cardiac tissues to characterize neurotrophin signalling elements (TrKB receptor) in adult homeostatic hearts from wild type and NT3-LacZ$^{-/+}$ mice, paying special attention to changes in cell composition at the coronary arterial walls.

Our results show that neurotrophin receptor TrkB expresses in sympathetic nerves as well as in coronary endothelium and smooth muscle cell at prenatal developmental stages. In the adult, nerves TrkB expression is reduced, but it remains conspicuous in coronary vessels, most especially in the adventitial layer. We also show that NT3 is expressed in the embryonic early myocardium but then this expression reduced in the adult, which retains NT3 coronary smooth muscle expression. Finally, our results show that the partial loss of the NT3 signal unbalances proliferation of coronary vascular cell, suggesting that NT3 is required to control basal cell proliferation in cardiac coronary vessels under normal conditions.

This is a collaboration between TERCEL groups RD16/0011/0017 and RD16/0011/0030. Suggested for poster presentation.

OSHICEL
TERCERA MESA DE PONENCIAS

SESIONES

LEUCEMIA LINFOBLÁSTICA AGUDA E INFILTRACIÓN EN EL SISTEMA NERVIOSO CENTRAL: EFECTO EN EL NICHO NEUROGÉNICO SUBVENTRICULAR. NODOS RD16/0011/0002 Y RD16/0011/0017

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La mejora de las estrategias terapéuticas en el tratamiento de la leucemia linfoblástica aguda (LLA) ha permitido que la tasa de supervivencia supere en la actualidad el 80%, sin embargo, todavía las recaídas, principalmente en el Sistema Nervioso Central (SNC), constituyen una de las principales causas de muerte infantil. Comprender los mecanismos por los que las células leucémicas penetran y sobreviven en el tejido nervioso dando lugar a posteriores recaídas representa uno de los principales desafíos de la enfermedad.

En este contexto, el presente trabajo analiza las principales rutas de entrada de las células tumorales, así como sus principales lugares de asentamiento dentro del parénquima nervioso. Nuestros datos indican que elevados niveles de expresión de BMP4 favorecen el desarrollo de la LLA en un modelo xenogénico, induciéndose un fenotipo más agresivo, y ponen de manifiesto la implicación de la vía de señalización canónica de BMP en la infiltración en el SNC.

El estudio de la localización de los infiltrados en SNC mediante técnicas de inmunofluorescencia y microscopia electrónica indica que, además de los acúmulos meníngeos, es posible observar grupos de células leucémicas en diferentes localizaciones del parénquima nervioso, cercanos en muchos casos a la piamadre y en correlación con el daño observado por RMN en la barrera glial-pial. Además, las células leucémicas que alcanzan el parénquima nervioso muestran quimiorresistencia a la terapia intratecal. La infiltración leucémica provoca una respuesta inflamatoria en el tejido nervioso que va acompañada de alteraciones en la citoarquitectura, muerte neuronal, degeneración de fibras nerviosas y edema. Este daño tisular tiene importantes consecuencias funcionales motoras y sensitivas como indican los test comportamentales realizados.

El estudio por citometría de flujo del nicho neurogénico de la zona subventricular (SVZ) de animales que desarrollan la enfermedad, indica que ésta es una de las localizaciones donde los blastos leucémicos se alojan. Esta colonización induce el bloqueo de la diferenciación de las *neural stem cells* (NSCs) alterando la neurogénesis. Estos datos sugieren que la SVZ podría actuar como santuario frente al tratamiento quimioterápico intratecal, favoreciendo recaídas posteriores en la enfermedad. Además, apoyan que la infiltración leucémica en el SNC podría contribuir al desarrollo de los problemas neurocognitivos detectados en los pacientes de LLA y atribuidos hasta ahora, principalmente, a los efectos secundarios de la terapia intratecal.

ENHANCED ANTI-INFLAMMATORY PROPERTIES OF HUMAN MESENCHYMAL STROMAL CELLS BY TRANSIENT CO-EXPRESSION OF CXCR4 AND IL-10. NODOS RD16/0011/0011 Y RD16/0011/0013

