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**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****:* Crosstalk between RhoGTPase signaling and cytoskeletal remodeling during cell migration

***Application Reference N°:*** 4377

1. **Purpose of the visit**

The main goal of the visit at the Netherlands Cancer Institute was to establish future collaboration between experimental group lead by Dr.Metello Innocenti and a modeling group of Systems Biology in Dublin, Ireland.

Both groups work to investigate the biochemical mechanisms underlying cell migration proccess.

1. **Description of the work carried out during the visit**

The work carried out during the visit can be divided in the experimental and modeling part:

-The model was constructed based on the preexisting data in Dr.Innocenti's group. The model is based on biochemical signaling during the F-actin polymerization. The model combines the biochemical activities of proteins involved in cytoskeletal reorganization and their effect on the cell morphology during cell motility (cells exhibiting filopodia, lamellopodia, both or none). The model will further be refined and tested upon the return to Dublin.

-Experiments were carried out to elucidate the interaction mechanism between RhoGTPase RhoA and its effectors mDIA1 and ROCK1. First, the localization and the number of interactions was assessed via Proximity Ligation Assay. Further, motility assays were done in different conditions (serum starved and EGF stimulated conditions and in presence or absence of ROCK inhibitor). Finally, after noticing a significant change in the directional migration, we investigated this effect by performing Dunn Chamber motility assays.

1. **Description of the main results obtained**

Migration is a translocation of the cell body, which underlies important cellular mechanisms such as wound healing and embryonic development but is also responsible for pathological conditions such as cancer invasion and metastasis [1]. The understanding of the underlying biochemical mechanisms controlling cell migration is crucial and can potentially establish ways to inhibit the disease progression.

Our study focuses on the biochemical control of cell migration by the activities of G proteins (GTPases), in particular by the most prominent RhoGTPase RhoA and its dowstream effector proteins mDIA1 and ROCK1. Both mDIA1 and ROCK1 mediate RhoA action on the actin cytoskeleton; mDia1 produces actin filamets by nucleation and polymerization, while ROCK is responsible for the induction of actomyosin bundles and contractility [2]. Hence, cell morphogenesis, adhesion and motility can be determined by the balance between these two effectors [2]. Though the interaction of RhoA and mDIA and ROCK has been extensively described, the interplay between these effectors depending on different cell localizations has never been investigated.

In the current study, we used Proximity Ligation Assay (PLA), which allowed to visualize the interactions between RhoA and mDIA/ROCK. We performed PLA in MDA-MB231 breast cancer cell line in different conditions. We used 3 different cell lines: the control cell line and two mDIA1 knockdown cell lines, which were developed previously in the Dr.Innocenti's group. Further we treated the mentioned cell lines with the ROCK inhibitor Y27563. These experiments were meant to show how the RhoA-mDIA and RhoA-ROCK interactions can be affected by inhibition of ROCK or mDIA1 respectively. Although we did not see the difference in the RhoA-ROCK interaction upon mDIA1 inhibition, our results show that number of RhoA-mDIA1 interactions greatly decrease (up to 60%) upon ROCK inhibition.

To asess the biological effect of the ROCK inhibition on RhoA-mDIA interactions we have performed various motility assays. These assays include serum starved and EGF stimulated conditions in presence or absence of ROCK inhibitor. The results show that upon ROCK inhibition the directional migration significantly increases, which might suggest that mDIA-RhoA plays an important role in the cell directionality during motility. Further assessment of this hypothesis is required.

[1] A.Lambrechts, M.Van Troys and C.Ampe, The international journal of biochemistry and cell biology, 2004

[2] S.Narumiya, M.Tanji, T.Ishizaki, Cancer Metastasis Rev., 2009

1. **Future collaboration with host institution (if applicable)**

This visit helped to establish collaboration between the experimental group of Dr.Innocenti at the Netherlands Cancer Institute and the modeling Prof.Kholodenko's group in Systems Biology Ireland. In future, we plan to collaborate on the project involving RhoA-mDIA and RhoA-ROCK interactions as well as developing a final model for Dr.Innocenti's data. Hopefully, this visit will establish a long term collaboration working on the project of cell migration.

1. **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)***

"Role of RhoA interaction with its effector proteins in directed migration" in progress (to be published in 2014)

1. **Other comments (if any)**

I would like to thank again the Quantissue granting committee for providing the financial support to pursue the internship at the Netherlands Cancer Institute.