Project description

The Regional Research Network on extracellular vesicles (EV)

Introduction

Extracellular vesicles (EV) have recently attracted increasing interest because of their potential function in cell-to-cell communication, thrombus propagation and implications in several diseases. Furthermore, the possible use of microvesicles as a source for biomarkers as well as in therapeutic applications [1] has even increased the popularity of these vesicles. Remarkably, it has been foreseen that the next generation of biomarkers for disease diagnostics and prognostics may heavily to come to lean on EV.

The overall goal of the EV network is to establish a platform for hitherto fragmented groups in the HSØ region interested in and working with EV, to strengthen the regional quality and production on EV research.

Most mammalian cells are able to release several types of sealed membrane particles of less than 1 μm into their extracellular matrix .The nucleic acid and protein content of these extracellular vesicles (EV) seems to reflect the nature and status of their cells of origin and thus, these vesicles may be considered to mirror their cellular phenotypes. Interestingly, the EV composition may differ in health and disease, making these vesicles tremendously attractive as a relatively non-invasive source of biomarkers since EV can be isolated from biological fluids.

To gain insight in health and disease, the isolation and analysis of EV is expected to gain momentum in the years to come. Despite increased interest in EV research, there are several important challenges to overcome in this new field, such as how the different vesicle types should be defined, purified and analyzed. We therefore believe the time is appropriate for uniting the groups in HSØ interested in this rapidly expanding field of EV research.

The Regional Research Network on EV focuses on a research field that has developed very fast as illustrated by the 25 participants attending the first international conference on this topic in 2005 and the 700 researchers that attended the first meeting of the newly launched International Society of Extracellular Vesicles (ISEV) in Gothenburg in 2012. Highlighting the importance of the field is also the very recent NIH Common Fund's Extracellular RNA Communication program ($130 M), which “will explore new ways in which cells communicate with each other using membrane enclosed extracellular ribonucleic acids (RNAs)” (http://www.nih.gov/news/health/jul2012/od-02.htm) and the European Network on Microvesicles and Exosomes in Health and Disease(ME-HAD) (http://www.cost.eu/domains_actions, approved Actions 2012), which focuses on basic understanding and translational potential of the EV. In accordance with these international initiatives, the Blood Cell Unit, Section for Research, Dept. of Medical Biochemistry, OUS-Ullevål aims at being the administrative coordinating group that will integrate research groups

working within the microvesicle field in the HSØ to establish a Regional Research Network on EV.

The network will be very useful among scientists working in the EV field. In this context, there are several important activities for the network such as to exchange knowledge, to evaluate instruments and materials, to develop and implement techniques and, last but not least, to generate collaborative projects. Furthermore, there is as of today no HSØ platform or network, in fact no Norwegian platform at all, to foster and integrate research activities in this new and important EV field.

The participants in the network are academic research groups and a research group from the industry. The academic groups belong to hospitals within The South-Eastern Norway Regional Health Authority: These groups work in different disease group entities such as cancer, sepsis, inflammation and brain disease. In additional international collaborators will support the activities of the network.

Our driving force will be that working together with various aspects of EV, in the frame of a network of coordinated knowledge exchange and collaboration, will ensure that the netted participants bring EV research in the HSØ region to the front in the years to come.

Establishment of The Regional Research Network on EV will provide several important advantages:

- Firstly, to comply with the Research Strategy of HSØ 2008-2012 (http://www.helse-sorost.no/fagfolk/forskning/forskningsmidler/Documents/forskningsstrategi_hso_2008-2012_final.pdf) the network will approach the high potential of EV as diagnostic biomarkers in different diseases by cooperating with frontline clinical research groups (see Figure 2) to extend and transfer basic knowledge "from bench to bed", ie.translational medicine (HSØ 2008-2012: "Aim: Increased cooperative research and translational medicine")

- Secondly, the network will focus on common challenges of EV characterization including pre-analytical handling and isolation procedures, unraveling size- and numbers, and describing vesiclecomposition. This may lead to various inter-disciplinary, collaborative projects.

