



## Commentary

Extracellular vesicles: Squeezing every drop of regenerative potential of umbilical cord blood<sup>☆</sup>Santiago Roura<sup>a,b,\*</sup>, Joaquim Vives<sup>c,d,e,\*\*</sup><sup>a</sup> ICREC Research Program, Health Science Research Institute Germans Trias i Pujol, Can Ruti Campus, Carretera de Can Ruti s/n, 08916 Badalona, Spain<sup>b</sup> CIBERCV, Instituto de Salud Carlos III, calle Monforte de Lemos 3-5, 28029 Madrid, Spain<sup>c</sup> Servei de Teràpia Cel·lular, Banc de Sang i Teixits, Edifici Dr. Frederic Duran i Jordà, Passeig Taulat, 116, 08005 Barcelona, Spain<sup>d</sup> Musculoskeletal Tissue Engineering Group, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Passeig de la Vall d'Hebron 129-139, 08035 Barcelona, Spain<sup>e</sup> Departament de Medicina, Universitat Autònoma de Barcelona, Passeig de la Vall d'Hebron 129-139, 08035 Barcelona, Spain

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## ABSTRACT

Collection, cryopreservation and transplantation of umbilical cord blood (UCB)-derived cells have become a popular option for regenerative medicine, not limited to the transplantation of hematopoietic cell progenitors only. Indeed, increasing evidence shows that extracellular vesicles (EV), which include a heterogeneous pool of membranous structures secreted by the vast majority of cells, can serve as powerful tools for cell-free therapy due to precise multifunctional molecular cargoes. In this issue, Hu et al. [1] described that EV extracted from UCB (UCB-EV) ameliorate bone loss in senile osteoporotic mice and promote *in vitro* osteoblast differentiation of bone marrow-derived Mesenchymal Stromal Cells through miR-3960-mediated signaling. These results envision the capability of UCB-EV of priming multipotent stem cells toward the osteogenic cell lineage and interfering on bone resorption processes. Although processing and manufacturing of EV-based products have to further develop major issues, we foresee that EV will soon become new therapeutic products supplied by UCB banks for treating human diseases, including bone-related conditions.

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The field of extracellular vesicles (EV) has grown remarkably in recent years, providing valuable proof-of-principle that heterogeneous pools of lipid-enclosed vesicles can be associated with a wide variety of cell types and human conditions or diseases [2]. EV specifically encapsulate and transfer bioactive molecular cargoes, including lipids such as membrane-associated and luminal proteins, mRNA, miRNA or non-coding RNA to recipient cells. Many studies are being carried out currently in order to identify the pathophysiological role of EV in body fluids and potential associations of their presence, levels and differential composition with disease-specific diagnosis and prognosis, and use them as source for the discovery of novel biomarkers in liquid biopsy. Increasing evidence also shows that a variety of secreted factors, including

EV, mediate in the immunosuppressive and trophic (restorative) functions exerted by multipotent Mesenchymal Stromal Cells (MSC). In brief, the exogenously supplied MSC home in on sites of injury or disease, and paracrinely modulate deleterious inflammatory responses and induce the migration of patient's own resident stem cells that construct the new tissue rather than serving to replace and regenerate damaged tissues [3,4]. Thus, the use of EV in cell-free therapeutic approaches is gaining traction for regenerative medicine [5].

Umbilical cord blood (UCB) has revealed as the most potentially unlimited source of reparative cells for use in multiple human conditions, from hematopoietic reconstitution to tissue regeneration [6–8]. In contrast to other cell sources, UCB is advantageous since its collection is safe and painless, and it can be preserved long term without loss of cell viability and function. UCB displays a low risk of transmitting viral infections or somatic mutations, and its use is not restricted by ethical issues [9]. However, low cell dosage and the possibility of delayed engraftment constitute key obstacles for the widespread use of UCB for hematopoietic reconstitution [10]. Therefore, on one hand, new treatment options such as the transplantation of double partially human leukocyte antigen–matched products is overcoming these limitations [11,12]; and, on the other hand, the search for alternative UCB-derived products such as platelets derivatives or EV will contribute to realize the full potential of UCB banks.

**Abbreviations:** DC, differential ultracentrifugation; EV, extracellular vesicles; miR, microRNA; MSC, mesenchymal stromal cells; RBC, red blood cell; SEC, size-exclusion chromatography; UCB, umbilical cord blood.

