PROJECT REPORT

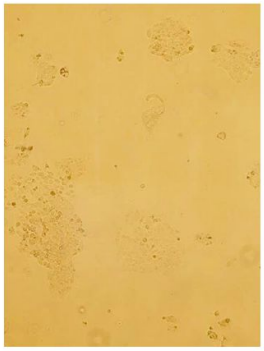
Grant type: ERC mobility, PI: Krisztian KVELL MD PhD, grant ID: 125828

Host research institution

ERC mobility grant provided support for 3 months. During this I have performed experiments in a foreign ERC-funded laboratory at the Max-Planck Institute of Münster, Germany (**see images below**) under the supervision of Prof. Hans Schöler (PI of host ERC Advanced Grant: Prometheus, project ID: 669168, duration: 2015-08-15 to 2020-07-31). Short duration and unfamiliar lab context usually limit realistic expectations, yet I have managed to perform the planned experiments. I have geared up the preliminary murine system to efficiently be used with human cells.

Switch to human cells instead of mouse cells

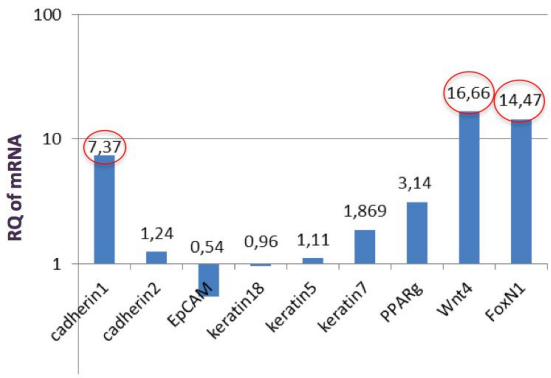
Previous experiments were performed on mouse thymic epithelial cell line (TEP1 with BALB/c origin). The DMEM-cultured fast growing cell line dates back 28 years from non-human species and disputable genetic integrity. For this reason I have changed to using human thymoma cells (benign thymic epithelial cell overgrowth, **see image on right**). The so-called 1889c cells originate from more recent human benign disease and has a well-characterized genotype and depository (DSMZ, Germany). As expected, 1889c cells grow slow and form island-like structures with human RPMI growth medium.



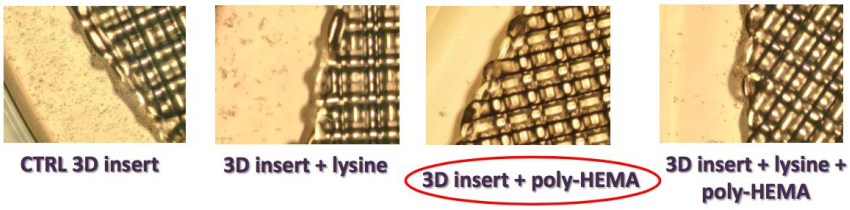
Adaptation to human T-cell expansion medium

Later experiments will concentrate on thymocyte (T-cell) differentiation starting from peripheral blood hemopoietic stem cells (HSCs). These cells have special growth factor requirements. Also, if eventual human application is in focus, cell cultures shall be performed using substances free from animal products (FCS). For this I have adapted 1889c cells to grow in serum-free medium optimized for T-cell differentiation (human T-cell expansion medium, Sigma/Merck). 1889c cells prefer to initiate cell growth first in RPMI medium, later human T-cell expansion medium may also be used for cultures.