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Mesenchymal stromal cells (MSCs) currently constitute the more frequently used cell type used in advanced therapies with different purposes, most of which are related with inflammatory processes. Although the therapeutic efficacy of these cells has been clearly demonstrated in different disease animal models and also in phase I/II clinical trials, very few phase III trials with MSCs have demonstrated the expected therapeutic efficacy. Aiming at improving the efficacy of these cells without compromising their safety profile, a transient expression of molecules involved in cell migration and anti-inflammatory effects were induced in these cells. To this end, human adipose tissue derived MSCs (AdMSCs) were transfected with monocistronic and bicistronic mRNAs carrying codon optimized versions of CXCR4 and IL10. MSCs transfected with either of these mRNAs expressed significant levels of these molecules, maintained the characteristic immunophenotype and differentiation capacity of MSCs and did not show detectable cytogenetic or genetic abnormalities. CXCR4-expressing MSCs showed an enhanced *in vitro* migration capacity compared to WT-MSCs. Similarly, IL10-expressing MSCs showed improved *in vitro* immunosuppressive properties as compared to WT-MSCs. Using a LPS-induced inflamed pad mouse model, MSCs co-expressing CXCR4 and IL10 mediated a significant reduction in the local inflammation of LPS-treated pads compared to WT-MSCs, that was revealed both by the thickness and also by the leukocytes infiltration in inflamed pads. Biodistribution assays showed an increased migration of both types of CXCR4-expressing MSCs in inflamed pads, as well as a reduction in the number of MSCs trapped in the lungs, compared to animals treated with WT-MSCs. Moreover, using a graft versus host disease mouse model, an increased survival rate was observed in recipients treated with IL10 modified MSCs, as compared to WT MSCs. Taken together, our results demonstrate that the transient expression of homing and/or anti-inflammatory molecules enhances the therapeutic potential of MSCs, suggesting that these new generation of MSCs should constitute a new step in the development of advanced therapies for the treatment of inflammatory diseases.

OPTIMIZED CART CELLS FOR IMPROVED THERAPIES AGAINST R/R AML AND ALL. NODO RD16/0011/0005

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Acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) are the most common form of acute leukemia in childhood and adults respectively. Despite good initial responses to conventional chemotherapy and hematopoietic stem cell transplantation, the prognosis of patients with R/R ALL or R/R AML is very poor. Therefore, there is still a clear unmet need for those patients being crucial the development of novel therapeutic strategies. Chimeric antigen receptor (CAR) T cell-based immunotherapy is emerging as one of the most promising advanced therapy for cancer treatment. CART therapies (especially those targeting CD19) have demonstrated impressive results but with relevant side effects (i.e. cytokine release syndrome, "on-target/off-tumor" toxicity...) and also imply a prohibitive cost for Health Authorities when considering their application in a progressively number of diseases. Our hypothesis is that the use of novel technologies, would contribute to ensure the viability of innovative, safe and efficient CART cell therapies for R/R ALL and AML. Thus, our aim is: i) to develop, select and fully characterize improved CART cells targeting CD33, which is expressed in up to 90% of AML blast, as well as ii) to implement a virus-free gene-transfer strategy based on Sleeping Beauty transposition, that would contribute to reduce the cost of CART manufacturing.

Thus, based on previously described antibodies against CD33 we have generated two different CARTs with 4-1BB costimulatory domain that showed strong *in vitro* cytotoxic effect. To improve our CART cells, we have modified several moieties of the CAR structure, generating twelve different CARs with different hinge-TMD regions. The best constructs showing specific activation along with reduced tonic signaling have been selected for further characterization using a NFAT/NFkB/AP1 triple reporter system in Jurkat cells. We have compared the antitumoral efficacy of these selected constructs performing *in vitro* killing assays against CD33 positive AML cell lines.

On the other hand, to implement the innovative Sleeping Beauty technology for CART cell production we have generated a CAR targeting CD19 based on the validated FMC63 clone (present in tisa-cel and axi-cel). This structure has been cloned into a pT2 transposon vector and initial experiments showed transduction efficacies of up to 45-55%, compatible with clinical applications. Currently, cell expansion, cytokine production and specific *in vitro* cytotoxicity and *in vivo* antitumoral efficacy against CD19 positive cells is being tested.

Finally, i*n vivo* experiments and additional genomic studies at single cell level are being performed in order to select the constructs that will be validated in a large-scale production at GMP level.

SECRETOMA DE CÉLULAS MADRE MESENQUIMALES PARA LA CICATRIZACIÓN DE ÚLCERAS CUTÁNEAS CRÓNICAS: PRUEBA DE CONCEPTO. NODO RD16/0011/0022

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Esta ponencia es fruto de la colaboración entre los grupos de Málaga (José Becerra) y Barcelona (Elisabeth Engel).

Las úlceras crónicas, representan en la actualidad una importante carga económica en el sistema sanitario de los países desarrollados y causan un impacto significativo en el bienestar de las personas que las padecen. Dado su alto nivel de prevalencia, se han convertido en una verdadera "epidemia silenciosa", asociadas al aumento de factores de riesgo de las sociedades modernas, tales como la edad avanzada, la obesidad y la diabetes.

