Stem Cells in Neuroendocrinology

Paris, December 7, 2015

Stem cells, which are found in several tissues in the adult body, retain the potential to divide and differentiate, providing a pool of new cells to replace those that are dying or damaged. Recent developments in growing and manipulating stem cells in the laboratory offer exciting prospects for regenerative medicine, with the production of specific cell types to repair malfunctioning tissues and organs. The recent discovery of stem cells in the pituitary gland, the master gland of the hormone system in mammals, has opened up the opportunity to apply these techniques to problems with endocrine regulation. At the Fondation IPSEN’s 15th annual colloquium on Endocrinology, held in Paris on December 7, 2015, an international panel of speakers reviewed the biology of these stem cells and their progeny, and progress in their uses as therapeutic tools. The meeting was organised by Donald Pfaff (The Rockefeller University, New York, USA) and Yves Christen (Fondation IPSEN, Paris, France).

The ultimate stem cell is the fertilised egg, which is totipotent: it will give rise to all the cell types found in the embryo and its supporting membranes. As the egg divides into a multicellular ball, each cell division produces one pluripotent stem cell that retains the capacity to divide, or proliferate, and one cell that proceeds down the differentiation path, first becoming a rapidly multiplying progenitor cell and later a fully differentiated cell that loses the capacity to divide (Inna Tabansky, The Rockefeller University, New York, USA). As the embryo differentiates, first into germ layers and later into specific tissues and organs, the stem and progenitor cells become multipotent: they can now produce only the cell types relevant to that structure. Each location in the embryo is characterised by a spatial and temporal ‘landscape’ of chemical signals that act on the genome to determine the fate of the cells. As differentiation proceeds, the progenitors are increasingly committed to become a specific cell type, for example, skin fibroblasts, gut endothelial cells or neurons, and all the genes that are not required by the specific cell type are inactivated.

Populations of stem cells have been discovered in various adult tissues, including the brain, teeth, bone marrow (the precursors of blood and bone cells), muscle, fat body and testes (Tabansky). These cells, which are rare and divide slowly, are multipotent: they are already limited to producing the cell types of the tissue in which they are found. When they do divide, like embryonic stem cells they give rise to two daughter cells, one of which retains its capacity to proliferate while the other becomes a rapidly
dividing progenitor cell that subsequently differentiates into specific cell types within that organ or tissue.

In the past decade, two powerful techniques have been developed for producing large numbers of stem cells in the laboratory: first, pluripotent cells can be harvested from early embryos; termed embryonic stem cells, they retain their capacity in vitro to proliferate and to differentiate into all the cell types of the body. The second method is to create pluripotent cells from skin fibroblast cells by reversing the process of differentiation. In vitro, these induced pluripotent stem cells (iPSC) can be induced to differentiate again into a whole range of body cells (Tabansky)². Both ESC and iPSC are being used to investigate basic stem-cell biology, such as identifying the chemical signals that drive the choice between proliferation and differentiation. Methods for producing particular types of differentiated cells have been developed (Lorenz Studer, Memorial Sloan Kettering Cancer Centre, New York, USA), with potential therapeutic uses, such as transplantation into the body to replace lost or damaged cells. Stem cells in vitro are also providing good models for investigating disease, testing pharmaceuticals and learning more about how stem cells contribute to the development of cancer.

About 7 years ago, multipotent stem cells were discovered in the pituitary gland, opening up the neuroendocrine system to the application of stem-cell techniques. The pituitary, known as the master gland of the endocrine system, produces hormones that control the release of peripheral hormones that regulate growth, metabolism, body temperature, sleep-wake cycles, reproduction, mineral balance, skin pigmentation and stress responses. But it does not do this alone: pituitary hormones are secreted in response to stimulation by the hypothalamus, an area in the base of the brain the size of a sugar-lump. The hypothalamus exerts its control in two ways. Chemical factors produced by neurons in the hypothalamus are transported down the pituitary stalk to the anterior lobe of the pituitary gland through a local network of blood vessels; in the anterior lobe they stimulate release of pituitary hormones. In contrast, the posterior lobe receives nerve fibres from cells in the hypothalamus that also run through the stalk: their terminals release oxytocin and vasopressin (anti-diuretic hormone) into the lobe, where they are stored until required. Some mammals also have an intermediate lobe, which secretes melanocyte-stimulating hormone, although in humans this division is rudimentary and often considered to be part of the anterior lobe. Together the hypothalamus and pituitary form the neuroendocrine circuit, which controls peripheral hormone production in response to internal and external conditions and through feedback signals from circulating peripheral hormones.

The stress response is a good example of this complex regulation: in stressful situations, neurons in the hypothalamus produce corticotropin-releasing hormone (CRH), which stimulates cells known as corticotrophs in the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the peripheral blood circulation. In turn, this causes the adrenal cortex to release the glucocorticoid hormone, cortisol, which increases metabolism, an essential response to meeting stress. As the circulating cortisol level rises, it feeds back to the hypothalamus and pituitary to limit the response by cutting down the release of CRH and ACTH. Not surprisingly, the system is more complex than this: CRH belongs to a family of peptide molecules that includes three types of...
urocortin, which bind to different degrees to the two known types of receptor for CRH (Alon Chen, Max Planck Institute, Munich, Germany). The urocortins seem to be involved in recovery from stress: mice in which the genes for the three urocortins have been inactivated still display symptoms of stress after 24 hours, unlike their normal counterparts. However, mice overproducing urocortin 3 unexpectedly show an increase in behavioural responses that resemble anxiety and depression. The system may be compensating for the altered gene or the urocortins may have different functions in the various brain areas where they are present – a warning of the care that is needed in interpreting results of gene alteration experiments that affect behaviour.

