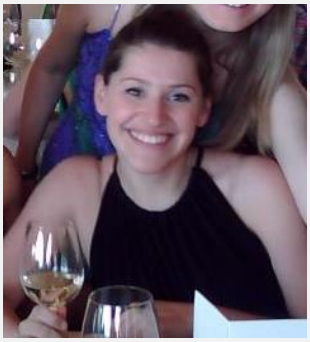
# ImmunoTools *special* Award 2014



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## Differentiation of mouse pluripotent stem cells to adipocytes and the involvement of vasculature in brown tissue biology

Obesity represents a health problem with vast impact in Europe. Mammals have two types of adipose tissue. White adipose tissue (WAT), hormone-sensitive, is used as a store of energy. Brown adipose tissue (BAT) is a highly vascularized organ, that when activated by the sympathetic nervous system is able to generate heat rather than ATP, through uncoupled oxidative phosphorylation. BAT is abundant in small rodents and newborn humans, and partly disappear in human adulthood. In the last two years BAT has been proposed as target for the prevention and/or treatment obesity in human. Recent studies have pointed to the importance of microvasculature in controlling metabolic function in adipose tissue. In particularly the diet-induced damage led to marked reductions in the vascular network of both BAT and WAT. Previous studies have shown that Vascular Endothelial Growth Factor A (VEGF-A) has a crucial role in controlling vascular network formation in adult tissues. The capillary rarefaction in BAT, due to a decrease of VEGF and its receptor VEGFR2 leads to the process called “BAT whitening”, that is the trans-differentiation of brown adipocytes to white adipocytes.

Embryonic stem (ES) cells are a robust system for the characterization of developmental mechanisms. ES cells derived from the inner cell mass of developing embryos have a great potential in regenerative medicine. ES cells can be maintained for a prolonged time without changes in their cellular characteristics in vitro (self-renewal), or differentiated to a wide range of cell types.

In our laboratory we study the differentiation of mES cells into adipocytes and endothelial cells. Through by the in vitro “hanging drop” technique we can drive the differentiation using a different mix of growth factors and cytokines. The interplay between adipocytes and endothelial cells and the role of angiogenesis in differentiation and maintenance of BAT will be investigated by changing cell culture condition from 2D to 3D. At different time point cell colture will be collected and analyzed by cytofluorimeter for the presence of differentiated brown adipocytes and endothelial cells. Also the expression of cytokines and growth factors will be quantified with ELISA kits.

During adipocytes and endothelial cells differentiation dramatic changes occur in cell morphology, cytoskeletal components and adhesion molecules. On this basis 2D and 3D (collagen or fibrin gel) colture will be analyzed by immunofluorescence in confocal microscopy with particular attention at fat lobules formed by endothelial cell and adipocytes. All these experiments will be performed in the absence or in the presence of different growth factors or extracellular matrix (ECM) to increase the amount of mES cells differentiated in mature endothelial cells and adipocytes.

High amount of differentiated endothelial cells may improve insulin sensitivity and BAT thermogenic response. It has been shown that increasing BAT mass through transplantation improves metabolism parameters in diet-induced obesity. Therefore the characterization of the molecular mechanisms in the development and differentiation of mES cells is of crucial importance for the development of new diagnostic and therapeutic strategies.