Las células madre mesenquimales (MSC) ejercen su efecto terapéutico de forma paracrina, a través de la secreción de multitud de factores bioactivos englobados bajo el concepto de secretoma. El empleo del secretoma como elemento terapéutico está abriendo interesantes perspectivas como tratamiento biológico en muy diversas patologías.

Se ha desarrollado un protocolo de obtención, pauta y método de administración controlada de secretoma de MSC para promover la curación de heridas crónicas de la piel, en un modelo murino. La administración local y controlada de secretoma promueve el reclutamiento y la colonización celular del lecho cicatricial, la síntesis de factores angiogénicos y de los componentes de la matriz extracelular que facilitarán la cicatrización de la herida.

A diferencia de las terapias basadas en células, el secretoma tiene grandes ventajas en el tratamiento de las úlceras crónicas en entornos clínicos. Proporciona baja inmunogenicidad, ausencia de riesgo tumorogénico y enormes ventajas logísticas, al permitir una rápida disponibilidad para el tratamiento, poder producirse y escalarse de forma sistemática, continua, así como almacenarse para poder ser formulado en la presentación farmacéutica más adecuada, por lo que a medio plazo, la tecnología desarrollada de liberación de secretoma podrá implementarse para diferentes indicaciones clínicas.

Nuestro propósito es desarrollar un sistema de liberación de los componentes del secretoma que proporciona las condiciones óptimas para facilitar la cicatrización de heridas y eliminar la necesidad de cambios frecuentes de apósitos. Como mecanismos de acción fundamental del mismo, nos basamos en estimular la proliferación y movilización de MSC o progenitores específicos del tejido, responsables de promover la reparación y la regeneración locales, así como potenciar la angiogénesis que permita el suficiente aporte nutritivo y de oxígeno, deficiente en esta patología.

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POSTERS

EX VIVO FUCOSYLTRANSFERASE VII TREATMENT INCREASES ADIPOSE MESENCHYMAL STEM CELL ANTI-INFLAMMATORY PROPERTIES UPON HCELL/CD44 LIGATION. NODO RD16/0011/0001

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BACKGROUND

Mesenchymal stem cells (MSCs) are a multipotent progenitor cell population distributed throughout all tissues. *In vitro*, MSCs display potent anti-inflammatory/immunomodulatory properties. However, all MSCs lack molecular effectors of cell migration, being incapable of actively infiltrating inflammatory sites. It has been shown that e*x vivo* enzymatic fucosylation of the molecule CD44 on MSCs generates the potent E-selectin ligand HCELL, enabling *in vivo* MSC migration to endothelial beds that express E-selectin (*i.e.,* bone marrow and inflamed tissues). However, apart from this augmented migration capacity, to date the immunobiology and functional properties of fucosylated MSCs has not been studied.

METHODS

Ex vivo fucosyltransferase VII (FTVII) treatment and mitogen proliferative assays were performed as described previously[1, 2]. Briefly, murine splenocytes were isolated from C57BL/6 and BALB/c mice spleen cell suspensions. Then, $1x10^5$ splenocytes were treated with 10 mg/ml concanavalin A to induce T cell proliferation. At the beginning of the cultures, murine AdMSCs (mAdMSCs) from C57BL/6 mice were seeded in wells at decreasing ratios. After 3 days of culture, cellular proliferation was measured by BrdU incorporation. In some experiments, unmodified AdMSCs (UmAdMSCs), fucosylated AdMSCs (FucmAdMSCs) or FucmAdMSCs followed by treatment with sialidase (sialFucmAdMSCs) were previously cultured in presence of different concentrations of murine E-selectin-IgG (mE-IgG) or hyaluronic acid (HA), following described protocols[3]. To block CD44/HCELL interactions to HA a purified anti-mouse CD44 antibody was used. To study the contribution of different anti-inflammatory molecules in the immunosuppressive properties of mAdMSCs, the TGFβ inhibitor SB-431542, the IDO inhibitor 1-methyl-DL-tryptophan (1-MT) or the iNOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) was added at the beginning of the cultures. Levels of TGFβ, IDO and nitric oxide metabolites in culture supernatants were measured by ELISA and nitrate/nitrite assay, respectively.

RESULTS

To analyze the effects of exofucosylation on immunoregulatory properties of mAdMSCs, we evaluated the capacity of UmAdMSCs and FucmAdMSCs to suppress mitogen-induced proliferation of both syngeneic and allogeneic murine T cells. Co-incubation with UmAdMSCs or FucmAdMSCs significantly inhibited mitogen-stimulated splenocyte proliferation in a dose-dependent manner from ratio 1:1 to ratio 1:20, with identical inhibitory effects using both types of mAdMSCs, in both syngeneic (Fig. 1A) and allogeneic contexts (Fig.1B).

