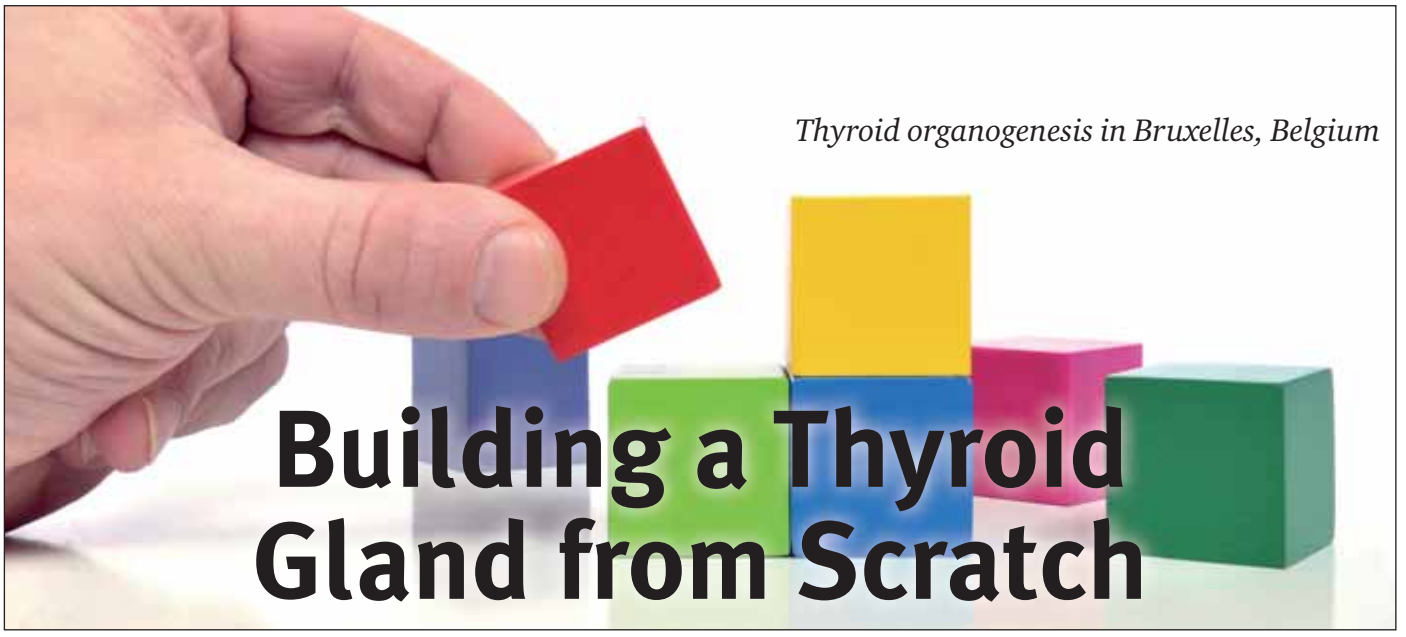

Thyroid organogenesis in Bruxelles, Belgium

Building a Thyroid Gland from Scratch

Photo: Fotolia/svort

Three-dimensional cell culture is the flavour of the month, right now. But you don't necessarily need fancy 3D scaffolds for this. Sabine Costagliola managed to create functional follicular organoids from embryonic stem cells, just by genetic manipulation and the addition of a hormone.

Iodine is a nutrient that is extremely easily taken up from the environment… if you are a fish. If you, however, happen to be one of those vertebrates that left the ocean around 400 millions years ago, you need a thyroid gland to gather and control your iodine levels, along with your growth, metabolism, heart function and brain development. The complexity of the three-dimensional arrangement of the thyroid cells and its biosynthetic capacity is intimidating. It has already been a daunting task to assess how the structure is formed *in vivo*, viz., as an invagination of the foregut endoderm. Any attempt to produce structures resembling a thyroid gland *in vitro* has been long regarded as science fiction, until now. Sabine Costagliola's group from the Université libre de Bruxelles turned science fiction into reality.

Biological alchemy

The rationale behind the controlled differentiation of stem cells is somewhat the reverse of the 2006 Nobel Prize. Gurdon and Yamanaka's work showed that adult cells can be rebooted to an undifferentiated state, in which the genome regains the potency to generate several cell types in the adult body. The transcription factor cocktail Oct-3/4, SOX2, c-Myc and Klf4 can turn adult cells into stem cells. But for regenerative medicine that's only half of the trick.

The next challenge is to take those undifferentiated cells and cast a biochemical spell to turn them into a specific cell type. The successful generation of a plethora of cell types from stem cells in the last decade has been witness to the power of this approach. However, if we talk seriously about regenerative medicine, we have to glance at a most ambitious goal: the regeneration of organs and body parts. That's a bit more complicated. The usual protocols for differentiation involve the expression of transcription factors or the exposure of cells to molecules that swing randomly in a media. Those two approaches tell the cells what needs to be changed in the genome but can't answer one primordial question that PhD students also often pose: how do we get organised spatially?

Take the thyroid follicles as an example; they are a spherical arrangement of polarised cells enclosing a colloidal central cavity, in which the thyroglobulin iodination takes place. How would you assemble such a structure from scratch?

