COST
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17103

Grantee name: Patricia Rico Tortosa

Details of the STSM

Title: **Deciphering the functional coupling of boron transporter/integrins/GFR in muscle cells.**

Start and end date: 17/10/2022 to 27/10/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

Cell differentiation studies. Human wild type (WT) and Duchenne muscular dystrophy (DMD) immortalised muscle cells have been seeded onto Fibronectin (FN)-coated glass substrates and the new bioengineered 2D platforms presenting boron (B) covalently bound in the solid-phase. Cells were seeded and induced to differentiate in absence of serum and supplementing the medium with insulin for 7 days. We compared the formation of myotubes with soluble borax added in the culture media and the cells differentiated onto the 2D platforms presenting B. We also compared the B effects in the presence or absence of FN. After culture, cells were fixed and immunostained against sarcomeric α-actinin to determine myotube formation. Images were visualised in fluorescent microscopy. We assessed the percentage of cell differentiation by image analysis techniques and imageJ software.

Co-localisation studies.

1-We have performed co-localisation studies using PLA DUOLINK assay. Cells were seeded onto FN-coated glass substrates for 3h in absence of serum. We have compared if co-localisation of NaBC1-integrins occurs in the presence/absence of B in the media. Co-localisation assay has been performed following manufacturer´s instructions. Images were visualised in confocal microscopy.

2-We have used fluorescent B (home-made biological tool B-FITC) for intracellular boron tracking. Muscle cells (WT) were seeded onto FN-coated glass substrates for 24h and 3 different concentrations of B-FITC were added to the cultures to determine the optimal B-FITC concentration to be used in future experiments. Live cell imaging has been performed by confocal microscopy after 30 mints incubation.

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[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

3-We have combined the B-FITC with diverse specific probes for intracellular organelles. We have combined B-FITC/mitochondria dye, B-FITC/endoplasmic reticulum dye and B-FITC/lysosome dye. Muscle cells (WT) were seeded onto FN-coated glass substrates for 24h. Live cell imaging has been performed by confocal microscopy after 30 mints incubation with the different dyes.

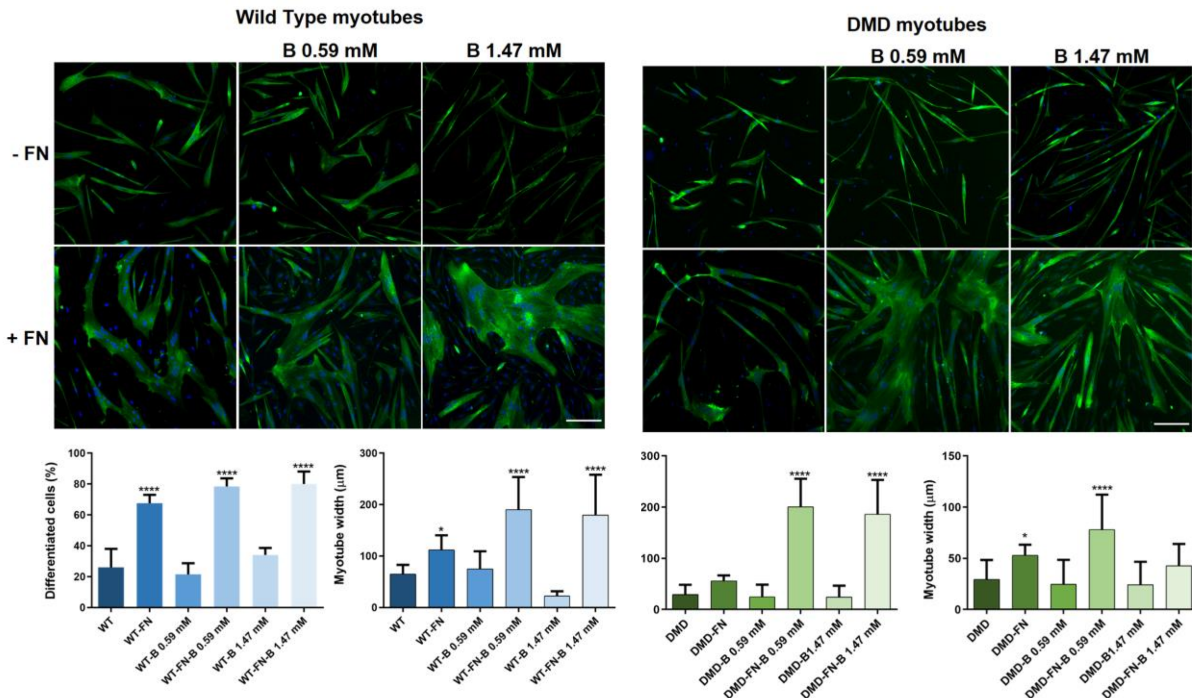
Actin flow measurements.