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However, co-incubation for 72h of FucmAdMSCs with mE-IgG, but not with isotype control IgG, significantly suppressed donor T cell proliferation in the mitogenic assay (Fig. 2A). Importantly, this effect was abrogated by elimination of E-selectin adherence by sialidase treatment of FucmAdMSCs (sialFucmAdMSCs), indicating that the observed augmented anti-proliferative effect is directly related to the capacity of HCELL to engage E-selectin. To determine whether this effect is secondary to ligation of CD44 itself, we performed this analysis using UmAdMSCs previously cultured in presence of the conventional CD44 ligand, HA. Also ligation of CD44 with HA induced a similar anti-proliferative effect, but, as expected, there was no boosting of UmAdMSC immunomodulation in presence of mE-IgG (Fig. 2A,B)**.** Importantly, the improved anti-mitogenic effect observed after E-selectin or HA engagement was abrogated in presence of a function-blocking anti-CD44 mAb treatment (Fig. 2A,B). To elucidate the relevant molecular effector(s) involved in the improved anti-mitogenic effects observed after E-selectin and HA ligation, we analyzed expression of TGFβ, IDO, nitrates/nitrites (*e.g.,* nitric oxide (NO) metabolites), each of which is reported to mediate immunosuppression. Engagement of HCELL via E-selectin and of CD44 via HA, in each case, profoundly boosts levels of TGFβ, IDO, and NO metabolites (Fig. 3 A,B,C). To further analyze the contributions of TGFβ, IDO and NO on the observed MSC anti-proliferative effect, we performed *in vitro* MSC:splenocyte co-cultures in presence of inhibitors of these molecules, with or without co-incubation with HA or E-selectin. Addition of SB-431542, 1-MT or L-NMMA in each case significantly rescued the proliferation of mitogen-stimulated splenocytes, and simultaneous use of all three inhibitors resulted in complete recovery of proliferation (Fig. 4). Collectively, these results indicate that these molecules play pivotal roles in the mAdMSC-mediated immunomodulatory effects on activated splenocytes.

CONCLUSIONS

These findings indicate that CD44/HCELL ligation to HA and E-selectin, respectively, unleashes MSC immunobiologic properties indicating that fucosylated AdMSCs may be a new safe and more effective cell therapy product for the treatment of immune-mediated disorders.

ACKNOWLEDGMENTS

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NON-CLINICAL ***IN VIVO*** SAFETY STUDY OF HUMAN UMBILICAL CORD LINING STEM CELLS AS INVESTIGATIONAL MEDICINAL PRODUCT FOR INTRAVENOUS INJECTION: TOXICOLOGY AND KINETICS OF BIODISTRIBUTION IN NOD/SCID MOUSE MODEL. NODO RD16/0011/0001

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BACKGROUND

Rheumatoid arthritis is a chronic systemic inflammatory disease that mainly affects the joints, and is clinically manifested by pain, joint stiffness, and synovitis, which leads to cartilage damage and bone erosion. Several preclinical and clinical studies have shown that cell-based therapy using mesenchymal stem cells (MSC) from different sources represents a promising therapeutic tool for treating rheumatoid arthritis. We hypothesized that intravenous infusion of umbilical cord lining stem cells (ULSC) may constitute a safe procedure with no significant related adverse effects that would rule out its later use in a clinical trial designed to evaluate its therapeutic efficacy in rheumatoid arthritis and other inflammatory diseases.

PURPOSE AND METHODS

This non-clinical *in vivo* study was developed using 7 to 9 weeks old males and females NOD/SCID mice. The safety and toxicity assay was performed by the transplant of two different doses of ULSC wild type ($5x10^4$ or $1x10^6$ cells/mouse) in a unique infusion. The animals were evaluated using the test based on the clinical score exam of Lloyd and Wolfensohn from 5 minutes after the transplant to the final of the study (4 months after the transplant), and the cell biodistribution and tumorigenicity was studied in the different organs obtained from the animals after sacrifice. For that, some sections from each organ were stained with hematoxylin/eosin and with anti-human Ki67, anti-human CD90 and anti-mouse CD3 and antibodies by immunohistochemical staining. Also, haematological and plasma biochemical analyses were performed to demonstrate the safety of the cell transplant.

To evaluate the kinetics of biodistribution of the cells *in vivo*, ULSC were transfected with the luciferase gene and intravenously injected into the animals in the same doses previously mentioned. After the injection of the luciferase substrate luciferine into the animals, the bioluminescence signal was evaluated *in vivo* by using an IVIS Lumina imaging system at different time points until the signal disappears. In some organ samples, real-time PCR assays was performed in order to look for human gene expression (β-actin and β-microglobulin) using murine GAPDH as a control of the assay.