- Thirdly, the network will get insight into where this rapidly expanding knowledge field is moving, by strengthening the links to other European MV groups, such as ISEV, the COST ME-HAD and the ISAC Microvesicle Analysis Interest Development Group.

Background

"Although it has long since been realized that eukaryotic cells release complex structures, termed membrane vesicles, into their extracellular environment, only in the past decade has been realized that these entities are not merely junk or debris indicative of cell death. Such microvesicles have recently demonstrated to be micromaps of their cell of origin and may be of both physiological and pathological relevance" (ME-HAD, http://www.cost.eu/domains_actions, approved Actions 2012, Pg 4/1147).

EV are small cell derived membrane vesicles released from almost all eukaryotic cells into their extracellular environment. EV comprise a whole range of different –vesicles including microparticles (MP) and exosomes (E). These nanosized structures (30 – 1000 nm in

diameter) can be found in biological fluids. EV originate from several distinct shedding and secreting processes in the cell, leading to specific subgroups of EV [2]. Global analysis of EV content indicates so far that the vesicles contain both molecules common to EV of different origins and other molecules specific to a given cell of origin.

In spite of increasing interest in EV, there is as of yet no consensus or guidelines as to how EV should be defined, purified, or analyzed.

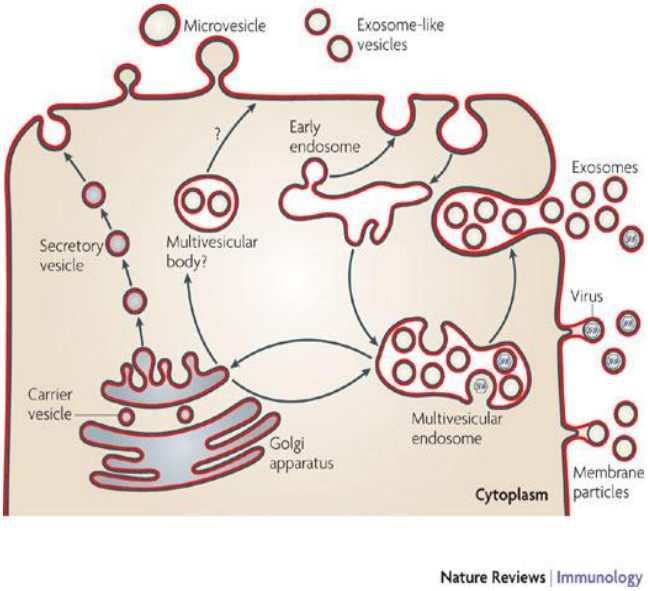

Figure 1: Different types of secreted membrane vesicles. C.Théry et al, Nature Reviews, Immunology Vol 9, August 2009

Exosomes (Exo) are so far the most widely studied lipid-based extracellular vesicles. These particles of approximately 40-100 nm are membrane-derived vesicles that are formed from the limiting membrane of the late endosome by invagination and budding [3].They accumulate in cytosolic multivesicular bodies (MVBs) and from there they are released after fusion with the plasma membrane, a distinct process that is not shared by other membrane-derived vesicles such as microparticles. Characterization of EVmembrane proteins, such as tetraspanins and integrins, is thus becoming increasingly important. Exosomes have been shown to be secreted from many different cell types, including inflammatory cells, stem cells, muscles, neurons, epithelial cells and tumor cells. Exosomes are released into the extracellular space and havebeen detected in several human biological fluids such as blood plasma and serum, urine, saliva, breast milk, semen, cerebrospinal fluid and bronchoalveolar lavage fluid [4].

Exo are proposed to have many distinct physiologic functions that may vary depending on their cellular origin i.e Exo have been implicated in immune regulation, blood coagulation, cell migration- and differentiation. Exohave also been implicated in the pathogenesis of disease such as tumor development, cardiovascular disease, neurodegenerative diseases and infections [5]. This has sparked the idea that examining Exo from body fluids for their structures and their content (composition) may be useful in the search for novel biomarkers. Perhaps Exo even may be employed as therapeutic tools.