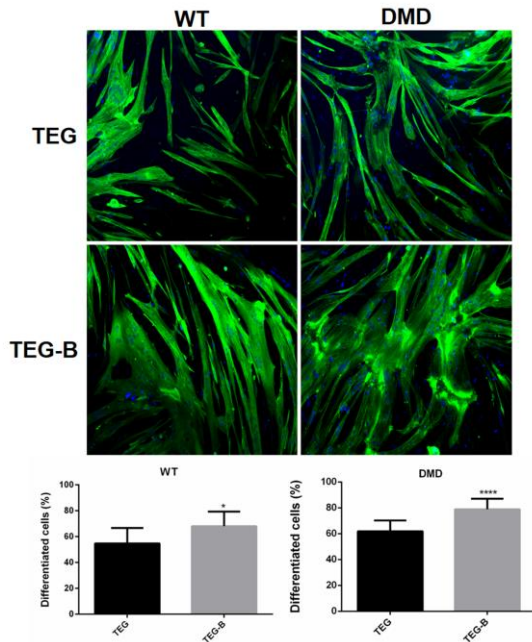
The retrograde actin flow, continuous centripetal movement of the cell peripheral actin networks, is widely observed in adherent cells. The retrograde flow is believed to facilitate cell migration when linked to cell adhesion molecules. Muscle cells were transfected with a plasmid expressing a GFP-actin-binding protein for 24h. After that, cells were seeded onto FN-coated glass substrates and soluble B was added when necessary. Cells were incubated for 4h. Live cell imaging for actin flow measurements was performed in a spinning disc confocal microscopy taking 1 picture every 2 seconds in a total of 4 minutes (120 pictures/cell).

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

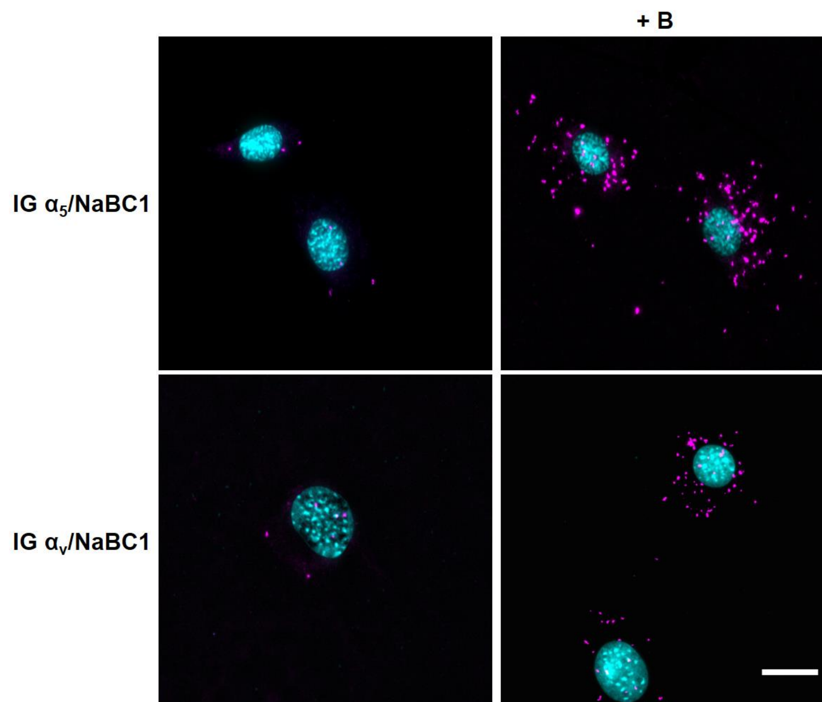
Our aim is to demonstrate that enhanced muscle differentiation occurs after the simultaneous activation of boron transporter (NaBC1) and FN-binding integrins. The results in the following figure show that the presence of two different concentrations of B in the culture media (0.59 mM and 1.47 mM respectively) together with FN strongly induce myogenic differentiation and myotube diameter in WT and DMD cells. Thus, simultaneous activation of NaBC1 and FN-binding integrins enhances muscle differentiation.