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Osteoporosis, which is related to various factors including menopause and aging, represents the most common chronic metabolic bone disease worldwide [13]. In particular, osteoporosis is characterized by low bone mass, deterioration of bone tissue and disruption of bone microarchitecture, leading to compromised bone strength and an increase in the risk of fractures of difficult management. Despite fracture prevention is the primary treatment goal for patients with osteoporosis, innovative therapies enhancing bone mass formation and inhibiting bone resorption are mandatory.

In this context, the study by Hu et al. attempted to assess the potential of EV extracted from UCB (UCB-EV) in reversing age-associated impairments such as those produced by senile osteoporosis. In essence, the authors hypothesized that miRNAs transported by UCB-EV exert beneficial effects concerning the balance of bone resorption and bone formation, which is essential for the maintenance of bone mass. Succinctly, the presented findings demonstrated that intravenous injection of UCB-EV alleviate age-related bone loss in old mice, as defined by increased trabecular and cortical bone mass, enhanced osteogenic activities and reduced osteoclast formation. Further evidence corroborated the promotion of osteoblast formation and the inhibition of osteoclast development when the UCB-EV collected were added to primary cell cultures of bone marrow-derived MSC. Of note, Hu et al. deciphered the underlying mechanism mediating the regulatory effect of UCB-EV on osteoblast differentiation. In particular, they described that this effect was mediated by miR-3960, which was much higher abundant in UCB-EV than any other miRNAs, in line with previous observations [14]. miRNAs are single stranded non-coding RNA comprised of 18–22 nucleotides which are central in genetic regulation, cell differentiation and development, including bone balance and osteoporosis [15]. However, in this study, specific miR-3960 inhibition could not affect the anti-osteoclastic effect exhibited by UCB-EV, suggesting that other molecules would contribute to this process. Although the study of miRNA involved in bone formation is still in its infancy, some molecules have already been linked to osteogenic pathways. In this regard, miR-1224 has been found to inhibit osteoblast differentiation from MSC and promote osteoclast differentiation from macrophages [16]. Moreover, osteogenic differentiation of MSC is stimulated by miR-22 upregulation [17,18].

In light of the data presented by Hu and co-workers, it is greatly expected that EV such as those collected from UCB units will be a choice for the treatment of senile osteoporosis in next decades. For that purpose, we envision that non-replicating EV would be favorable compared to cell-based therapeutic products in terms of low toxicity, biocompatibility, biological permeability, and ease of handling and cryopreservation, either in the autologous or allogeneic settings. However, the purity of EV is essential due to their size distribution, which ranges from 50 to 1500 nm. In general, it is well recognized that cells secrete essentially a mixture of EV comprising larger size heterogeneous microvesicles or shed microvesicles (100–1500 nm) and relatively homogeneous in smaller size exosomes (50–150 nm) [19]. There is a pressing need for a consensus in the field for using standardized isolation methods ensuring both 1) the removal of smaller EV from most of the contaminating soluble or non-vesicular particles or protein aggregates, and 2) the preservation of the vesicular structure and molecular messengers transported by EV. Only this will guarantee the success of novel EV-based therapies in humans.

Today, differential ultracentrifugation (DC) has been the most widely used laboratory methodology but alternative procedures such as size exclusion chromatography (SEC) have emerged to obtain purer EV preparations [20–22]. By using DC, high quantities of not EV-related molecules can be pelleted along with EV. In the study of Hu et al., SEC was not used as the EV isolation technique. Due EV-associated components might be masked by disproportionately large number of unspecific molecular cargo, attention should be paid when these erroneously considered 'EV-enriched preparations' are subjected to further experiments or analysis.

Remarkably, the findings by Hu and co-workers open the door to the design and development of novel UCB-derived therapeutic products. To

date, the majority of UCB collections, either in public or private banks, are red blood cell (RBC)-depleted prior to cryopreservation [23]. This process is crucial to reduce volume sample, storage space and the cytotoxicity caused by the thawing of RBC. Also, only an accurate RBC sedimentation guarantees high rates of stem or progenitor cell recovery provided that the mononuclear cell fraction (where stem cell populations reside) is needed for banking. In this context, the study of Hu et al. offers a valuable approach reinforcing the increasing trend toward UCB in assisting health care worldwide. We concur with the possibility that the yellow modified buffy coat, which is obtained at the top of the bag during the processing of UCB units, will also serve as source of multifunctional EV. This would greatly increase novel clinical uses of UCB-based cell-free products.

