PROGRAMME DE STAGE D'INITIATION À LA RECHERCHE BIOMÉDICALE DU CENTRE DE RECHERCHE CHU SAINTE-JUSTINE – ÉTÉ 2018

Centre de recherche CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant
Université de Montréal

Characterization of fibronectin mutations leading to scoliosis.

Numéro de l'offre de stage : No. 8

Équipe de recherche

Philippe Campeau, M.D.
Médecin généticien, CHU Sainte-Justine
Professeur adjoint de clinique, Département de pédiatrie, Université de Montréal
Axe de recherche : Maladies musculosquelettiques et réadaptation

Coordonnées

p.campeau@umontreal.ca

Description du projet

We recently identified a new genetic disease caused by fibronectin (FN) mutations, a developmental bone disease called spondylometaphyseal dysplasia (SMD, characterized by scoliosis and abnormal growth plates). Our goal is to better understand how the mutations cause disease, and whether fibronectin mutations also cause the more common scoliosis of adolescence.

For this purpose, ATDC5 cell will be stably transfected with the full-length FN constructs harboring patient mutations. This cell line can undergo chondrogenic differentiation. First, we will test by Western blotting and indirect immunofluorescence whether the mutation causes deficiency secretion. We will analyse by light and transmission electron microscopy the formation of the FN network We will determine by immunocytochemistry whether the mutant FN affects the formation of these networks including fibrillins, latent TGF-β binding proteins (LTBPs), and collagens I, II and III. We will therefore investigate the expression levels of these growth factors and their recptors at mRNA (qPCR) and protein (Western blot, immunofluorescence) levels, The rate and efficiency of chondrocyte differentiation will be indirectly assessed by analysis of gene expression; qPCR will be performed for markers of chondrogenesis (SOX9, COL2A1, and ACAN), as well as for markers of chondrocyte hypertrophy and terminal differentiation (COL10A1, MMP13, RUNX2). The differentiation will also be assessed by histology to determine the shape and alignment of the cells (H&E), the matric proteoglycans (toluidine blue) and the distribution of FN, both inside and outside the chondrocytes (by immunohistochemistry). These experiments should allow us to determine if the FN mutations found in SMD and in scoliosis lead to chondrocyte dysfunction, abnormal extracellular matrix composition and assembly, or both.

Mots clés

Maladies osseuses, matrice extracellulaire, génétique.