

Euroclast Newsletter April 2015

Here is a Euroclast newsletter to inform you about the latest developments within the consortium.

Update: progress meeting: “Defining the models”, in Kiel, Germany, 7-10th December 2014

We had our second Euroclast progress meeting, for the first time as a full consortium with all ESRs now in post. The meeting, held in Kiel, Germany, focused on finding the right model for studies and the ethical and research governance environment around use of different model systems. We discussed use of animal models in research, current technologies for generating a transgenic mouse model, analysing animal phenotypes and considerations regarding working with human subjects and human tissues and cells.



Well fed and watered in Kiel

Differences exist between the various countries in which Euroclast ESRs work and we were made aware of this during an interactive discussion session. A large part of the meeting was reserved for presentations by the ESRs of their research projects and results to-date. Feedback was provided by external expert Professor Tim Arnett from London and by all consortium members. Findings presented included:

- A subpopulation of resorbing osteoclasts have autophagy protein, LC3, localised to the ruffled border, even when cells are autophagy deficient. (Anh)
- Microarray gene expression data has allowed for the identification of novel key molecules involved in initiating and maintaining the resorption machinery. (Arjen)
- Immunogold labelling of HM20 resin embedded samples is not suitable for detection of the vesicular proteins LC3 and Plekhm1. An alternative approach, cryosectioning, may allow for detection of these by improving retention of antigenicity. (Emma)
- LIGHT, a transmembrane protein, is not a RANKL-independent stimulator of osteoclastogenesis. (Vikte)
- Cloning dilution of the RAW264.7 cell line resulted in subclones with different characteristics in response to RANKL treatment. Some subclones were RANKL-insensitive while others responded and formed multinucleated TRAP+ osteoclast-like cells. (Laia)
- Knockdown of a novel protein (undisclosed) disrupts the podosomal belt in osteoclasts on glass. (Sandra)
- Different human osteoclast precursors respond in distinct ways to IL-17A. (Sara)

- IL-1 β can stimulate multinucleation, as well as bone resorption in all three osteoclast precursor subsets, with each responding distinctly to this cytokine. (Yixuan)
- In an earlier experiment it was observed that the amount of V-ATPase 116 kDa subunit a isoform 3 (a3) in human osteoclasts appeared to be post-translationally regulated by lysosomal degradation and absence of glycosylations. Using osteoclast cell culture systems and various lysosomal inhibitors or glycosylation inhibitors we attempted to verify this initial observation but the inhibition of said processes did not appear to affect the amount of a3. (Henrik)
- Lysosomal protein, MFSD1, is highly expressed in osteoclasts. To assess whether MFSD1 is localised at the ruffled border, osteoclasts cultured on hydroxyapatite-coated coverslips will be stained for MFSD1 and examined by immunofluorescence. (David)
- Plasmatic lipoprotein Apolipoprotein E (ApoE) shows anti-inflammatory action on murine osteoclasts by inducing IL-10 expression. Moreover ApoE deficiency in murine osteoclasts results in increased expression of activity markers such as TRAP, cathepsin K, calcitonin receptor, MMP. (Giuliana)

We also established some collaborative activity between the consortium partners –

- Collaboration between the University of Aberdeen with the Göteborgs Universitet, and ACTA, VU University of Amsterdam and Karolinska Institutet: Vikte and Yixuan have provided SNX10 mutant and uPARAP mutant samples for Emma to analyse using TEM. Emma has also paid a work visit to the shared microscopy facility of ACTA in Amsterdam (housed in the Amsterdam Medical Centre, a different campus to that of ACTA) to learn cryosectioning for TEM from Nicole van der Wel. To verify that the immunogold labelling worked, she will be staining for TRAP with antibodies provided by Euroclast partner Göran Andersson (Karolinska Institutet, Sweden).
- Collaboration between ACTA, VU University of Amsterdam and Christian-Albrechts Universität zu Kiel: David has provided Sara with CD63 and LIMP-2 knockout mice samples for TEM analysis.
- Collaboration between ACTA, VU University of Amsterdam and the Karolinska Institutet: Sara went to the Karolinska Institutet to learn how to assess the adhesion of her cells in different matrices with the help from Anja Reithmeier.
- Collaboration between ACTA, VU University of Amsterdam and Radboud University, Nijmegen: The University of Amsterdam has been providing Giuliana dentine for research.

As part of the meeting and in between the workshops and lectures, ESRs were welcomed to the Molecular Imaging North Competence Centre where they received an extensive tour and demonstration of the state-of-the-art imaging equipment for preclinical imaging housed there. They also visited the laboratories of Paul Saftig and Markus Damme at Christian-Albrechts Universität zu Kiel. A mentoring session with a senior partner of the consortium (not the supervisor) allowed further bonding of ESRs with the other members of the Euroclast consortium.



An attentive audience at the MOIN in Kiel

ESRs visited the famous Lübeck Christmas market at the start of the meeting. To make sure supervisors were not left out; they repeated this seasonal treat at the end, showing the supervisors how to keep warm by sipping on *Glühwein* (mulled wine), a typical Christmas drink at the local Kiel market.



