

MEETING REPORT

UK–Russia Researcher Links Workshop: extracellular vesicles – mechanisms of biogenesis and roles in disease pathogenesis, M.V. Lomonosov Moscow State University, Moscow, Russia, 1–5 March 2015

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The UK–Russia extracellular vesicles (EVs) workshop was held at the Medical Center of the M.V. Lomonosov Moscow State University, Moscow, Russia, with 56 attendees from UK and Russian universities and research institutes. The program consisted of 6 research sessions and was focused on studies of EVs isolated from in vitro model systems or biological fluids, including blood and urine. The multidisciplinary program included presentations on mechanisms of EV biogenesis, the role of EVs in disease pathogenesis, the diagnostic value of EVs, including their quantitation and cargo load, as well as the clinical use of EVs in regenerative medicine. Methodological challenges imposed by the nanoscale size of EVs as well as targeted delivery approaches for therapeutics were considered in a separate session on technologies. The main aim of the workshop was to overview challenges confronting EV researchers and to facilitate knowledge exchange between researchers with different backgrounds and skills. Given the lack of definitive EV nomenclature, specific terms (*exosomes* or *microvesicles*) were only applied in the meeting report to studies that carried out full EV characterization, includ-

ing differential ultracentrifugation isolation approaches, comprehensive protein marker characterization, and single vesicle analysis (electron microscopy and nanoparticle analysis), to ascertain EV size and morphology following the International Society for Extracellular Vesicles standardization recommendations (1,2). In studies where characterization was not conclusive, the term EV is used.

Session 1, “Extracellular vesicles in cardiovascular pathologies,” gave us an idea of how these different vesicles might be implicated in the cardiovascular system, as either markers of pathology, mediators of cellular processes, or vehicles for delivery of proteins.

In the first talk, C. Shanahan (King's College London, London, UK) showed that previously described matrix vesicles, released by vascular smooth muscle cells in the vessel wall that initiate pathological calcification, are exosomes as defined by their size and morphology, electron microscopy, proteomic composition, and presence of exosome-enriched proteins. Notably, their release was regulated by sphingomyelin phosphodiesterase 3, suggesting that inhibition of this pathway may protect against calcification.

From the same laboratory, A. Kapustin showed that actin depolymerization and Rho-associated coiled-coil forming protein serine/threonine kinase (ROCK) activity were also required for exosome release by vascular smooth muscle cells.

Sean Davidson (University College London, London, UK) presented data concerning exosomes, isolated from the plasma by differential ultracentrifugation and thoroughly characterized for the presence of exosome-enriched markers, as well as their size distribution and morphology. This exciting research showed that such exosomes are able to protect the heart against ischaemia and reperfusion injury, such as occurs during a heart attack. Protection involved heat shock protein 27 (HSP27) phosphorylation by exosomal HSP70, in response to activation of Toll-like receptor 4 (TLR4) – part of the innate immune system. In another study examining the innate immune response, X. Li (Cambridge University, Cambridge, UK) showed that lactadherin was able to inhibit activation of the inflammasome and thereby limit post-ischaemic cerebral injury; however, as yet, the role of EVs in this process has not been explored.

Standing up for the positive role of large-sized EVs, L. Ayers (Oxford University Hospitals NHS Trust, Oxford, UK) presented a clinical study in 119 cardiac patients subject to a dobutamine stress, showing that EV release, as measured by flow cytometry, only occurs in healthy patients and not in disease, suggesting this is a protective normal physiological response. Supporting this potential protective role, I. Bogachev (Belgorod National Research University, Belgorod, Russia) showed that in an experimental model of endothelial dysfunction the total number of EVs was increased after acute dysfunction, but decreased after chronic dysfunction.

Turning to specific proteins, M. Heck (University of Edinburgh, Edinburgh, UK) described a fascinating odyssey of exploration of invadolysin, a serum protease with diverse roles in chromosomes and lipid droplets. E. Ferrer (Cambridge University, Cambridge, UK) showed that translationally controlled tumour protein is transported via EVs and contributes to the pathology of pulmonary arterial hypertension. M. Balatskaya (Lomonosov Moscow State University, Moscow, Russia) showed that T-cadherin is released on the surface of human platelet-derived EVs, attached via a glycosylphosphatidylinositol anchor.

