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RESEARCH ARTICLE

Isolation and *in vitro* cultivation turns cells from exocrine human pancreas into multipotent stem-cells

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Summary

Several research groups have reported on the existence and *in vitro* characterization of multipotent stem-cells from the pancreas. However, the origin of these cells remains largely unexplained. Here, we report that *in vitro* culturing itself can turn adult cells from human exocrine pancreas into a cell population with typical stem cell characteristics. A simple, yet reliable method enabled us to track cell fates: Combining automated continuous observation using time-lapse microscopy with immunocytochemical analyses, we found that a significant fraction of the pancreatic cells ($\approx$14%) can survive trypsination and displays a drastic change in the protein expression profile. After further cultivation, these cells give rise to a heterogeneous cell population with typical multipotent stem cell characteristics; i.e. they proliferate over long time periods and continuously give rise to specialized cells from at least two germ layers. Although we cannot exclude that a rare pre-existing stem cell-type also contributes to the final *in vitro*-population, the majority of cells must have been arisen from mature pancreatic cells. Our findings indicate that multipotent cells for regenerative medicine, instead of being laboriously isolated, can be generated in large amounts by *in vitro* de-differentiation.

Introduction

In mammals, stem-cells play a major role in at least three different physiological processes: (1) ontogenesis, (2) homeostasis, and (3) regeneration (Moore and Lemischka, 2006; Eming et al., 2007).

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Although all stem-cells share the common ability to give rise to specialized cell-types, they differ significantly in other aspects depending on which of the three processes they are involved in. Ontogenic stem-cells, for example, exist only transiently and do not posses the ability of continuous self-renewal, but rather give rise to many different types of progeny cells during development of the organism. In contrast, adult stem-cells, which are required for the homeostasis of tissues (e.g. the intestinal mucosa) fulfill their task for almost the entire life of the postnatal organism (Clarke and Meniel, 2006). These cells often divide in an asymmetrical manner, one daughter cell being identical to the mother cell, while the other eventually gives rise to terminally differentiated cell-types. The third type of stem-cell, which is involved in the regeneration of impaired or lost tissue, can be subdivided into different classes: (1) mobile pre-existing stem-cells that move into the regenerating region from the blood or lymph, (2) resident stem-cells that were present in the affected tissue region before the regenerative process began, and (3) stem-cells, that are generated on demand from differentiated cells by undergoing a regulated de-differentiation. The latter type of stem-cells, which is believed to be involved in tissue regeneration, such as of that of the liver (Cantz et al., 2008), occurs only transiently (Straube and Tanaka, 2006). When the process comes to completion, termination cues cause the regeneration-apparatus to shut down and the now dispensable stem-cells cease to exist (Martin and Parkhurst, 2004).

These considerations apply to the situation *in vivo*. However, when stem-cells are isolated and subjected to *in vitro* culture conditions, things change dramatically. Murine embryonic stem-cells, for example, which turn up only during a very short period of embryogenesis (days 3–4), can be maintained and propagated for indefinitely long periods in cell culture, when kept on a feeder layer and treated with leukemia inhibitory factor (Williams et al., 1988). Conversely, adult stem-cells, which self-renew for many years *in vivo*, start spontaneous differentiation when removed from their niche and put into a culture dish. To date, unlimited *in vitro* self-replication has not been achieved with adult stem-cells due to the lack of a means to suppress their spontaneous differentiation. Given these stark differences between stem-cells *in vivo* and *in vitro*, one may wonder whether stem-cells retain their identity when transferred to *in vitro* conditions, or if they just constitute an interesting, though often useful and well reproducible artifact (Hansson et al., 2007).

The issue of conserving cell identity becomes even more problematic when the stem-cells in question have been observed *in vitro*, but have no known equivalent *in vivo*. In this case, the usual validation of cell identity via cell replantation is not possible, because there is no sufficiently characterized reference cell-type to compare them with. Recently, we and others have reported on such a case (Kruse et al., 2004, 2006; Seaberg et al., 2004; Seeberger et al., 2006; Gorjup et al., 2009). Using simple isolation procedures, a heterogeneous cell population could be obtained from the pancreas which displayed all the essential characteristics of multipotent stem cells *in vitro*. These cell-lines have been thoroughly characterized by the aforementioned groups: They are long-term proliferative, while at the same time generating specialized cell-types from different germ layers. Thus, phenomenologically, they behave like stem-cells. However, the very existence of stem-cells in the pancreas, let alone multipotent stem-cells, which are able to cross germ layer boundaries, is still a matter of considerable debate (Yalniz and Pour, 2005; Choi et al., 2004; Dor et al., 2004; Jaenisch, 2004; Bouwens, 1998). It is even more surprising, that our cell-lines were derived from purified parts of the exocrine pancreas, whereas pancreatic stem-cells, if contained in the pancreas at all, are thought to reside in the pancreatic ducts (Bonner-Weir et al., 2004; Grapin-Botton, 2005).

There are three hypotheses on the ancestry of the obtained pancreatic stem cell-lines: (1) *Impurities/rare cells* – The isolation procedure could select for a very rare pancreatic stem cell-type, which is able to survive the drastic switch to cell culture conditions and has a differentiation potential that can overcome germ layer boundaries. A variation of this hypothesis assumes that motile stem cell-types, such as mesenchymal stem-cells from the blood, represent the actual origin of the cell-lines (Seeberger et al., 2006; Lin et al., 2006; Ruhnke et al., 2005). (2) *Malignant transformation* – Some cells may degenerate, begin to proliferate, and give rise to various specialized cells. In this case, the procedure of cell isolation would have resulted in the reproducible generation of cancer cell-lines, not stem cell-lines as believed hitherto. (3) *De-differentiation* – The isolated, terminally differentiated pancreatic cells dedifferentiate in cell culture, thereby acquiring a more plastic state that can proliferate and give rise to other specialized cell-types. Of course, a combination of the three different mechanisms of cell-line formation is possible as well.

In order to determine which of these hypotheses is most appropriate, we have combined automated