Cells can communicate directly with each other through cell–cell contact, or at a distance using secreted soluble mediators (eg. hormones, cytokines etc). Recently, a third mode of intercellular communication has been shown to be mediated by Exo, an emerging pathway with an unraveled potential. Excitingly, Exo contain both proteins and different RNAs (mRNA and microRNA), which can be shuttled between different cells, in fact over long distances [6]. Results so far indicate that exosomes from different cell origins and state of growth contain variable amount and composition of protein and RNAs. This could further delegate importance to the functional properties of specific Exo. At present, however, Exo variability could be the result of different isolation techniques used in different studies. Detailed characterization of exosomal content will therefore be an important task in the close future, albeit a difficult one. Thus, there is an urgent need to standardize methodology to isolate exosomes and characterize their content.

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MicroRNA (miRNA) has been identified as a fine-tuner in a wide array of biological processes, including development, biogenesis, metabolism and homeostasis. Deregulation of miRNAs has been shown to cause diseases, especially in cancer (ref) [7]. Recently, it was discovered that extracellular miRNAs circulate in the blood of both healthy and diseased persons. Most of the secretory miRNA are contained in different membrane coated vesicles, including Exo, or bound to RNA-binding proteins. However, the secretory mechanisms and the biological functions of extracellular miRNAs remain largely unclear, also in cancer.

Microparticles (MPs) are membrane-derived nano-fragments (50 to 1000 nm) that are active in coagulation and inflammation. They are released into the extracellular environment through a membrane reorganization and blebbing process followed by cell activation or apoptosis[8]. MPs constitute a storage pool of bioactive effectors with varied cellular origins, and as such are able to act as intercellular messengers [9]. In line with Exp, MPs are present in body fluids where they reflect normal tissue homeostasis, but undergo phenotypic and quantitative changes to play a pathophysiological role in several diseases frequently associated with thrombotic disorders. Upon cell activation the plasma membrane is reorganised with active externalization of phosphatidylserine and internalisation of phosphatidylcholine [10]. Phosphatidylserine is a signal for phagocytosis [11]. MPs, however, seem to survive longer than their parental apoptotic cell, probably because of their size allowing only smaller clusters of senescence signals. As of today, little is known on the effect of MP clearance on the haemostatic balance under physiological or pathological settings.
In addition to phosphatidylserine exposure, protein-lipid raft domains are formed and furnish the MP with its specificities and biological roles. MPs bear intracytoplasmic and membrane-bound effectors from the originating cells such as tissue factor (TF) [12] and CD-14 (monocytes) or glycoprotein $(GP)_{Iba\text{-}IX\text{-}V}$, P-selectin [13] or integrins (platelets). Very important in this connection is the TF bearing property of MP, since these procoagulant MP may trigger coagulation and promote thrombosis. Under bacterial infections, particularly in septic conditions, extensive MP- associated thrombosis may occur [14]. It should also be pointed out that recent evidence suggests certain MPs to carry the potential to support tissue remodelling through the binding of plasminogen activators and metalloproteinases [15].

In line with Exo, MPs are important mediators in intercellular communication, and function as actors and possible mediators in the interplay between thrombosis and inflammation. They can transfer organelles, mRNA, receptors and other proteins to target cells as well as deliver various cytokines. These multiple properties of MPs and their many possible cellular targets support the concept that MPs may have key roles in cell reprogramming and tissue remodelling [16].
Standardized procedures for MP measurement are not available yet. However, a consensus has been formed on blood sampling and MP isolation by serial centrifugation steps to avoid

exosome contamination. MPs can be analyzed through capture techniques (using immobilized annexin V, and insolubilized antibodies for phenotyping) combined with functional coagulation assays. Flow cytometry allows quantification and determination of cellular origin via the use of specific fluorescent antibodies and calibration beads. However, small EVs are still challenging to analyse, reflected by the development of new techniques (Atomic force microscopy, Nano-tracking analysis [17] etc.)

Aims

The overall goal of the EV network is to establish a platform for hitherto fragmented groups in the HSØ region interested in and working with EV.