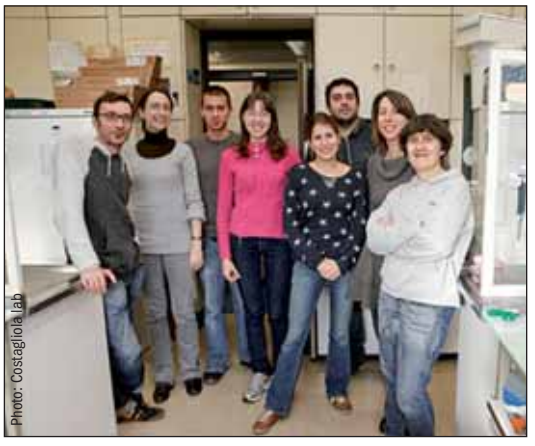

Photo: Costagliola lab

Sabine Costagliola (right) and her team just before casting a biochemical spell that turns embryonic stem cells into functional thyroid follicles

For Sabine Costagliola the challenge to understand the thyroid gland started early in her career. Initially driven by her immunology passion, she produced a wide array of antibodies against key players in the thyroid function, such as the thyrotropin receptor (THSR). Eventually, this immunological knowledge went on to be converted into *in vivo* experiments. In 1994, she generated BALB/c mice that, when immunised with the extracellular domain of THSR, recapitulated features of human thyroid diseases. By 2000, she had developed new animal models for autoimmune diseases that affect thyroid function, such as Grave's disease and thyroid eye disease. As she became a group leader at the Université libre de Bruxelles, her research expanded to include zebrafish and the use of one of the tools that the 21st century brought along: recombinant murine embryonic stem cells.

Careful cell differentiation

NKX2-1 and PAX8 are two transcription factors that are essential for the development and survival of the thyroid gland but they are not exclusively found in the thyroid tissue. What's extremely remarkable about them is that they are never co-expressed in other organs. "We explored whether the overexpression of NKX2-1 and PAX8 could promote differentiation of murine embryonic stem cells into Thyroid Follicular Cells (TFCs)" – that was the starting point for the Costagliola lab.

The group generated recombinant murine embryonic stem cells with an extra copy of the two transcription factors under the control of the doxycycline inducible promoter. Upon addition of doxycycline (Dox) both transcription factors were expressed to detectable levels by immunofluorescence. After the Dox treatment, the expression of several markers for

thyroid fate was analysed by quantitative reverse transcriptase-PCR. The response of the cells was astonishing; they switched on specific genes for follicular thyroid cells, such as the thyroid-stimulating hormone receptor and thyroglobulin. The combined expression of NKX2-1 and PAX8 thus led the cells to steer their gene expression into a thyroid direction. That was a step in the right direction but no matter how many times the group observed the cells at the fluorescence microscope, they were still on a monolayer. No intricate three-dimensional pattern formed. At this point, many other researchers would have abandoned the project. Not so Costagliola.

"We revised the treatment protocol on the basis of two critical observations." The first observation was the finding that the endogenous NKX2-1 and PAX8 genes were up-regulated by the Dox treatment. A system biologist would call this a 'positive feedback loop' – NKX2-1 and PAX8 boosted their own transcription. "We limited the Dox treatment to a three-day period." After that the group assumed the cells would run on their own NKX2-1 and PAX8. The second observation: "the robust expression of the thyroid hormone receptor indicated that the cells had acquired the ability to respond to the hormone" and therefore after the three-day Dox treatment, the cells were exposed to thyroid hormone.

The experiment was repeated with the modifications and after the transient overexpression of NKX2-1 and PAX8, the stem cells received hormonal treatment. The next time the group sat at the microscope, they contemplated, amazed, that there were not only cells on the plates but also rounded structures reminiscent of thyroid follicles. Were they functional?

Making hormones

"A follicular structure is considered to be a prerequisite for thyroid hormone biosynthesis" was the opinion of the group and they moved forward to test the maverick idea. Could those *in vitro*-generated spherical aggregates really go down the whole biosynthetic pathway?

Thyroid hormone biosynthesis requires at least three crucial steps. First, the iodine has to be grabbed from the external milieu and transported into the follicle, a task for the sodium iodine symporter (NIS). Second, thyroglobulin has to be synthesised and targeted to the inner cavity of the follicle, where the third step takes place: with the help of hydrogen peroxide, thyroglobulin is iodinated.

By immunostaining, Costagliola's group tested whether markers for the biosynthetic steps were present in the follicles. Remarkably, the localisation of NIS, thyroglobulin and other proteins, such as E-cadherin, was almost identical between the follicles and the control, adult thyroid tissue. This fact alone suggested that the follicles could be functional enough to perform the quintessential thyroid task: iodine organification. This process turns thyroglobulin into an iodinated protein, recognisable with antibodies raised against specific iodinated epitopes. After the staining, there was no doubt that the thyroglobulin in the luminal space of the follicle was being iodinated.

To confirm the results, the group ran the "classical" assay for iodine organification. After two hours of incubation in media containing the isotope I125, the total proteins were extracted and the incorporation of radioiodine was measured. Under these conditions, the *in vitro*-generated follicles managed to catch a fair amount of the I125 and put it into their proteins. The follicles not only looked like thyroid tissue, they could also function as such.

The final proof

Boosted by their *in vitro* results, the group was ready to perform the dream experiment of regenerative medicine, functional rescue *in vivo*. They injected mice with radioactive iodine to ablate the thyroidal gland – low levels of thyroxine (T4), the main thyroidal hormone, confirmed the murine hypothyroidism. Then they grafted their follicular organoids onto the athyroid mice, waited four weeks and measured the levels of T4 again. Astonishingly, the *in vitro* differentiated follicles rescued the levels of T4 in nine out of ten mice. Costagliola's group had successfully ablated and then restored the function of the thyroid gland from scratch.

These data have immediate implications for the treatment of hypothyroidism, a condition that affects one out of 3,000 humans. Furthermore, this remarkable research silences many critics of regenerative medicine. No fancy three-dimensional matrix was required for the production of thyroidal follicles. The pure manipulation of transcription factors and hormonal stimulation was sufficient to organise the cells in functional geometrical configuration. Thanks to the passionate work of groups like the Costagliola lab, the dream of generating full organs and body parts is one stage closer.

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