The second session, “Extracellular vesicles in oncology,” was focused on the potential role of EVs as cancer biomarkers. V. Aushev (N.N. Blokhin Russian Cancer Research Center, Moscow, Russia) reported high levels of several miRNAs (miR-205, -30b, and others) in tumour-associated blood-circulating EVs of lung cancer patients, with some of these miRNAs also selectively secreted by corresponding cells *in vitro*. EVs bound to the surface of blood cells were investigated in a comparative study of healthy women and breast cancer patients by S. Tamkovich (Institute of Chemical Biology and Fundamental Medicine,

Novosibirsk, Russia). Healthy women had smaller EVs (30–50 nm vs. 50–70 nm), and an overrepresentation of cancer-specific miRNAs (miR-103, miR-191, miR-195) found in erythrocyte-bound EVs compared to leukocyte-bound or plasma-circulating EVs. N. Yunusova (Tomsk Cancer Research Institute, Tomsk, Russia) reported on the role of circulating proteasomes, representing a multi-subunit complex with several proteolytically active sites, and EVs, providing intracellular and interstitial transfer of biologically important molecules, in the pathogenesis of ovarian cancer.

Some prospective therapeutic effects of EVs as well as novel delivery approaches were discussed in the third session, “Extracellular vesicles in tissue regeneration.” N. Iraci (University of Cambridge, Cambridge, UK) presented on cell-to-cell communication mediated by neural stem-cell-derived EVs in the context of inflammation. This work identified a novel mechanism of intercellular signalling via recycling of IFN- γ through IFNGR1 to activate Stat1-dependent signalling in target cells. Other topics in this session included unidirectional exchange of mitochondria from differentiated cells, such as cortical neurons, and human multipotent mesenchymal stem cells (D. Zorov, Lomonosov Moscow State University, Moscow, Russia), where signals either stimulate differentiation or reprogram metabolism. S. Skaalure (Imperial College London, London, UK) reported a variety of techniques for regenerative medicine, including biodegradable scaffolds to deliver cells to damaged tissues, as well as the use of EVs to deliver miRNA and therapeutic drugs to cell populations to direct cellular behaviour. The final speaker, N. Kalinina, focused on the paracrine role of EVs secreted by human mesenchymal stromal cells from adipose tissue in controlling angiogenesis as a therapy to promote tissue regeneration.

Many cells within the immune system release EVs, including exosomes, which can have either immune activating or inhibiting properties. Recently, pathogens have also been shown to release EVs capable of inhibiting immune responses. In session 4, “Extracellular vesicles in immunology and pathogens,” L. Smyth (King’s College London, London, UK) presented her findings on how exosomes isolated by differential ultracentrifugation from regulatory T cells (Treg) and defined by their size, morphology, and protein enrichment can inhibit T cell responses as well as dendritic cell function. She highlighted an inhibitory role for the ectoenzyme CD73, as well as miRNAs, present in Treg exosomes. The theme of miRNA and EVs was further discussed by R. Crossland (Newcastle University, Newcastle upon Tyne, UK), who highlighted a role for EVs in graft-versus-host disease and the use of EV miRNAs to monitor the onset of this disease. Lastly, stunning EM images, showing the release of streams of EVs from bacteria, were presented in 2 talks by D. Ross and I. Kudryakova (G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms,

Pushchino, Russia), followed by a discussion of why bacteria release EVs and the effect they may play in immune modulation.