The scientific aims of this network are:

- To strengthen the regional research quality and production on EV, and thereby increase the chances to detect and exploit biomarkers in different diseases
- To facilitate interactions between national and international EV groups, as well as encourage other research groups to include EV in their research field, leading to increased collaboration.
- To make possible the exchange of researchers with front international groups
- To initiate EV research processes to position the Network members for EU funding
- To initiate and coordinate cooperative EV projects between the network members:
  By taking advantage of the miscellaneous expertise within the network – we foresee that fruitful collaborative projects will emerge on the interphases between basal mechanisms, technology/ instrumentation and clinical aspects, further leading to translational research on EV
    - Characterization of EV secretion (size and number) derived from cancer cells and cell lines, found in serum/plasma, culture medium and urine
    - Characterization of EV (mRNA, microRNA, proteins, lipids, functionality) isolated from patient samples.
    - Establishment of new EV technologies (particle counters for size and number), bead technology (detection and isolation)

The administrative and organizing aims of the network are:

1. To coordinate ongoing and forthcoming research on EV by initiating registering of the current research activities in each group. Subsequently, a kick off meeting will be arranged to further elaborate on the topics in focus. Strategic plans will be discussed; agreements will be made as how to interact within the network and as to which digital platforms should be used to handle “daily” communication. Annual meetings will be planned (Table 1) and set in motion.
2. To establish a meeting calendar for the network, planning strategic and scientific meetings, as well as to assure network members participation in major international EV meetings/ workshops and to organize scientific exchanges with the two supporting groups (Table 1). This will make sure that the members of the network take part in research that will increase the knowledge on EV; consensus decisions etc.
3. To employ a network-funded engineer for technical assistance, support and handling of webpage issues. The tasks to be performed by this engineer will be discussed at the kick off meeting.

Implementation

The network will work according to the planned timeline (se Table 1), with regular strategic and scientific meetings, starting with a kick off meeting January 2013.
The network will establish a Management Committee that will be responsible for execution of meetings within the network
An interactive website will be established (within the 1. quarter of 2013) and information about activities and scientific breakthroughs will be spread through the website.

Contact has been established with knowledgable supporting groups (see Figure 2) in order to facilitate exchange of scientists from the netgroups.
In order to carry through the aims to initiate and coordinate cooperative EV projects between the network groups, the Network will, within short time, generate working groups based on previuos experience and availability to instrumentation for joint project development. The Network will together have possibilities to use instrumentation for characterization of EV secretion (particle counters (NanoSight) for size and numbers, flowcytometry for qualitative detection), EV content (microRNA, mRNA, protein, lipids), functionality (procoagulant and fibrinolytic properties), imaging and electron microscopy.

A network – funded engineer will be employed for technical assistance such as analysis related to extracellular vesicles research and maintenance of equipment (i.e. particle counters).This engineer will also be responsible for maintenance of the website, execution of secretary functions, and will be located at the Unit of Blood Cell Research at OUS-Ullevål.

Timeline – ***The Regional Research Network on EV***	*Year 1*		*Year 2*		*Year 3*	
	Spring	*Autumn*	*Spring*	*Autumn*	*Spring*	*Autumn*
Kick-off meeting	*x*					
Management Committee Meetings	***x***		***x***		***x***	
Generation of meeting calender	***x***		***x***		***x***	
Registration of ongoing MV research activities	***x***		***x***		***x***	
Summary of instrument facilities		***x***		***x***		***x***
Generation of working groups and joint project developement	***x***		***x***		***x***	
Webpage start-up	***x***	***x***				
Webpage up-date		***x***	***x***	***x***	***x***	***x***
Regional Annual Symposium	***x***		***x***		***x***	
Regional Workshop	***x***	***x***	***x***	***x***	***x***	***x***
International Conference with presentation of papers		***x***		***x***		***x***
International Workshop	***x***		***x***		***x***	
Researcher exchange with "in front" labs	***x***	***x***	***x***	***x***	***x***	***x***
Network-connected engineer	***x***	***x***	***x***	***x***	***x***	***x***
Annual report		***x***		***x***		***x***
Final report						***x***

Table 1. Timeline for The Regional Research Network on EV

Organization of the network

The network will consist of 5 academic research groups, 1 industrial research group and 3 supporting groups.

