Universidad de Navarra

Máster en Investigación Biomédica
Facultad de Ciencias

Research Project Proposal
Academic year 2016-2017

Project Nº 23

Title: Deletion of endothelial metalloproteinase-10 by CRISPR/Cas9 genome editing and analysis of functional effects

Department/Laboratory: Laboratory of Atherothrombosis, Program of Cardiovascular Diseases, Center for Applied Medical research, CIMA

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Summary

The fibrinolytic and matrix metalloproteinases (MMP) systems cooperate in thrombus dissolution and extracellular matrix proteolysis. We have demonstrated that MMP-10 has a profibrinolytic effect by enhancing tPA-induced thrombolysis, in vivo and in vitro. With the working hypothesis that endothelium can be a source for MMP10 and thus contribute to fibrinolysis, we propose the following objectives:

- Generate a human endothelial cell line with no MMP10 expression using CRISPR/Cas9 genome editing.
- Analyse coagulation and fibrinolysis in human whole blood in the presence of these cells and compare the results with parental endothelial cell line and with EA.hy926 cells overexpressing MMP10.

A CRISPR/Cas9 editing strategy will be planned. After MMP10 gene analysis, guide RNAs will be designed, synthesized and cloned in a vector containing Cas9 and GFP. Human endothelial EA.hy926 cell line will be transfected and transformants selected by flow cytometry and cell sorting. After clonal expansion, clones will be screened by immunoblotting and DNA sequencing. Blood coagulation and fibrinolysis will be analysed by thromboelastometry of human whole blood in the presence of endothelial cells grown on Cytodex carriers.

References

1. Orbe J, Barrenetxe J, Rodriguez JA, Vivien D, Orset C, Parks WC, Birkland TP, Serrano R, Purroy A, Martinez de Lizarrondo S, Angles-Cano E, Páramo JA. Matrix metalloproteinase-10 effectively reduces infarct size in experimental stroke by

enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor-mediated mechanism. Circulation. 2011;124:2909-19.

2. Martínez de Lizarrondo S, Roncal C, Calvayrac O, Rodríguez C, Varo N, Purroy A, Lorente L, Rodríguez JA, Doeuvre L, Hervás-Stubbs S, Angles-Cano E, Páramo JA, Martínez-González J, Orbe J. Synergistic effect of thrombin and CD40 ligand on endothelial matrix metalloproteinase-10 expression and microparticle generation in vitro and in vivo. Arterioscler Thromb Vasc Biol. 2012;32:1477-87.

3. Orbe J, Rodríguez JA, Sánchez-Arias JA, Salicio A, Belzunce M, Ugarte A, Chang HC, Rabal O, Oyarzabal J, Páramo JA. Discovery and safety profiling of a potent preclinical candidate, (4-[4-[[(3R)-3-(hydroxycarbamoyl)-8-azaspiro[4.5]decan-3-yl]sulfonyl]phenoxy]-N-methylbenzamide) (CM-352), for the prevention and treatment of hemorrhage. J Med Chem. 2015;58:2941-57.

POSSIBILITY OF PhD

YES*

* (PhD grant required)