Universität Basel

# Research Project

# Cellular and molecular analysis of thymus organogenesis and maintenance

## Third-party funded project

**Project title** Cellular and molecular analysis of thymus organogenesis and maintenance
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The thymus provides the physiological microenvironment for the development of T lymphocytes. Hence, its function is critical for the successful establishment and maintenance of the immune system's capacity to distinguish between vital self and injurious non-self. This competence is primarily instructed by thymic epithelial cells (TECs). However, the molecular and cellular mechanisms controlling thymus formation, homeostatic maintenance and function are at present only incompletely defined. Spontaneously occurring, monogenic loss and gain of function mutations in man have been highly informative in defining the mechanisms that establish and maintain the physiology of complex organ systems. Selecting two of these "experiments of nature" with relevance to thymus biology, we anticipate to gain unprecedented insight not only into the pathomechanisms of these selected primary immunodeficiencies but, equally importantly, advance our understanding of the genetic, epigenetic and biochemical mechanisms that underpin normal thymus biology. Specifically, this research proposal seeks (i) to characterize the mechanism by which naturally occurring mutations of the transcription factor FOXN1 gain a dominant negative function and consequently impair normal thymus biology (Specific Aim 1); (ii) to elucidate the role of Lin28A expression regulating TEC growth, metabolism and function (Specific Aim 2); and (iii) to reveal the role of adenylate kinase 2 (AK2) in maintaining a normal cell energy level and regular metabolic functions in TEC (Specific Aim 3).Working Hypothesis The proposed research is based on the testable hypotheses that TEC development and function are severely compromised (i) by mutations in the transcriptional activation domain of FOXN1, consequently disrupting the interaction with co-factors required for the correct DNA, (ii) the disruption of the Lin28A/let-7 axis; and (iii) by the disruption of adequate, AK2-controlled energy homeostasis.Specific Aims#1: To define the structure::function relationships of wild type and dominant negative forms of FOXN1.#2: To establish a role for the Lin28A/let-7 miRNA feedback axis in TEC biology.#3: To delineate the role of adenylate kinase 2 in TEC development and function. Experimental Design and MethodsWe will address these specific aims using mouse gene editing combined with state of the art molecular biology, cell biology, imaging and bioinformatic analysis. In particular, we have generated new mouse models to specifically probe the function of the three interrelated gene products, FOXN1, LIN28 and AK2, in TECs at distinct developmental stages. The chosen design of TEC-specific targeting of Lin28 and Ak2 avoids early embryonic lethality and thus allows the detailed investigation of their impact on TEC differentiation and function at distinct developmental stages, ranging from mid-gestation embryogenesis to senescence. Cytometry-based deep phenotyping, transcriptomic analyses at both population level and single cell resolution, and high-parameter single cell spatial imaging are tools to be employed to gain a better understanding of the consequences of the experimentally introduced genetic perturbations. Expected Value of the Proposed ProjectThe thymus plays a vital role

in generating a repertoire of functionally competent T cells and thus provides a unique function that relies on molecular mechanisms astonishingly unique from that of other epithelial organs. The proposed work focuses on two human primary immunodeficiencies that severely affect TEC function. Although their causative gene mutations have been identified, the precise cellular and molecular mechanism underpinning these pathologies remain to be defined. The proposed research will therefore provide not only novel insights into the cellular and molecular mechanisms that prompt this loss of TEC function but will also inform on the genetic control of regular TEC development and function. Insight attained from the outlined studies will therefore be relevant to the fields of immunology, developmental biology and regenerative medicine.

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