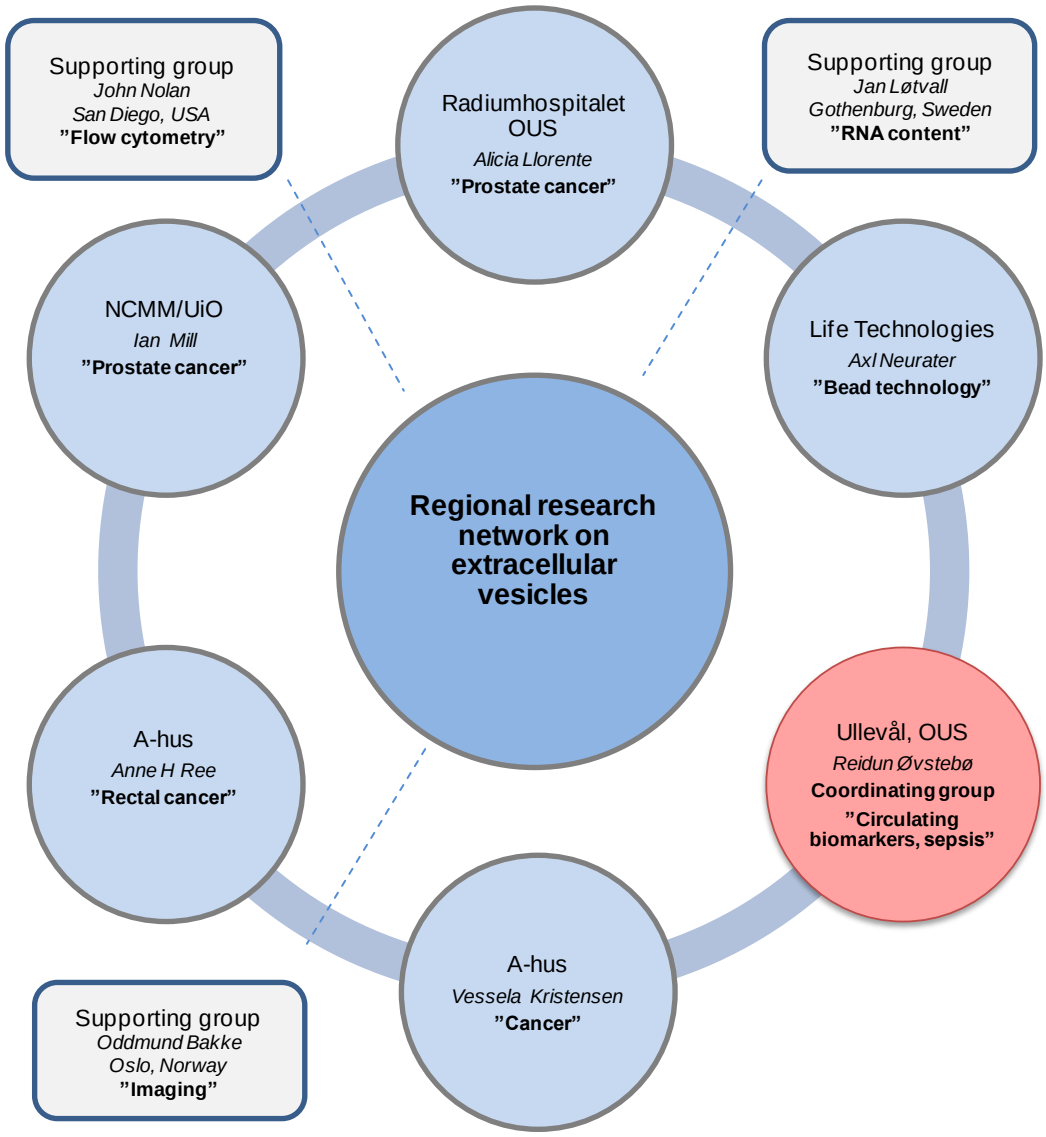

Figure 2. Organization of the Regional research network on extracellular vesicles

