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STUDY AND MANIPULATION OF THE EMBRYONIC EPICARDIUM PROGRAM, A NEW APPROACH FOR HEART REPAIR

Ofelia M. Martínez Estrada

Facultat de Biologia – Universitat de Barcelona

1. Summary

Heart failure constitutes one of the leading causes of death worldwide. Over the last 10 years numerous strategies involving stem cell transplantation therapy have been used for cardiac repair. However, the benefit is still small. The molecular mechanisms that take place during heart morphogenesis are usually compatible with those that take place during heart regeneration. Considering this fact, a major challenge exists in the identification of factors that mimic the local environment present during embryonic or early life heart development, which can exert their beneficial effects during heart repair. The epicardium plays a crucial role during embryonic heart development and also participates in adult heart repair. Given the enormous interest in this topic we performed transcriptomic analysis of freshly isolated epicardial enriched cells at different stages of heart development. This analysis covered the characterization of the early embryonic epicardium, the epicardial mesenchymal transition (EMT) and invasion through the differentiation and maturation of the epicardium. In order to elucidate the role of *Wt1* in this signature we also analysed the transcriptomic profile of epicardial enriched cells from epicardial specific Wt1KO mice. This project studied the importance of two pathways potentially involved in epicardium development regulated by *Wt1*: one that is implicated in epicardium maturation, and the other implicated in the formation and expansion of the epicardial derived cells. We hypothesized that the modulation of these pathways could be used to identify new therapeutic targets for heart repair following myocardial infarction.

2. Results

Despite the importance of the epicardium in heart development and repair, a detailed characterisation of the epicardium during different stages of heart development was absent. The study of two new pathways identified by the analysis of a transcriptomic profile of epicardial-enriched cells at different stages of development from control and epicardial-specific *Wt1* knockout (epiWt1KO) mice was one of the main research aims of our project. We have produced evidence that clearly demonstrates that the *Bmp4* pathway is involved in the maturation process of the epicardium which is characterised by

a morphological change of epicardial cells from a cuboidal to squamous phenotype. Transcriptome and cell morphology analysis of epicardial cells from epiWt1KO mice revealed a defect in the maturation of the mutant epicardium, which includes a sustained up-regulation of *Bmp4* expression and the inability of mutant epicardial cells to transition into a mature squamous phenotype. We identified *Bmp4* as a transcriptional target of *Wt1*, thus providing a molecular basis for the retention of a cuboidal cell shape observed in the *Wt1KO* epicardium. Accordingly, the inhibition of the Bmp4 signalling pathway in both *ex vivo* and *in vivo* rescued the epiWt1KO cuboidal phenotype. Our findings indicate the importance of the cuboidal to squamous transition in epicardial maturation, a process regulated by *Wt1* and an aspect of epicardial biology unidentified to date. We have also demonstrated that Bmp4 signalling is able to modulate the expression of genes differentially expressed during the course of epicardium development, especially genes abundantly expressed during the early stage. We hypothesize that some of those genes could be involved in the modulation of the immature epicardium phenotype.

We were also involved in the identification of the role of Slit2/Robo pathway in epicardium development. We found an increase in *Slit2* expression during the expansion of the epicardial derived cells (EPDCs). In addition, we also demonstrated that Slit2 is a transcriptional target of Wt1. The increased expression of *Slit2* during the expansion of EPDCs and the fact that its expression was downregulated in the *Wt1KO* epicardium (in which there is a defective epicardial EMT) suggested to us a potential positive role of this pathway in epicardial EMT. To our surprise the analysis of the epicardium of Robo1,2 KO mice revealed an expansion of the EPDCs. Our in vitro and ex vivo experiments demonstrated that Slit2 is able to inhibit epicardial cell proliferation and migration, which could be one of the main explanations of the expansion of the EPDCs observed in Robo1,2 KO mice. Interestingly, we also demonstrated the presence of a negative regulatory loop in which Slit2 inhibit the expression of Wt1. As part of this project, we have also demonstrated a correlation of Wt1 with the proliferation of the epicardium both in vitro and in vivo. All these findings therefore provide a putative molecular mechanism for the expansion of the EPDCS observed in the Robo1,2KO epicardium.

3. Relevance

This was a basic science project but with potential clinical implications. A major challenge in the epicardium biology lies in the identification of factors that mimic the local environment present during the embryonic heart development that can exert their beneficial effects during heart repair. During this project we have identified two novel pathways involved in epicardium biology: one in epicardium maturation and the other in the expansion of the EPDCs. Interestingly, both pathways are signalling pathways modulated by secreted proteins of epicardial cells, which made them very interesting candidates to be manipulated.

Studies in organisms that are able to regenerate have demonstrated that regeneration does not occur from the proliferation of just one cell type, but from the cooperation of different cell types in conjunction with an increase in neovascularisation and the modulation of the local inflammatory response. We hypothesize that the two pathways studied in this project could be considered as interesting candidates to be manipulated in order to improve the poor regeneration capacity of the damaged human heart.

4. Bibliography

Epicardial cell shape and maturation are regulated by Wt1 via transcriptional control of Bmp4. Velecela, V., Torres-Cano, A., Garcia-Melero, A., Ramiro-Pareta, M., Muller-Sanchez, C., Segarra-Mondejar, M., Chau, Y.Y., Campos-Bonilla, B., Reina, M., Soriano, F.X., Hastie, N.D., Martinez, F.O., Martinez-Estrada, O.M. Development. 2019. dev178723