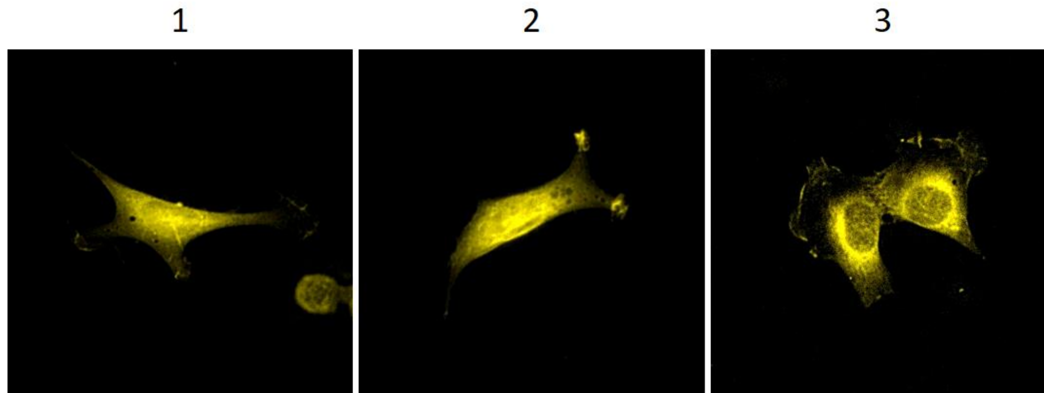


Next we cultured WT and DMD cells onto FN-coated 2D substrate platforms presenting B covalently-bound in the solid-phase. The results show that presence of B in the platforms strongly induce myogenic differentiation, suggesting a permanent activation of the NaBC1 transporter. Interestingly, the B concentration in the platforms is 0.046 μM, thus we accomplished NaBC1 activation in a more effective way compared to soluble B added in the culture media (concentrations of mM). Currently, we are developing new 2D material platforms presenting in the solid-phase cell adhesion domains and the combination of B and cell adhesion domains (RGD peptide), to demonstrate that enhancement of the muscle differentiation observed can be achieved due to the specific activation of NaBC1 and integrins.

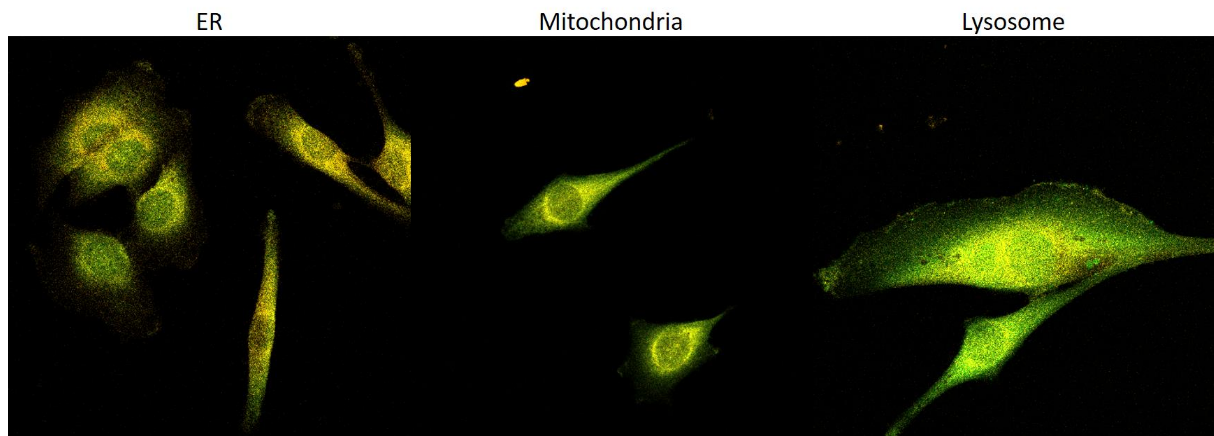
We have evaluated NaBC1 colocalisation with FN-binding integrins. The results show that active NaBC1 colocalises with active FN-binding integrins, indicating that a functional coupling could be produced between these two membrane receptors. For the immediate future we will perform other colocalisation experiments implicating NaBC1 and growth factor receptors essential in muscle tissue.