In conclusion, EV transfer functional proteins and nucleic acids between cells to modify the properties of their recipients. Collection, cryopreservation and banking of UCB-derived stem or progenitor cells have become a popular option not only for well-known hematopoietic stem cell transplantation but also for novel regenerative medicine-related indications, including damaged bone tissue remodelling. Our knowledge about bone homeostasis, which is mainly sustained by osteoclast-mediated bone resorption and osteoblast-mediated bone formation, has increased. In this context, the study of Hu et al. is relevant because of the demonstration that UCB-EV have the ability to ameliorate bone loss in senile osteoporotic mice and promote *in vitro* osteoblast differentiation. The role of EV in modulating osteogenesis *via* transfer of miRNA cargoes has also been reported using EV from MSC by others [24–26]. Taken together, these results reinforce the recognition of EV as active osteogenic mediators, so their translation into the clinical setting may lead to more efficient bone tissue regeneration products. Accordingly, we expect the addition of EV in the set of therapeutic armamentarium which are coming out of UCB collections worldwide in the next decade. However, before valuable EV-based products reach the clinics, some major concerns on EV processing and manufacturing are to be addressed. These include batch-to-batch heterogeneity of EV preparations, large-scale production following Good Manufacturing Practice and mandatory compliance with current regulatory requirements. Undoubtedly, the opportunity of having sufficient ability to overcome these barriers will be crucial to accelerate the development of EV-based strategies for the treatment of human diseases, particularly bone deficiencies.

### Conflict of Interest

The authors declare no conflict of interest.

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### Author Contributions

SR and JV: wrote the manuscript; final approval of the manuscript. All authors reviewed the manuscript.

