Diabetes: regeneration of the pancreas in the zebrafish

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How can we improve the treatment and quality of life of diabetic patients? One of the lines of the research studied involves the renewal of the beta cells of the pancreas which are responsible for the production of insulin. A team from GIGA at the University of Liège, led by Isabelle Manfroid and Marianne Voz, has just identified progenitor cells in the zebrafish which will make it possible to regenerate beta cells without the need for grafts or medicine.

Five hundred thousand diabetics in Belgium, more than 340 million in the world, more than 5 million deaths every year... Behind these frightening statistics, an entire community of researchers are actively studying the disease and working to find a solution to the problem. The members of the ZDDM laboratory (Zebrafish Development & Disease Models), are part of this community of researchers at the GIGA Research Institute at the University of Liège: "Our work focusses on diabetes: at the laboratory we have for many years been trying to understand how pancreatic beta cells are formed, these cells play a key role in the development of diabetes", says Isabelle Manfroid, one of the heads of the ZDDM laboratory in the Development, Stem Cells and Regenerative Medicine Unit of GIGA.

Diabetes is characterised by the destruction of pancreatic beta cells which secrete insulin, the hormone responsible for lowering sugar levels in the blood. There are two main types of diabetes, types 1 and 2. Type 1 is caused by a deregulation of the immune system which destroys beta cells: patients then become hyperglycaemic and have to inject themselves insulin for the rest of their lives. Type 2 is the result of insulin resistance: the tissues targeted by this hormone (such as muscle, the liver and adipose tissue which should normally consume glucose in response to insulin) no longer respond, which in turn causes hyperglycaemia.

In the long-term, the beta cells try to compensate for this resistance to insulin by trying to produce more of it but they become exhausted and die. The two main types of diabetes therefore involve hyperglycaemia and destruction of beta cells.

Other therapeutic options exist apart from the need for insulin injections for the life-span of the patient. "For serious cases, islets of Langerhans grafts are sometimes performed, but unfortunately, the life-span of the transplanted islets and the stabilisation of the disease are quite limited: at first, the patients concerned no longer need to inject themselves with insulin for a period of several months or even years, but then the graft no longer works and they become dependent on daily injections of insulin again. Another avenue of research that is currently in the development phase, aims to inject beta cells generated from human stem cells. All of these therapies have significant limitations and none of them actually cures the disease", she continues.
"Another therapeutic approach, which we have been actively working on, is to stimulate the regeneration of the patient's beta cells", she explains. "To do this, cells which are already present in vivo in the pancreas could be harnessed, these are pancreatic stem cells or progenitor cells capable of regenerating the beta cells. However, the existence of pancreatic stem cells in humans and even in mice (the traditional model used in laboratories) is shrouded in controversy because it is difficult to demonstrate their existence". How do pancreatic beta cells form in the zebrafish during embryonic development but also in the adult fish?

This is the question that has occupied the ZDDM laboratory for several years. The model of the zebrafish may seem exotic, yet it is very common in research circles. It is a freshwater fish of tropical origin, renowned for its ability to regenerate its own tissues. This latter property has particularly interested the team of GI GA researchers.

"This regeneration does not happen spontaneously in mice: they have to be injected with insulin, that takes up a lot of time and regeneration is incomplete. This ability is limited in humans too. We would like to understand how regeneration works in a successful system such as that of the zebrafish, if we can understand how it works we may be able to apply this to humans. This could take a long time of course. We really want to understand these regeneration mechanisms and, in time, learn to activate them in diabetic patients", she says hopefully.

For the first ten to twelve years, these studies were mainly focussed on embryonic development, therefore the formation of the pancreas where the beta cells are located. This part of the work was done by Marianne Voz. More recently, the laboratory has developed a regeneration model in the adult on which the study was done.
In an article which appeared in *BMC Biology* (1), the researchers provided a demonstration of pancreatic progenitors: these are not exactly stem cells (although the possibility has not been ruled out), they correspond to a slightly later stage in the differentiation of beta cells. Marianne Voz has identified these cells in the embryo: she showed that the cells that express nkx6.1, a gene coding for a transcription factor that is expressed very early in the embryonic pancreas, give rise to beta cells during embryogenesis.

Isabelle Manfroid and her team then looked at what happened in the adult: "*We saw that the cells that expressed nkx6.1 are pancreatic duct cells and that they have the ability to form pancreatic beta cells that are in regeneration. For example, after selective destruction of beta cells in the zebrafish, the latter can regenerate*"
them rapidly and spontaneously. We showed that the regenerated beta cells, or at least some of them, came from the cells that were expressing ntx6.1 in the pancreatic ducts”.

By combining and comparing their information, Marianne Voz and Isabelle Manfroid realised that the ntx6.1 cells, both in the embryo and in the adult, express certain genes in common and have similar properties: these ntx6.1 cells can form beta cells at both of these stages of life. ”Therefore, we find "embryonic" properties in the pancreatic duct cells in the adult: the latter explain a gene-battery present in the embryo which we know are necessary for the formation of beta cells. This was the first time that we were able to demonstrate this property”, adds Isabelle Manfroid.

**From the zebrafish to the mouse?**

To carry out this work, the GIGA researchers created a tool which makes it possible to indelibly mark cells. ”We made the progenitor cells fluorescent, and, based on this property, we were able to isolate them from the embryo or the adult and then analyse their transcriptome, that is to say, we were able to see which factors and which genes they express, over time or in different conditions, like regeneration. Thanks to this very powerful tool, we can mark cells, monitor them and see what happens at a molecular level. It is a real breakthrough”, says a delighted Marianne Voz.

Now that these progenitor cells have been demonstrated, how do the two researchers plan to continue their work? "In the case of the adult, as far as I am concerned", says Isabelle Manfroid, "0nce we have identified the cells that enable regeneration and make it possible to create new beta cells, we then need to understand why and how. Once we have understood these mechanisms, or at least some of them, we will be able to use mice (a model in which it is also possible to perform beta cell ablation) to see whether we can stimulate these mechanisms by using pharmacological compounds, for example. Over the next five years, the main task will be to really elucidate these mechanisms, the transition to using mice as a model could take up to five or six years. Demonstrating that it is possible to transpose the procedure to mammals is a key point".

"For us, explains Marianne Voz, "what is important is to understand what role this ntx6.1 factor plays in the ducts in the embryo. Understanding how it works in terms of the development of the organism”.

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