Enhanced growth on 3D biodegradable scaffold



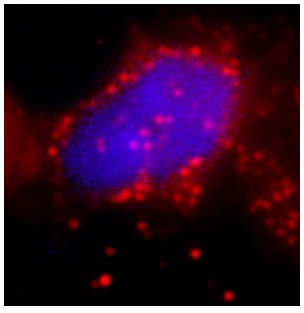
Human primary-derived cells often loose identity and dedifferentiate when grown in 2D monolayer cultures. To maintain (thymic epithelial) cellular identity 1889c cells were grown on 3D scaffold. According to qPCR measurements (**see image on left**) when 1889c cells are grown on 3D scaffold several markers of epithelial identity (FoxN1, Wnt4, Cadherin-1) are up-regulated compared to standard 2D monolayer cultures confirming preserved identity. 1889c cells tend to divide faster when grown in 3D conditions. Of note, biomaterial of the 3D scaffold was PCL (poly-caprolactone) that is both biocompatible and biodegradable (to lactic acid over time) appointing PCL as an appropriate candidate for scaffold constituent. Moreover, during initial experiments a significant proportion of 1889c cells exited the 3D scaffold and continued growing on host well plastic in 2D monolayer. As his is undesirable, I have forced 1889c cells for preferential growth on 3D PCL scaffold by pre-treating host well plastic surface with poly-HEMA to prevent 1889c cell adherence to host well plastic. Poly-HEMA treatment efficiently forced the growth of 1889c cells over 3D PCL scaffold (**see image below**).

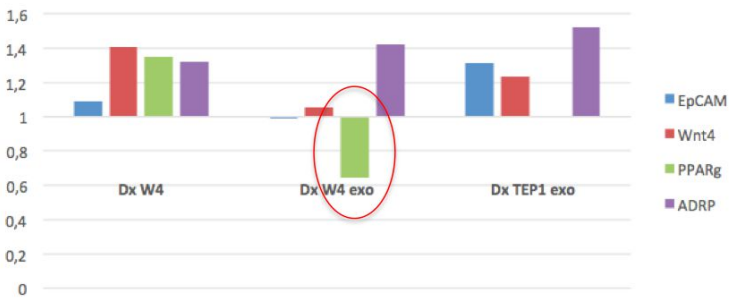


		Cell	Numbers / mL	
3D	30 000 cells	-	290 000 cells	-
2D	105 000 cells	15 000 cells	-	-

Exosome-mediated enhancement of thymic epithelial identity

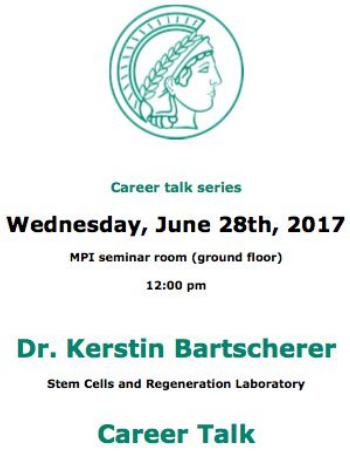
Previous experiments suggested that the Wnt4 glycolipoprotein can efficiently preserve thymic epithelial cell identity. It has been suggested that Wnt4 travels in microvesicles (including exosomes). For this I have enriched Wnt4-containing exosomes from thymic epithelial cells producing recombinant human Wnt4 (generated earlier by myself). When these were applied in culture it significantly suppressed PPARgamma expression (known to weaken thymic epithelial identity), providing firm proof of preserving thymic epithelial cell identity via Wnt4-containing exosomes (**see images below**).





Acquiring ERC-related soft skills

Besides the above mentioned scientific achievements, ERC soft skills have also been gained by participating ERC career talk seminars and ERC interview trainings. During ERC career talk seminars current ERC grant-holder PIs provided lecture on what specific circumstances of their career were believed to help to win their ERC grants (**see image below**). Then ERC interview trainings provided insight into the second (oral) round of ERC application. During this stage candidate PIs need to give a short talk on their scientific proposal. This was then analyzed by experts providing advice and hints.



Networking

During my stay at the Max-Planck Institute of Münster, Germany I have interacted with the local immunologist community. As a result two abstracts have been submitted to a Research Topic currently open under my co-guest editorship at Frontiers in Immunology (Immune tolerance and regulation / The role of PPARgamma in immune homeostasis) (**see images below**). I have also interacted with researchers of the neighboring CIM (Cells in motion cluster) and CeNTech (nano-biotechnology institute).