RESULTS

Our findings show overall safety and tolerability of ULSC injected intravenously in a single dose—either $5x10^4$ or $1x10^6$ cells/mouse—in a NOD/SCID mouse model with final evaluation time point at 4 months. As an immediate systemic

adverse event, death due to pulmonary embolism or venous thromboembolism occurred in few mice within a few seconds after intravenous infusion with high dose of ULSC. This adverse event is usually due to the larger size of the cells together with their high capacity of aggregation, but independent of the source or cell type. Most of the mice displayed no body weight loss neither alteration in any parameter assessed by the clinical score at any time point. Macroscopic examination of the organs revealed in few mice evidence of enlarged thymus and/or spleen, and subsequent microscopic examination of histology and immunohistochemistry determined the diagnosis of T cell lymphoma with positive immunolabeling with anti-mouse CD3 antibody, a common pathology in this mouse strain. Quantitative PCR assays using oligos against the human β-actin and β2-microglobulin genes provided proofs against the presence of human DNA in these tissues. Mice transplanted with ULSC-Luc^{+} showed a bioluminescent signal predominantly in the lungs, which remained until day +3 post-infusion, and then began to spread to the lower abdomen and ribs which could correspond to the last section of the large intestine and/or gonads and kidney. No residual signal was observed in any of the organs analyzed after the sacrifice of the animals.

CONCLUSIONS

Based on the results obtained in this non-clinical study, intravenous infusion of human umbilical cord lining stem cells in NOD-SCID mice may be considered a safe procedure, at least in this strain of animals and at a final time point of 4 months. The production of ULSC on a clinical scale and under GMP conditions could constitute a new therapeutic approach to investigate in clinical studies for an indication such as rheumatoid arthritis.

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This study has been funded by DaVinci Bioscience LLC (Yorba Linda, California, USA) and the Spanish Net of Cell Therapy (TerCel), RETICS subprogram of the I+D+I 2013-2016 Spanish National Plan, project RD16/0011/0001 (to J.M.M), funded by Instituto de Salud Carlos III and co-founded by FEDER "una manera de hacer Europa".

RESPINE, UN ENSAYO CLÍNICO EUROPEO PARA EL TRATAMIENTO DE LA DEGENERACIÓN DISCAL CON CÉLULAS MESENQUIMALES ALOGÉNICAS. NODO RD16/0011/0003

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El dolor lumbar en la parte baja de la espalda es muy frecuente. Un 75% de la población lo ha sufrido alguna vez y, aunque un 90% de estos pacientes se curan en 3 meses, un 10% se cronifica, lo que supone unos 67 millones de personas afectadas en Europa (5-6 millones en España). La enfermedad tiene un enorme impacto en la calidad de vida y origina gastos sanitarios de más de 12.000 millones de euros anuales. En muchos casos la enfermedad se debe a la degeneración del disco intervertebral. El tratamiento de referencia es la fusión vertebral, de la que se realizan unas 1.000 intervenciones anuales en España, pero puede causar alteraciones de la movilidad y degeneración de otros discos. Utilizando como base nuestro estudio anterior (*Noriega et al., 2017*) propusimos, junto con otros grupos europeos (que incluyen dos más de TerCel), y coordinados por la Universidad de Montpellier, el proyecto RESPINE, que contempla un ensayo clínico multicéntrico con 112 pacientes en el que participan 8 hospitales de 5 países de la UE, y que ha sido financiado por el programa Horizonte 2020. El proyecto RESPINE pretende llevar esta innovadora terapia con células mesenquimales alogénicas a la clínica, y mejorar la sintomatología y la calidad de vida de los pacientes con dolor lumbar originado por degeneración discal. El proyecto incluye también paquetes de trabajo dedicados a la investigación de los mecanismos de acción de las células mesenquimales, y especialmente al estudio de las respuestas inmunes y la seguridad. El procedimiento celular es simple y no invasivo, fácil de llevar a cabo por los médicos clínicos de los hospitales de Salud Secundaria y los Centros Clínicos europeos. Finalmente, RESPINE pretende, bajo el liderazgo de investigadores europeos, lograr una buena relación coste-eficacia que haga accesibles estos tratamientos en la UE. Recientemente hemos demostrado la factibilidad y seguridad del tratamiento con células madre mesenquimales, (MSV). Hubo, además claros indicios de eficacia, que llegó hasta el 71% con células autólogas (*Orozco et al., 2011*) y al 40% con alogénicas (*Noriega et al., 2017*). Hubo también mejora significativa de la hidratación de los discos, medida por Resonancia Magnética. El procedimiento se está poniendo en marcha también en la Clínica Universitaria de Navarra (TerCel-Pamplona) y *Orozco et a*l (TerCel-Teknon-Barcelona) han obtenido confirmación de los resultados con Células autólogas en tratamientos compasivos (*Soler Rich et al, 2015*). Por ello pensamos que la terapia con células mesenquimales podría ser una alternativa válida para el tratamiento de la degeneración discal, pues es más simple y conservadora que la cirugía de fusión vertebral, preserva la biomecánica y consigue una mejora del dolor igual o mayor que el tratamiento convencional. Por todo ello, sería muy interesante realizar un ensayo clínico multicéntrico con una cohorte más numerosa.

