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ISEV2022 Abstract Book

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OWP2.05=PT11.04 | Controlled release of epigenetically-activated extracellular vesicles from a gelMA/nanoclay composite hydrogel to promote bone repair

Kenny Man[1]; Inês A. Barroso[1]; Mathieu Y. Brunet[1]; Ben Peacock[2]; Angelica S. Federici[3]; David A. Hoey[3]; Sophie C. Cox[1]
[1]University of Birmingham, Birmingham, United Kingdom; [2]NanoFCM Co., Ltd., Nottingham, United Kingdom; [3]Trinity College Dublin, Dublin, Ireland

Introduction: Extracellular vesicles (EVs) have garnered growing attention as promising acellular tools for bone repair. Although EVs potential has been shown, issues associated with their therapeutic potency and short half-life in vivo hinders their clinical utility. Epigenetic reprogramming with the histone deacetylase inhibitor Trichostatin A (TSA) has been reported to promote the osteoinductive potency of osteoblast-derived EVs. Gelatin methacryloyl (GelMA) hydrogels functionalised with the synthetic nanoclay laponite (LAP) have been shown to enhance the retention of bioactive factors. This study investigated the potential of utilising a GelMA-LAP hydrogel to improve local retention and control delivery of epigenetically-activated EVs as a novel bone repair strategy.
Methods: GelMA/nanoclay composites were fabricated by combining 5wt% GelMA with different concentrations of LAP (0.5, 1 and 2 wt%) prior to visible light crosslinking. The hydrogels compressive modulus, shear-thinning behaviour, 3D printing fidelity and osteogenic potency was evaluated. EVs were derived from 5 nM TSA-treated or untreated osteoblasts over a 2-week period. EVs size, morphology and concentration were assessed via nanoflow cytometry and transmission electron microscopy. Isolated EVs were incorporated within the composites and their release kinetics were determined using the CD63 ELISA. Human bone marrow stromal cells (hBMSCs) osteogenic differentiation within the EV-functionalised hydrogel was evaluated by biochemical and histological analysis.
Results: LAP incorporation improved GelMA compressive modulus, shear-thinning properties and 3D printed shape fidelity in a dose-dependent manner when compared to LAP-free gels. Moreover, hydrogels containing LAP increased hBMSCs mineralisation capacity (1.41-fold) over 14 days. EV release kinetics from these nanocomposites were strongly influenced by LAP concentration with significantly more vesicles released from LAP-free constructs. EVs derived from TSA-treated osteoblasts (TSA-EVs) enhanced proliferation (1.09-fold), migration (1.83-fold), and mineralisation (1.87-fold) of hBMSCs when released from the GelMA-LAP hydrogel compared to the untreated EV gels. Importantly, the TSA-EV functionalised GelMA-LAP hydrogel significantly promoted encapsulated hBMSCs extracellular matrix collagen production (≥1.3-fold) and mineralisation (≥1.78-fold) in a dose-dependent manner compared to untreated EV constructs.
Summary/Conclusion: Taken together, these findings demonstrate the potential of combining epigenetically-activated EVs with a nanocomposite photocurable hydrogel to enhance the therapeutic efficacy of acellular vesicle approaches for bone repair.

OWP2.06=PT11.15 | The role of Notch2 in osteoclastogenic and angiogenic potential of extracellular vesicles in multiple myeloma

Domenica Giannandrea[1]; Natalia Platonova[1]; Michela Colombo[1]; Mara Mazzola[2]; Valentina Citro[1]; Raffaella Adami[1]; Filippo maltoni[1]; Silvia Ancona[1]; Vincenza Dolo[3]; Ilaria Giusti[3]; Andrea Basile[4]; Anna Pistocchi[2]; Laura Cantone[5]; Valentina Bollati[6]; Lavinia Casati[1]; Elisabetta Calzavara[7]; Mauro Turrini[7]; Elena Lesma[1]; Raffaella Chiaramonte[1]
[1]Department of Health Sciences, Università degli Studi di Milano, Milano, Italy; [2]Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milano, Italy; [3]Department of Life, Health and Environmental Sciences- University of L'Aquila, L'Aquila- Italy, L'Aquila, Italy; [4]Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Milano, Italy; [5]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milano, Italy; [6]Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy, Milano, Italy; [7]Division of Hematology, Valduce Hospital, Como, Italy

Introduction: Multiple myeloma (MM) is still uncurable due to the crosstalk between MM cells and bone barrow (BM) cells. Extracellular vesicles (EVs) play a key role in the pathological communication between MM and BM. Notch2 receptor and Jagged1 and 2 are overexpressed in MM, triggering Notch pathway also on BM population and inducing their pro-tumorigenic activity. Here, we investigate the effect of MM-derived EVs in eliciting the pro-tumoral effect of BM in MM progression and the role of vesicular Notch2.
Methods: MM cells constitutively inhibited for Notch2 (MMN2KD-EVs) and the shed EVs (MM-EVs) were characterized for Notch receptors by Western blot, for size and number by nanoparticle tracking analysis and electronic transmission microscopy, and for their uptake in recipient cells by confocal and flow cytometry. The transfer of Notch2 via EVs was evaluated by an in vitro system of sending cells expressing HA-tagged Notch2, their EVs and receiving cells. Notch pathway activation was assessed by Notch reporter assays in vitro (HeLa cells) and in vivo (transgenic zebrafish embryos). The pro-tumorigenic effect of MM-EVs and MMN2KD-EVs were evaluated by measuring their osteoclastogenic and angiogenic potential in vitro. To confirm the role