The Management Committee Group will consist of:

Reidun Øvstebø, PhD, The Unit of Blood Cell Research, Oslo University Hospital, Ullevål
Alicia Llorente, Dr.Philos, Dept. of Biochemistry, Institute for Cancer Research, Oslo University Hospital, Radiumhospitalet
Axl Neurater, MSc, Cellelular and molecular Research department, Life Technologies AS
Vessela Kristensen, PhD, Professor, Cancer Research Group, Akershus University Hospital
Anne Hansen Ree, PhD, Professor, Department of Oncology, Akershus University Hospital
Ian Mills, PhD, Prostate Cancer Research Group, NCMM, University of Oslo

Added value of the Network

This Network brings together basic science in academia, clinical research and research within industry. Laboratory experiments and clinical studies may be carried out in parallel with key persons engaged in both activities. The clinical research groups have large, existing biobanks that may be exploited after hopefully successful experiments in the laboratory.

Publication issues and innovation

Articles in highly ranked international journals will be prioritized, as well as extensive participation and dissemination of results in international congresses related to EV.
As for innovations all participants in the network will be encouraged to “think innovation potential” in their research. Emphasize will be put on this, and selected research findings will be focused and discussed at our network meetings. Also, the innovation potential within EV research is already recognized, as shown by the patents from some of the groups in the network.

Previous financial support to the network

None

Reference List

1. Roberson CD, Atay S, Gercel-Taylor C, Taylor DD: Tumor-derived exosomes as mediators of disease and potential diagnostic biomarkers. *Cancer Biomark* 2010, 8: 281-291.

2. Thery C, Zitvogel L, Amigorena S: Exosomes: composition, biogenesis and function. *Nat Rev Immunol* 2002, 2: 569-579.

3. Denzer K, Kleijmeer MJ, Heijnen HF, Stoorvogel W, Geuze HJ: Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 2000, 113 Pt 19: 3365-3374.

4. Gallo A, Tandon M, Alevizos I, Illei GG: The majority of microRNAs detectable in serum and saliva is concentrated in exosomes. *PLoS One* 2012, 7: e30679.

5. Skog J, Wurdinger T, van RS, Meijer DH, Gainche L, Sena-Esteves M *et al*.: Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol* 2008, 10: 1470-1476.

6. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ: Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011, 29: 341-345.

7. Kosaka N, Ochiya T: Unraveling the Mystery of Cancer by Secretory microRNA: Horizontal microRNA Transfer between Living Cells. *Front Genet* 2011, 2: 97.

8. Lechner D, Weltermann A: Circulating tissue factor-exposing microparticles. *Thromb Res* 2008, 122 Suppl 1: S47-S54.

9. Morel O, Toti F, Hugel B, Freyssinet JM: Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol* 2004, 11: 156-164.

10. Connor DE, Exner T, Ma DD, Joseph JE: Detection of the procoagulant activity of microparticle-associated phosphatidylserine using XACT. *Blood Coagul Fibrinolysis* 2009, 20: 558-564.

11. Freyssinet JM: Cellular microparticles: what are they bad or good for? *J Thromb Haemost* 2003, 1: 1655-1662.

12. Key NS, Mackman N: Tissue factor and its measurement in whole blood, plasma, and microparticles. *Semin Thromb Hemost* 2010, 36: 865-875.

13. Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E *et al*.: Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J Exp Med* 2003, 197: 1585-1598.

14. Nieuwland R, Berckmans RJ, McGregor S, Boing AN, Romijn FP, Westendorp RG *et al*.: Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood* 2000, 95: 930-935.

15. Varon D, Shai E: Role of platelet-derived microparticles in angiogenesis and tumor progression. *Discov Med* 2009, 8: 237-241.

16. Bobik A: Circulating microparticles. Markers or participants in carotid artery remodelling? *J Hypertens* 2010, 28: 673-675.

17. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P *et al*.: Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine* 2011, 7: 780-788.