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More details in: ***https://cordis.europa.eu/project/rcn/207207/factsheet/en***

IN VIVO GENOME EDITING STRATEGIES FOR THE TREATMENT OF PRIMARY HYPEROXALURIA TYPE 1. NODO RD16/0011/0005

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Sequence-specific endonucleases, specially CRISPR/Cas9 systems, have substantially increased the efficiency and specificity of genome editing, enabling the precise modification of the genome. Genome editing technologies not only facilitate deciphering the contribution of a specific mutation(s) in particular genetic disease but also represent an invaluable tool for the development of innovative therapeutic strategies for genetic diseases. In our group we are interested in the use of CRISPR/Cas9 systems as therapeutic approaches for treatment of Primary Hiperoxaluria type 1 (PH1), an inherited autosomal rare metabolic disease, caused by deficiencies in the hepatic alanine:glyoxylate aminotransferase (AGT). We have demonstrated that CRISPR/Cas9-mediated substrate reduction therapies (SRTs) represents a promising therapeutic option for PH1. Our previous results clearly indicate that glycolate oxidase (GO) targeting reduces urine oxalate levels and kidney damage without toxic symptoms. In this work, we have extended this therapeutic strategy to target other enzymes of the glyoxylate metabolism that would be widely applicable not only for PH1 but also for other PH subtypes. We have developed CRISPR/Cas9 systems targeting *Ldha* gene that encodes the hepatic lactate dehydrogenase (LDH). A single administration of AAV8 therapeutic vectors drastically reduced LDH levels in the liver of PH1 and PH3 mice, also reducing urine oxalate levels and kidney damage without toxic symptoms. Genome wide off-target analysis revealed the safety of this approach with no indel detection in the liver of treated animals. Altogether, our data provides evidence that *in vivo* genome editing technologies would provide new tools for improved and more universal therapeutic approaches for PH.

EFFECT OF BMP2 OVEREXPRESSION IN APPENDICULAR SKELETON DEVELOPMENT. NODO RD16/0011/0005

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Bone morphogenic proteins (BMPs) are multifunctional growth factors which belong to the TGF-β superfamily of proteins. BMPs have an important role in development and participate in the growth of almost all organ systems. Among the BMP family, BMP-2 has important roles in cardiac and joint development, endochondral bone formation and bone maintenance and repair. In fact, *BMP2* expression during early stages of fracture healing is compulsory for starting the reparative process. Here we evaluate the effects of the overexpression of *Bmp2* over osteochondral development.

Transgenic mice conditionally overexpressing BMP2 (Rosa26-Bmp2^{GOF}) were crossed with transgenic mice expressing Cre recombinase under the control of Prrx1 promoter (Prrx1-Cre). With this strategy, mice offspring would overexpress *Bmp2* in their limb buds, calvaria and rib cage. Appendicular skeleton structure was analyzed employing micro CT, while articular tissue will be analyzed by histology.

Overexpressing *Bmp2* during the embryonic development of the limbs generates a lethal phenotype in almost every mutant mouse. From an offspring of 28 mice analyzed (with an expected mendelian segregation of 50% of them overexpressing *Bmp2*) only one animal carried a copy of the recombinase Cre, as detected by genomic PCR. The Prrx1-BMP2^{GOF} mouse presented small hindlimbs in comparison with his WT littermates. Furthermore, micro CT analysis demonstrated that *Bmp2* overexpression caused important bone abnormalities, including changes in femur and tibia morphology with fused bone elements and abundant bone exostosis. Morphometric analysis showed that overexpressing *Bmp2* results in enlarged diaphyseal bone diameter with reduced mineral density.

TISSUE ENGINEERED SCAFFOLDS FOR MIMETIC AUTOGRAFTS. NODO RD16/0011/0005

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Bone tissue has an intrinsic regenerative capacity. However, this regeneration can be compromised, leading to delayed fracture healing and nonunion. Due to the scarcity of bone tissue that can be used as autograft, tissue engineering strategies arise as a sound solution by using biocompatible materials functionalized with cells and morphogens.