of vesicular Notch, the effect of MM-EVs or EVs from the BM of MM patients was assessed in the presence of a g-secretase inhibitor (GSI), which affects Notch activation (Informed consent and IRB of Insubria approval was obtained n. 1/2018.).
Results: MM-EVs carry and transfer Notch2 increasing Notch signaling in recipient endothelial cells and osteoclasts. MM-EVs induce the osteoclast formation and angiogenesis in a Notch2 dependent way. GSI effectively decreased the osteoclastogenic and angiogenic potential of MM-EVs in vitro and the angiogenic effect of EV from MM patients' BM.
Summary/Conclusion: These results suggest that targeting Notch pathway may hamper the pro-tumorigenic activity of MM-EV in the BM.

OWP2.07=PT11.02 | CD63 Regulates Cholesterol storage within endosomes and its distribution via exosomes

Roberta Palmulli[1]; Mickael Couty[2]; Frederik Verweij[3]; Stéphanie Charrin[4]; Maharajah Ponnaiah[5]; Melissa C. Piontek[6]; Florent Dingli[7]; Wouter H. Roos[6]; Eric Rubinstein[8]; Marie Lhomme[9]; Anatol Kontush[9]; Damarys Loew[7]; Graça Raposo[10]; Guillaume van Niel[11]
[1]Department of Pathology, University of Cambridge, Cambridge. CB2 1QP. United Kingdom, Cambridge, United Kingdom; [2]Université de Paris, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, F-75014 Paris, France, paris, France; [3]Université de Paris, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, F-75014 Paris, France, Paris, France; [4]Centre d'Immunologie et des Maladies Infectieuses, Inserm, CNRS, Sorbonne Université, CIMI-Paris, 75013 Paris, France, Paris, France; [5]National Institute for Health and Medical Research (INSERM), UMR-ICAN 1166, Paris, France, paris, France; [6]Faculty of Science and Engineering Molecular Biophysics — Zernike Institute for Advanced Materials Nijenborgh 4 9747 AG Groningen The Netherlands, Gronigen, Netherlands; [7]Institut Curie, PSL Research University, LSMP - Curie CoreTech Mass Spectrometry Proteomics, Paris, France; [8]Centre d'Immunologie et des Maladies Infectieuses, Inserm, CNRS, Sorbonne Université, CIMI-Paris, 75013 Paris, France, paris, France; [9]National Institute for Health and Medical Research (INSERM), UMR-ICAN 1166, Paris, France, Paris, France; [10]Institut Curie CNRS, PARIS 05, France; [11]Institute of Psychiatry and Neurosciences of Paris, INSERM U1266, France, Paris, France

Introduction: Exosomes are extracellular vesicles (EV) of endosomal origin that function as a clearance cellular pathway and in intercellular communication. CD63 is a tetraspanin enriched on intraluminal vesicles of multivesicular endosomes (MVEs) and is hence commonly used as exosomal marker and for functionalizing exosomes. Yet, the role of CD63 in Intra-Luminal Vesicles (ILVs) and exosomes biogenesis remains unclear.
Methods: To determine the role of CD63 in intracellular trafficking and ILVs biogenesis we generated in vitro cell lines KO for CD63 by CRISPR editing in HeLa and melanoma MNT1 cell lines. We isolated extracellular vesicles by size exclusion chromatography (SEC) for phenotypic analysis and differential ultracentrifugation (UC) for functional assays. We combined lipidomic and proteomic analysis to live cell imaging, photon and electron-microscopy coupled to immunolabeling to decipher pathways where CD63 is involved in generated KO cell lines and derived extracellular vesicles.
Results: We report here that depletion of CD63 neither impairs size and distribution of EV isolated by SEC nor enrichment of canonical exosome marker. OMICs analysis showed reveals changes in lipid composition, in particular cholesterol between Wild-Type (WT) and CD63 KO cells. This led us to study the role of CD63 in cholesterol sorting into ILVs and exosomes. Live cell imaging of cholesterol trafficking and staining of cholesterol enriched microdomains by EM show that CD63 controls sorting of cholesterol to ILVs and exosomes. In absence of CD63, cholesterol is redirected to the Trans-Golgi Network using actin-related tubulation from MVEs. In feeding assay we show that ILVs can serve as endosomal storage entities for cholesterol and exosomes can be used as alternative source of cholesterol by recipient cells.
Summary/Conclusion: Overall, these results demonstrate a role for CD63 in sorting cholesterol to ILVs and exosomes, a process consistent with the roles previously attributed to CD63. Our work also establishes ILVs and exosomes as an alternative source of cholesterol for cells that would complement that provided by lipoparticles.
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OWP2.08=PT11.06 | Fluorescent Nanoprobes Based on Goat Milk Small Extracellular Vesicles for In Vivo Detection of Inflammation Processes

María Isabel González[1]; Ana Santos-Coquillat[2]; Agustín Clemente-Moragón[3]; Mario González-Arjona[2]; Gorka Sobrino[3]; Virginia Albaladejo-García[2]; Héctor Peinado Selgas[4]; Javier Muñoz[5]; Pilar Ximénez Embún[4]; Borja Ibañez[3]; Eduardo Oliver[3]; Manuel Desco[2]; Beatriz Salinas[6]