Glühwein in Kiel and Lübeck at the Christmas markets

All in all this was a successful meeting, we learnt a lot and are grateful for the feedback received from our external expert Professor Tim Arnett on our research.



In front of the MOIN in Kiel



Lunch in the Maritim Hotel in Kiel

Euroclast members attended the 4th Joint Meeting of European Calcified Tissue Society (ECTS) and the International Bone and Mineral Society (IBMS) in Rotterdam, the Netherlands held from 25-28th April 2015

ECTS-IBMS 2015 provided an opportunity to share findings from the Euroclast consortium with the rest of the scientific world.

- Laia Mira Pascual presented the poster 'Tartrate resistant acid phosphatase 5a – a coupling factor between osteoclast and osteoblast with potential growth factor activity?'
- Yixuan Cao presented her poster 'IL-1B differently affects osteoclastogenesis of distinct

subsets of osteoclast precursors'

- Sara Sprangers presented her poster 'Different human osteoclast precursors respond in distinct ways to IL-17A'
- Anh Tran presented previous work from the University of Western Australia. The poster is on the 'Uptake and vesicular trafficking dynamics of degraded bone matrix in osteoclasts'.

Emma McDermott also attended to promote the consortium at the Euroclast poster at the consortium corner. Showcasing EU consortia is a new feature of ECTS and we were one of the very first consortia to feature in this way at the meeting in Prague last year. Emma and Anh made the poster which provided an opportunity to signpost the presentations of Euroclast during the conference and allow an opportunity to make delegates aware of what we do.

Vincent and Miep attended an EU consortium meeting during ECTS in which plans for future events at ECTS for larger consortia within Europe were discussed. ECTS is keen to attract the consortia to its meetings and is looking to facilitate interactions of consortia with its delegates. Six consortia were present at the meeting including a newly started ITN on bone pain. It is likely that there will be opportunities for Euroclast at the forthcoming ECTS conference in Rome in 2016 to hold a meeting as part of the ECTS programme. Details will be discussed over the coming month as the ECTS programme develops further.

Next Euroclast progress meeting "Gathering the tools" in Aberdeen and Braemar 13-19th June 2015

The ESRs will be coming to Aberdeen for an imaging workshop at the Institute of Medical Sciences to learn about the various imaging tools at the facility (e.g. SEM, TEM, confocal, flow cytometry) and how to apply these to osteoclast research.

The other consortium members will then arrive and we will travel west to hold our progress meeting near Braemar, a small village about 60 miles from Aberdeen, in the Cairngorm National Park at the Mar Lodge estate. A primary theme in this meeting will be public engagement, including the use of social media in research. There will also be a focus on scientific writing for the general public. ESRs will give a poster or oral presentation of their latest findings at the mid-term review meeting where the EU programme manager and an external expert will assess our progress.

News from the ESRs

David Massa López went to Göteborgs Universitet in Sweden to receive training in differentiating osteoclasts from bone marrow macrophages and has applied his new knowledge to research back in Kiel.

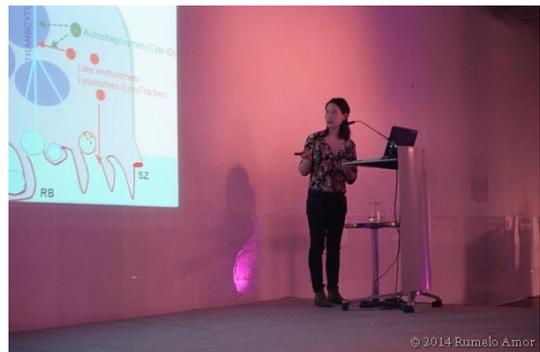
Arjen Gebraad has developed a method involving the use temperature-responsive culture plastics to detach osteoclasts differentiated from PBMCs and reseed them on a new substrate, without trypsinization. This novel technique will enable him to evaluate the effect of various substrates on mature, resorbing osteoclasts, without compromising cell surface proteins.

Sandra Segeletz will remain in Aberdeen after the Euroclast meeting in June for her secondment at the Institute of Medical Sciences. With assistance from Emma, she will be analysing samples by TEM.

Meetings attended by ESRs:

Yixuan Cao attended the Dutch Association for Calcium and Bone metabolism meeting last November. She has also been busy analysing adserverin knockout mice samples from collaborators in Toronto.

Anh Tran and Emma McDermott attended the first joint Scottish Microscopy Group and Microscopy Society of Ireland Symposium last November where they showcased work from the University of Western Australia and University of Aberdeen, respectively. Anh was selected to present her work and won best overall oral presentation.



Anh in front of a giant cell at the SMG meeting in Glasgow: could it be our friend the Euroclast?

In the next issue...

In our next newsletter we will have an update on the presentation of Euroclast at the ECTS/IBMS meeting in Rotterdam, an update on our third consortium meeting and more news and results from the ESRs.

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