**Research Networking Programmes**

**Short Visit Grant ☐ or Exchange Visit Grant ☒**

*(please tick the relevant box)*

# Scientific Report

**The scientific report (WORD or PDF file – maximum of eight A4 pages) should be submitted online within one month of the event. It will be published on the ESF website.**

***Proposal Title:*** Tension Sensing at the Neural Crest

***Application Reference N°:*** 4505

## 1) Purpose of the visit

The aim of this exchange visit was to analyse the precise role of Nedd9 in controlling neural crest cells (NC) collective migration in vivo, we focus on this issue since Nedd9 has the potential to work as a mechanosensor of the substrate during collective neural crest cell migration in vivo. A secondary aim was to strength the collaboration between Dr Elias H Barriga and Prof Roberto Mayor's lab at UCL.

## 2) Description of the work carried out during the visit

At the beginning of the exchange we focused on setting up the working concentrations of Nedd9 mRNA and Nedd9 morpholino (Nedd9MO), used in our gain- and loss-of-function experiments, respectively. We also test several constructs and probes that were then used to analyse the results obtained from our gain- and loss-of-function experiments. Once the optimal experimental conditions were obtained, we focused on the analysis of Nedd9 function during NC collective migration.

## 3) Description of the main results obtained

Nedd9 is essential for neural crest cell migration in vivo.

Our loss-of-function experiments confirmed that Nedd9 is essential for neural crest cells migration in vivo; we analysed the expression of several neural crest migration markers (Twist, Snail2, FoxD3) by in situ hybridization after blocking the translation of Nedd9 with an anti-sense morpholino (Nedd9-MO) and observed that the cells injected with Nedd9MO (Nedd9-morphant cells along the text) where not able to migrate. The specificity of the morpholino was assessed in a rescue experiment, where the co-injection of Nedd9 mRNA+Nedd9-MO restored the migration of neural crest cells. We also determined that the induction of the neural crest was not affected by the Nedd9-MO.

Nedd9 loss-of-function impaired cell dispersion and chemotaxis.

Neural crest cell migration involves the interaction of several cellular behaviors (Barriga and Mayor, 2015), thus we decide to test which of them where being affected by the loss-of-function of Nedd9. The first behaviour that we analysed was dispersion; in order to migrate, the neural crest performs epithelial-to-mesenchymal transition, an event described to occur at the onset of neural crest cells migration that prepare the cells to delaminate and collectively migrate. Hence, we perform cell dispersion assays as a measure of the capacity of the cells to perform epithelial-to-mesenchymal transition. Very interestingly we observe that the loss of function of Nedd9 completely blocks the dispersion of the neural crest cells, as demonstrated by the Delanoi triangulation maps. These results suggest a role for Nedd9 in controlling neural crest EMT, but we still to analyse if the cells do not disperse because there is an effect on cell-cell adhesion, cell motility, or both.

Other behaviour that has been well described during the migration of neural crest cells is their capacity to perform collective chemotaxis, briefly, NC cells express the chemokine receptor Cxcr4 and the NC surrounding tissues secrete its cognate ligand Sdf-1 (Theveneau et al., 2010). In our assay we confront neural crest cells explants that express Cxcr4 to migrate against a bead that has been soaked with Sdf-1. Consequently with the non-migratory phenotype observed in vivo and with the impaired dispersion phenotype, the capacity of the NC to migrate towards the chemoattractant source was drastically reduced, strongly suggesting that Nedd9 is also essential for neural crest directional migration in vivo.

Nedd9 loss-of-function reduces single cell motility.

In order to better understand the effects of the Nedd9 loss-of-function on the collective migration of the neural crest, we decide to analyse the behaviour and motility of individual cells. For such a reason we explanted the neural crest from the embryos and we dissociate the cells by culturing them in low calcium medium. We observed that the loss-of-function of Nedd9 reduce the motility of the neural crest cells, as evidenced when quantifying the migratory trajectories, cell velocity, and migrated distances of Nedd9 morphant cells.

Nedd9 loss-of-function reduces NC cell adhesion to the substrate.

The deduced protein structure for Nedd9 shows that it can bind to cell-substrate adhesion molecules such as Integrins, FAK, Src, among others, and potentially promote cell adhesion to the substrate. Hence we decided to analyse the capacity of control and Need9-morphant NC cells to attach to the substrate. For such a reason we explant and culture the NC in inverted dishes and quantified the explants for each conditions before and after incubation. Consequently with our predictions, Nedd9 morphant cells display reduced adhesion to the substrate. We also analyse cell morphology and focal adhesions formation in Nedd9-morphant NC cells, observing that the formation of these structures was drastically reduced, in comparison to control cells. These results suggest that Nedd9 could be controlling the capacity of the cells to interact with its surrounding environment by promoting focal adhesion formation and perhaps working as a mechanosensor of the migratory substrate, due to its homology to p130-CAS (Swada et al., 2006).

**4) Future collaboration with host institution (if applicable)**

This stay resulted in a fruitful collaboration among Dr Elias Barriga and Prof Roberto Mayor, we will keep collaborating in order to understand how the mechanical cues may participate or control the collective migratory behaviour of the neural crest cells in vivo and to elucidate the role of Nedd9 as part of the molecular mechanism that integrate these mechanical cues into the Neural Crest cells. Very importantly for the continuation of this project, I recently join Dr Guillaume Charras's Lab at the London Centre for Nanotechnology (LCN), UCL. With Dr Charras we applied and obtained funding to

pursuit postdoctoral training at LCN, UCL (EMBO-LTF fellowship and a Marie Curie postdoctoral grant), ensuring the collaborative work on the subject of this QuanTissue exchange for at least 3 years.

**5) Projected publications / articles resulting or to result from the grant *(ESF must be acknowledged in publications resulting from the grantee's work in relation with the grant)***

We publish two articles in wish we acknowledge to the Quantissue Exchange Grant 4505:

1. "Elias H Barriga and Roberto Mayor. (2015) Embryonic Cell-Cell Adhesion: A key player in collective neural crest cells migration. Curr Top Dev Biol, 112; 301-323. (http://dx.doi.org/10.1016/bs.ctdb.2014.11.023).

2. Elias H Barriga, Paul A Trainor, Marianne Bronner and Roberto Mayor. (2015). Animal models for studying neural crest development: is the mouse different? Development, In press.

In 2015 we will submit a research article where the results obtained from the exchange grant will be included and the Exchange grant 4505 will be acknowledge. The prospective tittle for this article is: "Nedd9 works as a Neural Crest mechanosensor".

**6) Other comments (if any)**

References:
Barriga et al. J Cell Biol 2013 Vol 27;201(5):759-76.
Barriga and Mayor Curr Top Dev Biol, 2015. Vol 112; 301-323.
Swada et al. Cell. 2006 Vol 1;127(5):1015-26.
Theveneau et al. Dev Cell 2010 Vol 19; 39-53.