SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: BM1406
STSM title: Understanding the role of lysosomal P2X4 purinergic receptor in breast cancer cell invasiveness
STSM start and end date: 10/06/2017 to 18/08/2017
Grantee name: Stéphanie Chadet

PURPOSE OF THE STSM:

(max.200 words)

The STSM, entitled "Understanding the role of lysosomal P2X4 purinergic receptor in breast cancer cell invasiveness", aimed to work on a collaborative scientific project integrated in the COST BM1406 program: IONCHAN-IMMUNRESPON, for which the two PIs, Drs Ruth Murrell-Lagnado (University of Sussex, UK) and Sebastien Roger (University of Tours, France) share common interest and complementary expertise. The purpose of the STSM was to allow Dr. Stéphanie Chadet, who is a post-doctoral researcher in the laboratory of Dr. Murrell-Lagnado, to learn new techniques and perform a set of experiments in order to produce a first manuscript on this work.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

The STSM was initially for 1 month period. However, we had to extend the period by 40 days in order to obtain the needed results.

Dr. Chadet performed a serie of critical experiments to assess the role of the P2X4 purinergic receptor in breast cancer cell functions and its cross-talk with P2X7 receptor. Especially, she evaluated the effects of P2X7 activation in intracellular and lysosomal pH changes. She analysed the role of the P2X4 receptor in these processes, which are associated with lysosomal exocytosis and cathepsin release.

The different parts of the work that have been carried out during the STSM are listed below:

1. Discussions about the results that have been obtained from both teems and generation of a detailed plan of action for how to take this project forwards
2. Measurement of cytosolic pH using fluorescent probes in time-lapse under different conditions to assess the role of P2X7 and P2X4 in pH regulation.
3. Measurement of lysosomal pH using fluorescent probes in imaging flow cytometry in order to unravel the molecular mechanism triggering P2X4 activation
4. Measurements of cathepsins activity in spectrofluorimetry using specific fluorogenic substrate and specific RNAi targeting P2X4 (siCTL/siP2X4), to understand the role of P2X4 in the exocytosis of lysosome-associated proteases.
5. The use of Matrigel-coated transwells and spheroid assays in 3D matrices to assess cell invasiveness.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

During the STSM, we succeeded in answering several critical questions. We have shown that P2X4 receptor is involved in the regulation of the intracellular pH, which is associated with lysosomal pH modifications, a key process that regulates lysosome fusion to the plasma membrane, exocytosis and protease release.

The main results obtained during the STSM are:
- P2X7 receptor activation triggers an intracellular acidification.
- This effect is prevented when P2X4 in knocked-down.
- P2X7 receptor activation leads to lysosomal alkalinization.
- P2X4 is involved in the P2X7-mediated cathepsin release and activity.
- We confirmed the role of P2X4 receptor in cell invasiveness.

FUTURE COLLABORATIONS (if applicable)

The two PIs, Drs Ruth Murrell-Lagnado and Sebastien Roger, have recently submitted a grant application for a project to look at the role of lysosomal P2X4 purinergic receptors in promoting breast cancer cell invasiveness and metastases.
This scientific mission allowed them to have interactive discussions, to address crucial questions and precise the orientations of the collaborative project.
In addition, the results obtained in Tours during the STSM are of great importance to draw up the first manuscript.
Moreover, the RML group has a collaboration with MedImmune who will provide some novel P2X4 antagonists that have recently been developed to treat inflammatory pain. These will be tested for their ability to inhibit breast cancer cell invasiveness, initially using in vitro assays. This falls within the objectives of the Action which is focused on drugs targeting ion channels involved in immune responses.