ImmunoTools *special* Award 2018

Andries Hijlke Groen, PhD-student

Supervisor: Prof. Dr. Robert Coppes

Department of Cell Biology, University of Groningen,
University Medical Center Groningen, Groningen,
The Netherlands

Construction of functional thyroid tissue from primary murine and human thyroid organoids

Thyroid cancer incidence is increasing and is now the fifth leading cause of cancer in women the United States. A total of 56,870 cases are expected to be diagnosed in 2017 in the United States. The high incidence combined with the high 10-year survival of 90-95% leads to a high prevalence of thyroid cancer survivors. Most of these patients depend on daily thyroid hormone substitution therapy (levothyroxine) for the treatment of hypothyroidism after removal of the thyroid gland. Almost half of these patients, however, suffer from unbalanced hormone levels due to the thyroid hormone replacement therapy, leading to fatigue, constipation, weight increase and potential cardiovascular diseases or osteoporosis. Replenishment of these cells by stem cell therapy could represent a more physiological and durable solution to unbalanced hormone substitution. Proof-of-concept of this strategy has first been demonstrated with embryonic stem cells and pluripotent stem cells, which are regretfully not yet usable in the clinic. Therefore, adult stem cells could represent a valuable alternative to treat these hypothyroid patients.

In our current study we are focusing on the identification and modulation of pathways that are critical for the survival and the regenerative ability of our thyroid (cancer) stem cells, with the aim of augmenting the regenerative potential of adult human thyroid gland stem cells and to further develop our thyroid tumor organoid model to provide a foundation to study both the physiology and pathophysiology of the thyroid gland.

To study the regenerative potential of the thyroid gland cells we isolated, cultured and propagated primary murine and human thyroid tissue as organoids. Thyroid organoids transcriptomically expressed multiple stem cell markers, and major thyroid gland lineage markers Nkx2-1, Thyroglobulin and T4. Upregulation of several stemness markers, proliferation markers and several cyclins was observed after prolonged passaging, without expressing thyroid tumor specific patterns. Induced maturation of murine and human organoids in vitro resulted in tissue resembling mini-glands that abundantly expressed major thyroid gland markers and released T4-hormone. (Xeno-)transplantation of dissociated organoids underneath the kidney capsule of athyroid mice resulted in the generation of hormone-producing murine and human thyroid- resembling follicles and increased survival. In parallel, differentiated and medullary human thyroid cancer organoids could be cultured and propagated

with a clear distinction in marker expression profile. These studies provide the first proof of principle that primary thyroid gland- derived organoids can be cultured and are able to develop into a functional mini-gland, suggesting potential applicability for thyroid gland generation.

However, modulation of pathways, such as Notch or BMP/TGF-beta, may enhance the self-renewal potential of our thyroid gland stem/progenitor cells. Therefore, recombinant human proteins from these key pathways from ImmunoTools would be a huge benefit for the optimization of our 3D *in vitro* long-term culture system, and we apply for the ImmunoTools *special* AWARD 2018 in order to maximise the regenerative potential of both our human thyroid gland stem cells and thyroid tumor cells via pathway modulation, and expedite the journey of these cells towards a cellular therapy for hypothyroidism and a foundation for further studies towards the treatment of thyroid cancer.

ImmunoTools *special* AWARD for **Andries Hijlke Groen** includes 16 reagents

recombinant human cytokines: rh Activin-A, rh BDNF, rh GDNF, rh IGFBP-4, rh IGF-I, rh IGF-II, rh KGF/FGF-7, rh KGF-2/FGF-10, rh Noggin, rh SCF, rh SDF-1alpha/CXCL12a, rh SHH, rh TGF-beta3, rh TNF-alpha, rh VEGF-121, rh VEGF-A/VEGF-165

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