Are adult pancreatic beta cells formed by self-duplication or stem cell differentiation?

Introduction

Researchers have long been interested in how tissues produce and maintain the correct number of cells in order to maintain homeostasis. It is known that tissue turnover can occur as a result of the duplication of existing differentiated cells or the differentiation of stem cells.

There are two broad types of stem cells found in humans and other mammals—embryonic stem cells and adult stem cells. In a developing embryo, stem cells can differentiate into all of the specialized tissues of the organism. Once the organism is born, adult stem cells act as a repair system, replenishing specialized cells and also maintaining the normal turnover of regenerative organs (blood, skin and intestinal tissues).

It is thought that there might be adult pancreatic stem cells intermingled with the mature, differentiated cells inside the islets of Langerhans of the pancreas or in the bone marrow. The question is, do these adult pancreatic stem cells exist and if so, do they differentiate into insulin-producing β cells?

Background

Researcher Dr. Douglas Melton and his colleagues at Harvard University performed an experiment to determine the source of pancreatic β cells in adult mammals. They developed a method for distinguishing stem-cell-derived β cells from those produced by pre-existing β cells. Pre-existing β cells were defined as fully-differentiated and producing insulin. An adult stem cell that will differentiate to become a beta cell does not fit this definition because it would not be fully differentiated and producing insulin at the start of the experiment.

To be able to find out about the source of beta cells, this is what Dr. Melton and his colleagues did:

- They genetically engineered a mouse model with β cells that possessed a marker. A marker is a gene or a DNA sequence with a known location on a chromosome. Markers can be used to track inheritance. What made the marker used by Dr. Melton’s team unique was that they could switch it on.

- In this experiment, a chemical, tamoxifen, was injected into the mice. This caused a chemical to be produced by mouse β cells possessing the marker. These cells are described as labeled. Cells lacking the marker do not express the chemical and are not labeled. This makes it possible for researchers to determine which islet cells possess the marker and which do not. When a stain is used, the labeled cells become blue. The unlabeled cells do not. In this activity, unlabeled β cells are light in color (white) and labeled β cells are dark (blue).
Since the marker is part of the genetic makeup of the mouse, it is passed on to cells produced through the mitotic division of existing β cells. Beta cells produced through the differentiation of stem cells would lack the marker and are not affected by tamoxifen.

The type of investigation conducted by Dr. Melton and his colleagues is referred to as a pulse-chase analysis. It examines a cellular process occurring over time. It does this by exposing β cells to tamoxifen. This exposure to tamoxifen is followed by the production of the chemical that labels the beta cells. This is the pulse phase of the experiment. The time spent waiting for existing β cells to be replaced by other β cells is the chase.

**The Experiment**

The diagram below represents an islet in the pancreas. Only β cells are shown. Dr. Melton defined an islet as a group of 10 or more β cells. The β cells are represented by small circles. The tamoxifen pulse leads to the expression of the label in insulin-expressing β cells present at the time of the injection. Any cells produced by these cells through mitosis will also express the label. The change is permanent.

When mice were analyzed immediately following the injection of tamoxifen, only about 50% of the β cells were labeled. Dr. Melton and his colleagues concluded from this that the procedure was only about 50% efficient. What they did discover was that every islet contained labeled beta cells.

Depending on the source of new β cells, Dr. Melton predicted that after the passage of various intervals of time, one of three outcomes would be possible:

1. Within existing islets, new β cells would be derived entirely through the mitotic division of existing β cells.
2. New islets would be derived entirely from stem cells
3. Within existing islets, stem cells would replenish β cells.

1. A, B, and C each represent one of the three outcomes predicted by Dr. Melton. For each one, indicate which outcome it represents and support your answer.
Dr. Melton and his colleagues analyzed the mice at 0, 4, 6, 9, and 12 months after tamoxifen injection. To determine whether entirely new islets were formed during the chase period, the number of islets containing labeled β cells was compared to the number counted during the pulse. The graphs below represent what they found.

**Average percentage of islets and beta cells per mouse expressing the label**

2. Which outcome(s) (A, B, or C) does this observation support? Explain.
In addition to islets containing 10 or more β cells, Dr. Melton also included small clusters containing fewer than 10 β cells in his study. He thought that the small clusters might be newly formed, stem cell-derived β cells that were in the process of becoming islets. Such a model predicts that clusters found after a long chase would contain no labeled β cells because they would have come from adult stem cells.

In the pulse group, 78% of the clusters contained labeled β cells. After a chase period of 12 months, half the life expectancy of a mouse, it was found that 82% of the clusters contained labeled cells.

3. What evidence does the small cluster analysis provide regarding the formation of new islets from stem cells over a 12-month period?
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4. Do these results of the small cluster analysis support model A, B, or C. Explain.
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One possible explanation of the results is that there is very little β cell turnover during the chase period. Through a cell-sorting analysis, Dr. Melton learned that the number of β cells increased 6.5 fold between 3 and 12 months of age. He estimates that 98% of the beta cells in a mouse chased for 9 months were born after the pulse.

5. Using evidence from Dr. Melton’s pulse-chase analysis to support your answer, explain which cell type—adult stem cells or differentiated β cells—should be chosen to investigate as a possibility for cell-based therapy for type 1 diabetes.
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6. (a) Identify two problems associated with the use of differentiated β cells for possible cell-based therapies for type 1 diabetes.
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   (b) Identify two problems associated with using adult stem cells for possible cell-based therapies for type 1 diabetes.
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