Our objective is to design engineered autografts capable of efficiently treat fracture nonunion. For this purpose, we designed polycaprolactone (PCL) based mimetic autografts composed of an inner cylindric scaffold, produced by PCL extrusion, providing mechanical stability and an osteoconductive environment. Additionally, we created a mimetic autograft (MA) by the addition of an exterior thin and highly porous fibrillar tube of PCL, synthesized by melt electrospinning writing (MEW), mimicking the periosteum. To evaluate their regenerative capacity, these scaffolds were placed in critical size femur defect model in rats and compared with rats were the defect was left untreated (Empty). Ten weeks after surgery μCT and histological studies were carried out.

At the μCT level, structural mimetic PCL scaffolds, devoid of cells and morphogens, showed no significant differences in healing or bone formation (Empty group, 11.47 ± 4.93 mm^3; MA, 14.95 ± 3.09 mm^3, $p = 0.1711$). Histological analysis demonstrates that MEW PCL mimicking periosteum enhances bone growth and present good implant integration, but insufficient for successful healing.

In conclusion, acellular mimetic autografts need to be optimized by functionalization with morphogens (BMP-2, BMP-7) and/or mesenchymal progenitor cells.

ADVANCED THERAPIES FOR THE TREATMENT OF PH1: CELLULAR REPROGRAMMING AND GENE EDITING. NODO RD16/0011/0011

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Primary Hyperoxaluria Type 1 (PH1) is an inherited rare metabolic disease causing urolithiasis, nephrocalcinosis, interstitial fibrosis and, in the final progression of the disease, renal failure. It is caused by a deficiency in the alanine:glyoxylate aminotransferase that is expressed in liver peroxisomes and is involved in the glyoxylate metabolism. Early diagnosis and rapid onset of supportive therapies are critical for maintaining long-term renal function, but when a reduction in oxalate levels is not achieved, the only potentially curative treatment is organ transplantation. Thus, the research of new therapeutic options for the treatment of these patients appears as a high priority.

We propose the combination of cellular reprogramming (by direct reprogramming or differentiation of PH1-derived iPSCs) and site-specific gene correction for the generation of autologous phenotypically healthy induced hepatocytes (iHeps) and hepatocyte-like cells (HLCs) from fibroblasts and from mononuclear cells of PH1 patients. Three-dimensional cultures of corrected iHeps on decellularized liver matrixes will be optimized as a tool for disease modelling. The capability of corrected iHeps to regenerate damaged mouse liver *in vivo* will be evaluated as a potential cellular source for regenerative purposes.

Gene editing of *AGXT* gene has been addressed by homologous-directed repair (HDR). For the correction of *AGXT* mutations we have designed specific gene editing tools that address the gene correction by two different strategies. In the first strategy, a specific mutation correction (c.853T-C, p.I244T) has been obtained after recombination with ssODN harboring wild-type sequence. In the second strategy, the whole corrected cDNA of the *AGXT* gene has been inserted near the starting codon of the endogenous gene, what constitutes an almost universal correction strategy for mutations causing PH1. Off-target analysis is being conducted.

Cellular reprogramming and *in vitro* differentiation protocols has been designed to obtain iHeps and HLCs. An *in vitro* characterization of reprogrammed cells has been conducted that demonstrated hepatic function, including up-regulation of genes involved in the glyoxylate metabolism. Moreover, PH1-HLCs exhibited a reduced activity of AGT enzyme, in comparison to WT-HLCs, and this reduced activity was recovered after specific gene correction.

The development of these advanced therapies is essential to provide alternatives to current treatments for metabolic diseases in which the improvement of *in vitro* models that resemble more accurately the human pathophysiology could lead to more efficient therapies, in the short and medium term. In the long term, these strategies could be useful as alternative cellular source to replace endogenous deficient hepatocytes with functional corrected cells.

NATURAL ESTROGENS ENHANCE HUMAN HEMATOPOIETIC ENGRAFTMENT IN A XENOGENIC TRANSPLANTATION MODEL. NODO RD16/0011/0011

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Hematopoietic Stem Cell Transplantation (HSCT) is routinely used to reconstitute hematopoiesis after myeloablation, being the most commonly-used cell therapy. HSCT efficacy and multilineage reconstitution can be limited by inadequate HSPC (Hematopoietic Stem and Progenitor Cell) number, poor homing, engraftment, or limited self-renewal. As recent evidence indicates that estrogens are involved in regulating the hematopoiesis, we sought to examine whether natural estrogens (estrone or E1, estradiol or E2, estriol or E3 and estetrol or E4) modulate human HSPCs. Our results show that human HSPC subsets express estrogen receptors differently. Additionally, these natural estrogens have distinct impact on human HSPCs in vitro. We found that both E2 and E4 expand human HSPCs and that E4 was the best tolerated and less toxic estrogen for human HSPCs. Furthermore, we show that a short-term treatment of immunodeficient mice transplanted with $hCD34^+$ cells with E2 or E4 improves human hematopoietic engraftment. Human cord blood $CD34^+$ cells ($CB\text{-}CD34^+$) were transplanted into sublethally irradiated immunodeficient NSG mice. Three days after transplantation, mice were treated for four days with daily subcutaneous doses of E2, E4 or vehicle. Human hematopoietic engraftment was evaluated in the BM of transplanted mice at four months later. E2 and E4 estrogens increased the proportion of $hCD45^+$ cells without modifying the proportion of myeloid and lymphoid lineages. Significantly, animals treated with either estrogen had significantly higher levels of human HSPCs.