The first speaker in the session “Extracellular vesicles in systemic disorders and nephrology” was R. Dragovic (University of Oxford, Oxford, UK), who presented an overview of the role of EVs in normal pregnancy and pre-eclampsia. The syncytiotrophoblast (STB) layer of the placenta releases both microvesicles and exosomes – defined based on their sedimentation properties, size, and protein marker enrichment – into the maternal circulation. These microvesicles and exosomes carry pro-inflammatory, anti-angiogenic, and pro-coagulant factors that may contribute to the maternal systemic inflammatory response observed in pre-eclampsia. Notably, STB exosomes and microvesicles may differ functionally and show immunosuppressive and pro-inflammatory effects, respectively. Hyperphosphatemia is a well-known cardiovascular risk factor. N. Abbasian (University of Leicester, Leicester, UK) reported that elevated phosphate can induce the formation of procoagulant endothelial EVs through an elevation of intracellular P_i concentration, which directly inhibits phosphoprotein phosphatases, triggering a global increase in phosphorylation and cytoskeletal changes. W. Oosthuizen and E. Morrison (University of Edinburgh, Edinburgh, UK) presented a new mechanism of intercellular signalling within the kidney and showed that EVs can be internalised by collecting duct cells, with this uptake regulated by a vasopressin/V2 receptor-mediated process of clathrin-dependent endocytosis. Interestingly, the site of vasopressin action was shown to be basolateral, but EV uptake was from both apical and basolateral compartments. Normal human urinary exosomes were the subject of T. Gracia’s talk (University of Cambridge, Cambridge, UK). She showed enrichment for innate immune proteins in exosomes isolated from urine by differential ultracentrifugation, including antimicrobial proteins and bacterial and viral receptors, and that urinary exosomes potently inhibit growth of uropathogenic and commensal *E. coli* and induce bacterial lysis. A repertoire of microRNAs has also been identified in urinary exosomes, raising speculation around their potential as biomarkers and targets for treatment. Finally, G. Sharonov (Lomonosov Moscow State University, Moscow, Russia) elegantly discussed the effects of receptor clustering within microdomains of the plasma membrane. He showed that small clusters facilitate signal transduction whereas intermediate clusters anchor actomyosin fibres to the membrane and promote EV budding.

Session 6, “Modern technologies to study extracellular vesicles,” featured novel technologies used to study nano-sized objects. S. Bertazzo (Imperial College London, London, UK) highlighted how various electron and light microscopy techniques could be superimposed to provide new insights into EV biogenesis. E. Khomyakova (Research Institute of Physical-Chemical Medicine,

Moscow, Russia) discussed theoretical considerations as well as experimental results comparing differential ultracentrifugation to rate-zonal centrifugation using a sucrose gradient for the purpose of optimizing EV isolation. D. Ter-Ovanesyan (Harvard University, Cambridge, MA, USA) presented his work on high-throughput RNA sequencing of exosomes isolated by differential ultracentrifugation from cell media and recent applications to the profiling of RNAs. V. Burdakov (Petersburg Nuclear Physics Institute, St Petersburg, Russia) presented work on the use of EV as a drug delivery vehicle for short interfering RNAs. E. Evtushenko (Moscow State University, Moscow, Russia) discussed his work on optimizing the NanoSight Nanoparticle Tracking Analysis instrument (Malvern Instruments Ltd, Malvern, United Kingdom) to give quantitative and reproducible measurements of EV size and abundance. I. Pintre (University of Manchester, Manchester, UK) presented her work on the synthesis of new bio-inspired nanoparticles for drug delivery. Finally, J. Welsh (University of Southampton, Southampton, UK) discussed advances in using flow cytometry to analyze single EVs and highlighted important considerations in optimizing flow cytometers for this purpose.

In conclusion, the meeting highlighted that EVs are implicated in a variety of normal and pathological processes, from protecting prokaryotic cells to the delivery of a wide range of cargos that improve or deteriorate systemic or local pathological conditions. Circulating EVs are attractive non-invasive diagnostic markers, potentially predictive of cardiovascular disease, cancer, renal disease, systemic conditions, or immune responses, with specific subsets of EVs, or their cargos, elevated in pathological conditions. Data presented about combinations of EVs capable of stimulating biological effects and local controlled delivery approaches put a focus on EVs as prospective tools in regenerative medicine. Yet, despite these promising clinical applications, questions addressing why and how cells release EVs remain poorly investigated and require further multidisciplinary and innovative studies.

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