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Soutenance de thèse

Specific features of the aortic endothelium in a murine model of Marfan Syndrome.

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Podosome formation in aortic endothelial cells exposed to TGFb has been well described *in vitro* (in tissue culture dishes) and *ex vivo* (in living aortic vessel segments). The aim of the project was to go to the next step and demonstrate the occurrence of podosomes *in vivo* in response to endogenous TGFb. For this purpose, we chose to use a genetically engineered mouse model, which spontaneously presents high TGFb levels in the aorta, reasoning that this would be a favorable environment for these structures to appear. Marfan mice represent the murine model of the human disease Marfan syndrome. Similar to the human disease, a mutation in the fibrillin-1 gene (C1039G/+) leads to enhanced TGFb levels in the aortic wall as well as in circulating blood. We therefore used the Marfan mouse model in search of podosomes in the aortic endothelium.
"En face" viewing of the endothelium stained for filamentous actin, cortactin, Tks5 adaptator protein and MT1-MMP metalloprotease detected podosome rosettes with features similar to those detected in the *ex vivo* situation. Podosome rosettes were found in both descending and ascending aorta. Analysis of the underlying tissue with collagen IV staining revealed a basement membrane scattered with staining-free patches, most likely corresponding to collagen IV degradation. We propose that podosome rosettes are involved in basement membrane degradation in this mouse.
To examine the consequences of fibrillin-1 deficiency in endothelial cells and confirm the data obtained *in vivo* in Marfan mouse aorta, we used two *in vitro* approaches. First, we set up a protocol to isolate aortic endothelial cells from Marfan aortas, second, we depleted fibrillin-1 from BAE cells by siRNA silencing. Isolated Marfan aortic endothelial cells retained *in vitro* the TGFb

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activated phenotype and formed functional podosomes without any exogenous stimulation. TGFb levels measurements in fibrillin-1 depleted aortic endothelial cells confirmed that fibrillin-1 deficiency triggers an increase in active, cell associated TGFb, which in turns, leads to podosome formation in endothelial cells.
Finally we studied other alterations caused by the fibrillin-1 defect at the endothelial cell level *in situ*. Topological analysis of the Marfan mouse aortic endothelium monolayer revealed cell blebbing, numerous filopodia and showed altered cell-cell junctions.
At the ultrastructural level, transmission electronic microscopy revealed that the Marfan mouse endothelium had an appearance dramatically distinct from that observed in control littermates. The elastic lamina, weakened by fibrillin-1 deficit, disappeared in some places. The vessel wall also showed abundant extracellular matrix proteins. Endothelial cells presented an activated or apoptotic phenotype.
These studies provide the first demonstration for the occurrence of endothelial podosomes *in vivo* and suggest their involvement in vascular physiopathology.
In addition, they provide evidence that the aortic endothelium is profoundly altered in the murine model of Marfan syndrome.

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