Collectively, our data support a new application of estrogens to improve the hematopoietic recovery after HSCT. This application may have particular relevance to enhance the hematopoietic recovery after myeloablative conditioning and when limiting numbers of HSCs.

COMBINACION DE RUXOLITINIB Y LINFOCITOS T REGULADORES COMO TRATAMIENTO DE LA ENFERMEDAD INJERTO CONTRA HUESPED CRONICA. NODO RD16/0011/0035

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INTRODUCCIÓN

La enfermedad injerto contra huésped crónica (EICHc) postrasplante se asocia a un alto riesgo de morbilidad y mortalidad. En el presente ensayo clínico, establecemos un método de enriquecimiento de linfocitos T reguladores CD4+CD25+CD127- (Tregs) infundidos en fresco junto a terapia concomitante con ruxolitinib, inhibidor de JAK1/2, con el fin de explorar el potencial sinérgico de ambas estrategias en el tratamiento de pacientes con EICHc.

OBJETIVOS

Nos planteamos evaluar la sinergia entre ruxolitinib y Treguladores tanto en un modelo preclínico como en un ensayo clínico (proyecto TREGeneration, Programa Europeo Horizonte 2020) en pacientes con EICHc refractarios a corticoides que no han obtenido remisión completa bajo tratamiento con ruxolitinib.

MATERIALES Y MÉTODOS

27 ratones Balb/C ($H2^d$) fueron trasplantados con esplenocitos de donantes C57J ($H2^b$) con disparidad en el MHC y asignados a 4 grupos de tratamiento en el día 28 postrasplante: 1) Ruxolitinib, 2) Tregs, 3) Ruxolitinib + Tregs y 4) Vehículo..
En base a los resultados del estudio preclínico se plantea un estudio multicéntrico, prospectivo. Se incluyen 12 pacientes con EICH moderada o grave bajo tratamiento con ruxolitinib. Se establecieron 3 cohortes de 3 pacientes que recibieron Nivel A: 0.5 x10e6 Treg/kg, Nivel B: 1x10e6/kg y Nivel C: 2 x10e6/kg. La purificación se llevó a cabo mediante enriquecimiento secuencial de células Tregs en 2 pasos utilizando el sistema CliniMACS (Miltenyi): 1) Co-depleción de CD8+/CD19+, seguido de: 2) Selección positiva CD25+.

RESULTADOS

El modelo murino muestra una mayor efectividad del tratamiento combinado con ruxolitinib y T reguladores en la evolución del score clínico de EICH que también se relaciona con una mejora significativa de la supervivencia en los ratones que reciben ambos tratamientos.
En el ensayo clínico se incluyeron un total de 12 pacientes (pts), de los cuáles se infundieron 11 (Nivel A: 3 pts, Nivel B: 3 pts, Nivel C: 5 pts). No se alcanzó toxicidad limitante de dosis. A un máximo de un año de seguimiento, los eventos adversos fueron mayoritariamente de grados 1 y 2. El único evento adverso grado >3 consistió en una necrosis avascular en contexto de inmunosupresión postrasplante no relacionado con el tratamiento. De 11 pts evaluables, 5/11 pre-

sentaron algún grado de mejora en el score NIH de EICHc, 10/11 redujeron la inmunosupresión y 7/11 presentaron mejoría en la escala de puntuación de síntomas de EICH. La supervivencia global de la serie fue del 100%. La mediana de seguimiento fue de 24 semanas (rango 4-48).

CONCLUSIONES

En combinación con ruxolitinib, la infusión de linfocitos T reguladores es segura y tiene eficacia clínica en pacientes afectos de EICH crónica moderada o severa refractarios a corticoides. Los resultados son concordantes con los obtenidos en el modelo murino, cuyos resultados preliminares muestran mayor efectividad del tratamiento combinado con ruxolitinib y T reguladores en la evolución del score clínico usado para evaluar la EICH.eguladores en la evolución del score clínico usado para evaluar la EICH.

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