

On the 18th and 20th of September, the course "Extracellular vesicles: implications in biomedicine" co-organized by Universidad Internacional Menéndez Pelayo (UIMP) and the Grupo Español de Investigación e Innovación en Vesículas Extracelulares (GEIVEX) met more than 70 participants from 18 different nationalities. The course was hosted in Palau de Pineda, the UIMP University headquarters in Valencia and was supported by the COST Action European Network on Microvesicles and Exosomes in Health and Disease (ME-HAD).

Wednesday, 18th

On Wednesday 18th morning, the course was started with an opening ceremony by the Principal of *Universitat de València*, **Dr. Esteban Morcillo**, the director of the Valencia-UIMP university headquarter, **Dr. Agustin Domingo** and **Dr. Antonio Marcilla**, director of the course.



During the opening conference **Graça Raposo**, from Department of Cellular Biology, Institute Curie (Paris) introduced the concept of extracellular vesicles (EVs) from a historical perspective. Dr. Raposo commented on the pioneering works by the lab of Rose M. Johnstone in the early eighties, where the extracellular vesicles were for the first time described as a mechanism by which the transferrin receptor is shed during reticulocyte maturation. Then, she reviewed her own discoveries on the possibilities of anti-tumor therapy using dendritic cell (DC) derived exosomes and tumour derived exosomes, and highlighted the results of the Phase I trials using DC-derived exosomes.

Maria Mittelbrunn, from de National Centre of Cardiovascular Research (Madrid), made an overview on the different technical approaches to work with EVs. Since EVs can be isolated from all biological fluids and cells culture medium, it is very important before starting the purification to take into account some fluid specific factors. Then, she presented the different strategies for EVs purification (ultracentrifugation, polymeric precipitation, density gradients, immune capture, etc.), she warned about the presence of some contaminants in EVs samples (virus, mycoplasma, protein aggregates, lipoproteins, etc.) and the "pros and contras" of the actual methods of analysis (western blot, electron microscopy, flow cytometry) and quantification and sizing (nanotracking analysis, resistive pulse sensing). She finished with some clues and tips for working with EVs RNAs.

Hector Peinado, from the Weill Cornell Medical College, Cornell University (New York), presented interesting results of how exosomes from highly metastatic melanomas could increase the metastatic behavior of the primary tumor by educating bone marrow progenitors towards a prometastatic phenotype and by inducing vascular leakiness at pre-metastatic niches. The receptor tyrosin kinase MET has an important role in the tumorigenesis of melanoma-derived exosomes.

Juan Manuel Falcón, from Centro de Investigación Cooperativa en Biociencias CIC-BioGUNE, (Derio, Vizcaya) has been working for many years using metabolomics approaches for studying extracellular vesicles as biomarkers for hepatic function. Dr. Falcón's group described and characterized the proteome and transcriptome of hepatic EVs, and showed how the protein and RNA content of these EVs varies upon exposure to hepatotoxic drugs. Hepatic EVs can be transferred to rat liver stellate-like cells and promote their activation, they can modulate the metabolism as well as the proliferation rate of recipient cells. The data presented indicated that EVs might become a new source for non-invasive candidate biomarkers for hepatic disease.

Francesc Borràs, from IVECAT-Instituto de Investigación Germans Trias I Pujol (Badalona, Barcelona) reviewed the state-of-the art of the use of extracellular vesicles as a source of alloantigens for inducing immunological tolerance. He discussed on the importance of choosing different cells that can be used for obtained EVs with tolerogenic potential, including their maturation state, administration route and concentration of the EVs. The aim of Dr. Borràs laboratory is the study of therapeutic approaches using EVs for the treatment of autoimmunity and transplantation.

Thursday, 19th

On Thursday 19th morning, the trainees participated in practical sessions that took place at School of Pharmacy (*Universitat de València*, Campus de Burjassot). During this practical session, the students, with the help of Dolores Bernal, Maria Trelis, Alex Adan, Antonio Marcilla and Maria Mittelbrunn purified EVs from plasma, T lymphoblast cell line (control and overexpressing CD63-GFP) and the parasite *Fasciola hepatica*, using three different approaches: Filtration and Ultracentrifugation, magnetic isolation (using Miltenyi MicroBeads and MS Columns) and polymeric precipitation (Exo-spin™ technology). After isolation, EVs were analyzed by MACSQuant analyzer (Miltenyi-Biotec) and were quantified and sized using Nanotracking Analysis (with the help of Roberto Ghiandoni from Nanosight) and qNano (by Dimitri Aubert from IZON technology).



In the afternoon, **Marca H. Wauben** from the Department of Biochemistry and Cell Biology, Utrecht University (Netherlands) presented the possibilities and limitations for the analysis of nanosized cell-derived vesicles. In a very didactic talk, she explained that although EVs have tremendous opportunities for clinical applications, where a major technical problem is the individual analysis of nano-sized vesicles. Most of the analysis methods (Western-blotting, proteomics, capture assays, ELISA) are Bulk-based analysis methods. But since there are mixed populations of EVs, the challenge nowadays is to develop new techniques that allow the analysis at single-particle level. Dr. Wauben's lab has recently developed a high resolution flow cytometry-based method that allow the analyses of the number and composition of nano-sized vesicles at single-particle level.