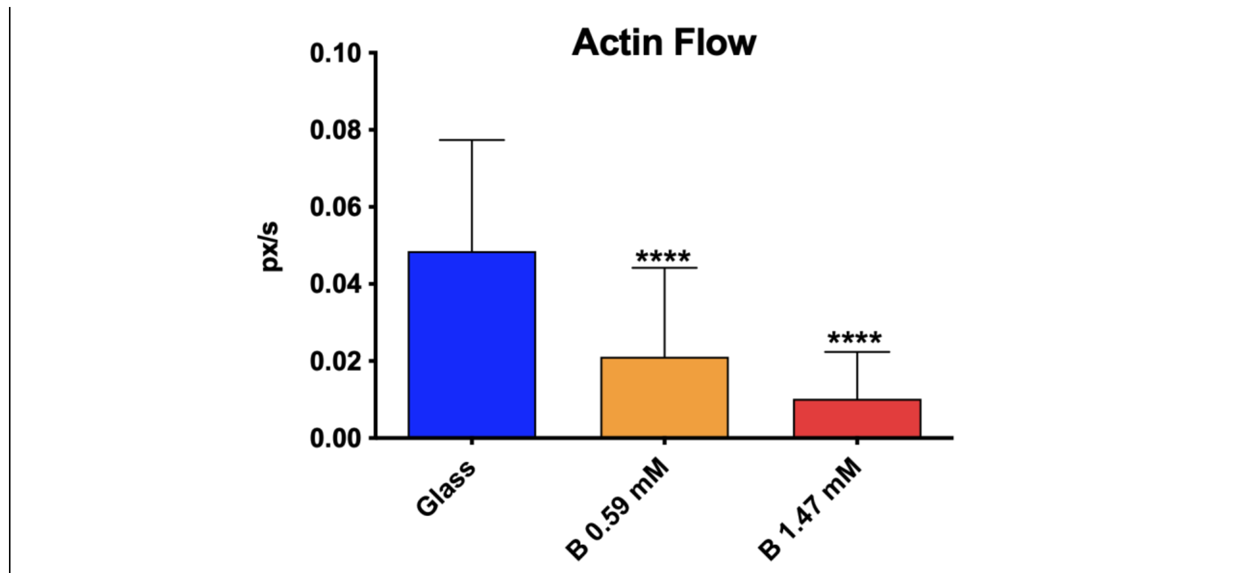
We have analysed B intracellular location using the new generated chemical molecule corresponding to the conjugated B-FITC. After addition of 3 different amounts of the labelled B, we visualised live cells by confocal microscopy. We observed that fluorescent B penetrates inside the cells and particularly accumulates near cell nuclei (probably endoplasmic reticulum) and in cytoplasmic structures that remind to focal adhesion plaques (cell points for cell attachment). We decided to use for further experiments the concentration of labelled-B number 2 that gave the best cell staining and avoided cell toxicity.



We then co-stained muscle cells with B-FITC and different staining against subcellular organelles (endoplasmic reticulum-ER, Mitochondria and Lysosome). After 30 minutes of dye incubation we visualised live cells by confocal microscopy. The results indicate that B localise in the ER, mitochondria and lysosome (yellow in the merged pictures), suggesting the possibility of the existence of intracellular B transporters not described to date. We will further explore this finding.



Finally, we performed actin flow measurements in order to demonstrate that NaBC1 acts as a mechanotransducer protein besides controlling boron homeostasis. Actin flow measurements indicate the cell peripheral actin networks movement and is an indirect measure of the force of the cell attachment to the substrate. The analysed results show that the presence of B strongly reduced actin flow movement, indicating that active-NaBC1 enhances cell attachment to the substrates, and that cells are exerting more and stronger forces in the presence of B. Overall the results suggest that NaBC1 is acting as a mecanotrasducer after activation. Immediate future experiments of nanoindentation of cells in the absence/presence of B and in cells seeded onto 2D platforms that permanently stimulate NaBC1 will be performed to further elucidate the changes in tension of the cell membrane after NaBC1 stimulation. If successful, we will describe a novel and important function for NaBC1 transporter related to cell mechanosensing mechanisms not described to date.


Actin Flow
px/s
0.10
0.08
0.06
0.04
0.02
0.00
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Glass
B 0.59 mM
B 1.47 mM