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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****:* “Identifying mechanotransduction pathways triggering epicardial development in zebrafish embryos”

***Application Reference N°:*** 4661

1. **Purpose of the visit**

We have described flow forces inside the pericardial cavity of the zebrafish embryo as essential for PE development (Peralta et al. 2013). In the absence of a beating heart, PE clusters do not form, suggesting a crucial role for pericardial flow in triggering PE development. The mechanism through which this regulation is mediated, however, is unknown. Our aim is to understand how mesothelial cells from the pericardial cavity and PE cells sense flow forces within the cavity. Our hypothesis was that primary cilia might be involved in mechanotranduction of pericardial flow floreces in pericardial mesothelial and PE cells.

Our objetives were:

1. To characterize presence and dynamics of ciliated cells in the pericardial wall and the PE in presence and absence of heartbeat.

2. To study the function of cilia in pericardial mesothelial and the PE.

3. To test the role of Ca-signalling in mechanotransduction in pericardial mesothelial and the PE.

4. To compare the forces needed to activate mechanotransduction in normal condition with cardiac heart contractility mutants with altered heartbeat. We will performe that assays with optical tweezers.

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

Using the Tg(beta-actin:arl13b:GFP) (Borovina et al. 2010) a primary cilia reporter line, we have characterized the presence of cilia in pericardial an PE cells protruding into the pericardial cavity in vivo. We have created a cilia map at 48 hpf, before PE cluster appear, and at 55 hpf, when the clusters are formed. We have analyzed cilia behaviour in presence and absence of heartbeat.

To study the function of cilia in epicardial development, we have injected zebrafish embryos with ift88 morpholino, disrupting ciliogenesis. Afterwards we performed proliferation analisys in pericardia cells doing immunohistochemistry against phosphohistone h3.

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

We have observed that cilia distribution inside the pericardial cavity is not homogeneously distributed. Cilia distribution remains constant during PE cluster formation (between 48 hpf and 55 hpf). We divided the pericardial cavity in different regions for the analysis and the mapping cilia. We realized that cilia movement is specific of the region where they are located and their closeness to the cardiac tube. This might mean that cilia are signaling different depending on their location and the forces acting upon them. Stopping the heartbeat by BDM treatment, we have confirmed that cilia are primary cilia (lack of autonomous movement). Moreover, cilia movement is coupled with the heartbeat. On the other hand, the analysis of proliferation in embryos inyected with ift88 morpholino have shown an important decrease in the number of pericardial proliferative cells. The epicardial layer at 72 hpf in morphants is compound by lower number of cells than control embryos.

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

We will continue the colaboration with Julien Vermot's lab for the publication of the data. We will perform in vivo calcium imaging of pericardial cells with the aim of correlating calcium-signalling to cilia movements and pericardial cell fate.

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)***

"Pericardial flow forces act on pericardial cells to control proliferation and PE formation through primary cilia in the zebrafish". Peralta M, Rua-Ferreira R, Boselli, F, Vermot J, Mercader , N.

(In preparation).

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