## References

- [1] Hu Y, Xu R, Chen C-Y, Rao S-S, Xia K, Huang J, et al. Extracellular vesicles from human umbilical cord blood ameliorate bone loss in senile osteoporotic mice. *Metabolism* 2019;95:93–101.
- [2] Shah R, Tushar P, Freedman JE. Circulating extracellular vesicles in human disease. *N Engl J Med* 2018;379:958–66. <https://doi.org/10.1056/NEJMr1704286>.
- [3] Monguió-Tortajada M, Roura S, Gálvez-Montón C, Pujal JM, Aran G, Sanjurjo L, et al. Nanosized UCMSC-derived extracellular vesicles but not conditioned medium exclusively inhibit the inflammatory response of stimulated T cells: implications for nanomedicine. *Theranostics* 2017;7:270–84. <https://doi.org/10.7150/thno.16154>.
- [4] Caplan AI. Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med* 2017;6:1445–51. <https://doi.org/10.1002/sctm.17-0051>.
- [5] Rani S, Ryan AE, Griffin MD, Ritter T. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* 2015;23:812–23. <https://doi.org/10.1038/mt.2015.44>.
- [6] Jaing TH. Umbilical cord blood: a trustworthy source of multipotent stem cells for regenerative medicine. *Cell Transplant* 2014;23:493–6. <https://doi.org/10.3727/096368914X678300>.
- [6] lafolla MA, Tay J, Allan DS. Transplantation of umbilical cord blood-derived cells for novel indications in regenerative therapy or immune modulation: a scoping review of clinical studies. *Biol Blood Marrow Transplant* 2014;20:20–5. <https://doi.org/10.1016/j.bbmt.2013.09.010>.
- [8] Ilic D, Miere C, Lazic E. Umbilical cord blood stem cells: clinical trials in non-hematological disorders. *Br Med Bull* 2012;102:43–57. <https://doi.org/10.1093/bmb/lds008>.
- [9] Stewart CL, Aparicio LC, Kerridge IH. Ethical and legal issues raised by cord blood banking - the challenges of the new bioeconomy. *Med J Aust* 2013;199:290–2. <https://doi.org/10.5694/mja12.11668>.
- [10] Roura S, Pujal JM, Gálvez-Montón C, Bayes-Genis A. Quality and exploitation of umbilical cord blood for cell therapy: are we beyond our capabilities? *Dev Dyn* 2016;245:710–7. <https://doi.org/10.1002/dvdy.24408>.
- [11] Kang HJ, Lee JW, Kim H, Shin HY, Ahn HS. Successful first-line treatment with double umbilical cord blood transplantation in severe aplastic anemia. *Bone Marrow Transplant* 2010;45:955–6. <https://doi.org/10.1038/bmt.2009.247>.
- [12] Wallet HL, Sobh M, Morisset S, Robin M, Fegueux N, Fürst S, et al. Double umbilical cord blood transplantation for hematological malignancies: a long-term analysis from the SFGM-TC registry. *Exp Hematol* 2013;41:924–33. <https://doi.org/10.1016/j.exphem.2013.05.297>.
- [13] NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. Osteoporosis prevention, diagnosis, and therapy. *JAMA* 2001;285:785–95. <https://doi.org/10.1001/jama.285.6.785>.
- [14] Hu R, Liu W, Li H, Yang L, Chen C, Xia ZY, et al. A Runx2/miR-3960/miR-2861 regulatory feedback loop during mouse osteoblast differentiation. *J Biol Chem* 2011;286:12328–39. <https://doi.org/10.1074/jbc.M110.176099>.
- [15] Chen J, Qiu M, Dou C, Cao Z, Dong S. MicroRNAs in bone balance and osteoporosis. *Drug Dev Res* 2015;76:235–45. <https://doi.org/10.1002/ddr.21260>.
- [16] Li B, Wu PP, Fu WW, Xiong Y, Gao YB, Deng GY, et al. The role and mechanism of miRNA-1224 in the *Polygatum sibiricum* polysaccharide regulation of bone marrow-derived macrophages to osteoclast differentiation. *Rejuvenation Res* 2019. <https://doi.org/10.1089/rej.2018.2126>.
- [17] Huang S, Wang S, Bian C, Yang Z, Zhou H, Zeng Y, et al. Upregulation of miR-22 promotes osteogenic differentiation and inhibits adipogenic differentiation of human adipose tissue-derived mesenchymal stem cells by repressing HDAC6 protein expression. *Stem Cells Dev* 2012;21:2531–40. <https://doi.org/10.1089/scd.2012.0014>.
- [18] Du L, Nong MN, Zhao JM, Peng XM, Zong SH, Zeng GF. *Polygatum sibiricum* polysaccharide inhibits osteoporosis by promoting osteoblast formation and blocking osteoclastogenesis through Wnt/beta-catenin signaling pathway. *Sci Rep* 2016;6(32261). <https://doi.org/10.1038/srep32261>.
- [19] Willms E, Johansson HJ, Mäger I, Lee Y, Blomberg KE, Sadik M, et al. Cells release subpopulations of exosomes with distinct molecular and biological properties. *Sci Rep* 2016;6(22519). <https://doi.org/10.1038/srep22519>.
- [20] Gardiner C, Di Vizio D, Sahoo S, Théry C, Witwer KW, Wauben M, et al. Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. *J Extracell Vesicles* 2016;5:32945. <https://doi.org/10.3402/jev.v5.32945>.
- [21] Böing AN, van der Pol E, Grootemaat AE, Coumans FA, Sturk A, Nieuwland R. Single-step isolation of extracellular vesicles by size-exclusion chromatography. *J Extracell Vesicles* 2014;3. <https://doi.org/10.3402/jev.v3.23430>.
- [21] Lozano-Ramos I, Bancu I, Oliveira-Tercero A, Armengol MP, Menezes-Neto A, Del Portillo HA, et al. Size-exclusion chromatography-based enrichment of extracellular vesicles from urine samples. *J Extracell Vesicles* 2015;4:27369. <https://doi.org/10.3402/jev.v4.27369>.
- [23] Roura S, Pujal JM, Gálvez-Montón C, Bayes-Genis A. The role and potential of umbilical cord blood in an era of new therapies: a review. *Stem Cell Res Ther* 2015;6(123). <https://doi.org/10.1186/s13287-015-0113-2>.
- [24] Wang X, Omar O, Vazirisani F, Thomsen P, Ekström K. Mesenchymal stem cell-derived exosomes have altered microRNA profiles and induce osteogenic differentiation depending on the stage of differentiation. *PLoS One* 2018;13:e0193059. <https://doi.org/10.1371/journal.pone.0193059>.
- [25] Otsuru S, Desbourdes L, Guess AJ, Hofmann TJ, Relation T, Kaito T, et al. Extracellular vesicles released from mesenchymal stromal cells stimulate bone growth in osteogenesis imperfecta. *Cytotherapy* 2018;20:62–73. <https://doi.org/10.1016/j.jcyt.2017.09.012>.
- [26] Li Y, Jin D, Xie W, Wen L, Chen W, Xu J, et al. Mesenchymal stem cells-derived exosomes: a possible therapeutic strategy for osteoporosis. *Curr Stem Cell Res Ther* 2018;13:362–8. <https://doi.org/10.2174/1574888X13666180403163456>.