Edit Buzás, from the Department of Genetics, Cell- and Immunobiology at Semmelweis University (Budapest, Hungary) explained the role of extracellular vesicles during inflammation. EVs can drive inflammation and autoimmunity by multiple mechanisms because of their content of bioactive molecules. Since they contain PAMPs (pathogens associated molecular patterns) and DAMPs (danger associated molecular pattern; like HSPs, S100 proteins and miRNAs), they can act as a danger signal that trigger inflammation. They carry inflammatory lipid mediators and cytokines, and can participate in tissue destruction. Finally she presented data that support the use of EVs as biomarkers for autoimmune diseases.

After the conferences, a round table moderated by Antonio Marcilla, included the participation of Marca H. Wauben, Edit Buzás, Graça Raposo, Roberto Ghiandoni, José Manuel Garcia-Verdugo, and Maria Jesús Vicent, which discussed about the following topics:

- 1- Nomenclature, origin, markers
- 2- Isolation Methods
- 3- Biofluids vs Culture medium
- 4- Contaminants
- 5- Storage (before or after exosome isolation)
- 6- Methods for analysis
- 7- Methods for quantification & sizing
- 8- How to manipulate EVs (fluorescent labeling, incorporate RNA)
- 9- RNA content. Organisms without sequenced genome available.
- 10- Clinical applications: biomarkers, vaccination, and treatment.

What we know today about the emerging role of the extracellular vesicles in important physiological and pathological process is just the 'tip of the iceberg'. Systematic approaches such as transcriptomics, proteomics and metabolomics, coupled with individual analysis of nano-sized vesicles, will be particularly important in the next years for characterizing the different EVs subsets and understanding the role of these EVs. It was a consensus that in the next 2-3 years a concise nomenclature and Standard Operational procedures for the isolation and characterization of EVs from different cells and sources will be achieved unifying and significantly advancing this growing field.

The day finished with a joint informal dinner at Palau de Pineda, where trainers and trainees communicate in an informal way and shared experiences.

Friday, 20th

The use of EVs for drug delivery is one of the most exciting and promising areas of research in EVs field. **Maria Jesús Vicent** from Centro de Investigación Príncipe Felipe (Valencia) made an interesting overview about the potential use and applications of EVs for drug delivery. In parallel, she also presented alternative platforms using polymers leaving the interesting possibility of combining in the future EVs and polymers for drug-targeted delivery.

The interplay of host-pathogen EVs and their role in infectious diseases was presented by **Hernando del Portillo**, ICREA Research Professor at Centro de Investigación en Salud Internacional (CRESIB) (Barcelona). He started presenting the data from helminths where Dr. Marcilla's laboratory demonstrated the secretion of exosome-like vesicles by parasitic trematodes and their potential role in the communication between the parasites and the host. Then, Dr. Portillo reviewed data from other host-parasite interactions including *Trypanosoma cruzi*, *Trichomonas vaginalis* and *Plasmodium vivax*. Interestingly, in the latter, it has

been shown by his own group that reticulocyte-derived exosomes from mice infected with malaria contain parasite proteins, and are involved in the induction of protective immune responses against the parasite, indicating these EVs can be a novel vaccine and therapeutic strategy.

The pivotal role played by extracellular vesicles in immune responses was presented in the closing conference by **Francisco Sánchez-Madrid** (Hospital de la Princesa and National Center of Cardiovascular Research, Madrid). During cell-cell interactions, molecules (proteins and RNA) can be exchanged through different structures, such as nanotubes and gap junctions, trans-endocytosis and, as shown by Dr. Sanchez-Madrid group, the transfer of EVs. The immune synapse (IS) is a highly-organized device for communication between immune cells. During the formation of an IS, the T lymphocytes polarize the microtubule organizing center (MTOC) and the secretory machinery towards the antigen presenting cell. Recently, the IS was shown to support the unidirectional transfer of RNA by the shuttling of exosomes from the T cell to the antigen presenting cell.



After the course joint picture, the closing ceremony was headed by the Principal of Universitat de València, **Dr. Esteban Morcillo**, the director of the Valencia-UIMP university headquarter, **Dr. Agustín Domingo**, as well as **Dr. Antonio Marcilla**, director of the course, and **Dr. Maria Mittelbrunn**, Secretary of the course. They made a balance of the three days and thanked the authorities, sponsors (COST Action BM1202; Nanosight, Miltenyi-Biotec and Bonsai-Advanced Technologies) and participants for their support and help.



LINKS:

<http://www.uimp.es/blogs/valencia/2013/09/17/los-mejores-especialistas-se-reunen-en-valencia-para-tratar-sobre-vesiculas-extracelulares/>

<http://www.uimp.es/blogs/valencia/2013/09/18/el-rector-de-la-universitat-de-valencia-inaugura-el-curso-sobre-vesiculas-extracelulares-en-la-uimp/>

<http://www.uimp.es/blogs/valencia/2013/09/19/4737/>

<http://www.uimp.es/blogs/valencia/2013/09/20/se-clausura-el-seminario-%e2%80%9cextracellular-vesicles-implications-in-biomedicine%e2%80%9d/>

<http://www.uv.es/uvweb/universitat/ca/llista-noticies/professor-antonio-marcilla-dirigeix-valencia-seminari-sobre-vesicules-extracelulars-1285846070123/Noticia.html?id=1285891090591>

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