Neuroendocrine stem cells

Pituitary stem cells have the potential to give rise to all the types of hormone-producing cell in the gland (Studer; Hidetaka Suga, Nagoya University Hospital, Nagoya, Japan; Hugo Vankelekom, University of Leuven, Leuven, Belgium; Cynthia Andoniadou, King’s College, London, London, UK). In mice, they are found in niches bordering the cleft between the anterior and intermediate lobes and are characterised by the marker proteins Sox2, a transcription factor generally associated with stem cells, and nestin, a neurofilament protein found in neural stem cells (Vankelecom). Proliferation and differentiation in vitro and in vivo into pituitary-hormone producing cells confirms that these Sox2+ cells are pituitary stem cells (Andoniadou; Vankelecom; Karine Rizzoti, The Francis Crick Institute, London, UK). Their function is still being established. They may play a role in the neonatal maturation of the pituitary, as the niches are more prominent in newborn mice than in adults and contain more cells that differentiate more rapidly in vitro (Vankelecom). In adults, the pituitary stem cells are mostly quiescent and contribute only at a very low rate, if at all, to turnover of hormone-producing cells (Andoniadou; Vankelecom; Karine Rizzoti). In contrast, in a mouse model, the stem cells are activated after injury to the pituitary, so they may be involved in regeneration; however, efficiency is less than 100% and declines with age (Vankelecom). The stem cells are also activated when a peripheral gland is injured, as when the adrenal glands are experimentally removed (Rizzoti).

In the hypothalamus, a class of cell termed tanycyctes behave like neural progenitor cells. Found in the sub-ventricular zone, where most neurons in the brain are generated, they are Sox2 and nestin-positive cells that both proliferate and differentiate into several types of neuron. Neurogenesis in the hypothalamus is associated with regulation of body weight and feeding control but in a rather complex way (Seth Blackshaw, Johns Hopkins University School of Medicine Baltimore, USA; Rizzoti). High fat diet and deficiency of leptin, a hormone suppressing appetite, stimulates neurogenesis in the median eminence below the base of the third ventricle, in female but not male mice (Blackshaw). In contrast, neurons derived from tanycytes in other areas of the hypothalamus inhibit weight gain (Blackshaw; Rizzoti).

Whether a stem or progenitor cell continues to proliferate or becomes committed to differentiation – and then what type of cell it becomes – depends on intracellular signalling pathways that are only now being elucidated (Andoniadou; Jacques Drouin, Institut de Recherches Cliniques de Montréal, Montréal, Canada). In Sox2+ stem cells,
proliferation is promoted by the Wnt signalling pathway, commonly active during development. Stimulation of this path increases the level of \(\beta\)-catenin, an intracellular protein that activates transcription of genes controlling cell division (Andoniadou). Differentiation is triggered by molecular switches that use different combinations of transcription factors. For instance, pro-opiomelanocortin (POMC), a precursor of several hormones, is made in two types of cell in the pituitary: one produces \(\alpha\)-melanocyte-stimulating hormone (\(\alpha\)-MSH), which regulates skin pigmentation; the other secretes ACTH, the hormone that stimulates the adrenal cortex to produce cortisol. Progenitors of \(\alpha\)-MSH cells are characterised by Pax7, a selector gene, and the transcription factor Tpit, whereas the cells destined to produce ACTH have Tpit but not Pax7. In contrast, precursors of the cells secreting reproductive hormones have neither Pax7 nor Tpit (Drouin). Pax7 functions as a pioneer transcription factor, a type of protein molecule that has the unique ability of implementing a new cell fate. The coiled DNA strand of inactive genes is wrapped by compacted chromatin that prevents gene expression. Pioneer factors like Pax7 open up the compact chromatin, a process known as remodelling, permitting Tpit and other transcription factors to access the DNA and activate the programme of gene expression that results in the cell producing \(\alpha\)-MSH.

**Growing and grafting stem cells**

Neuroendocrine stem cells are almost impossible to harvest from humans. Instead, mouse and human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) are being used to produce hypothalamic and pituitary cells in vitro for both experimental and therapeutic purposes (Tabansky). With the correct combination of molecular factors, hESCs will produce all three developmental lineages that give rise to neural tissue; both hypothalamic neurons and hormone-secreting pituitary cells have been derived from these cultures – although these include cells that secrete more than one hormone, which is not the case in vivo (Studer). This method uses cells cultured on the surface of a dish; an alternative approach is to use three-dimensional floating cultures to recapitulate pituitary development (Suga). First, hypothalamic tissue and oral ectoderm (tissue that will become the lining of the mouth) are induced from hESCs or iPSCs, which provides the conditions for initiating the pituitary primordium. Unlike the flat culture method, specific signal molecules are used only to induce hypothalamic and oral ectoderm cells; further development works best in a medium free from serum and growth factors. The pituitary progenitors in such cultures differentiate into cells secreting a range of hormones in vitro; when cultured tissue containing ACTH-secreting cells was grafted into the kidney capsule of a mouse lacking a pituitary, cortisol secretion, motor activity and vitality were all restored and survival time improved.

The aim is, of course, to produce cells that will become functionally integrated when grafted into host tissue. As a proof of principle, dopamine neurons derived from hESCs in vitro grafted into a mouse model of Parkinson’s disease have resulted in recovery of motor functions, with such success that clinical trials in humans are scheduled for 2017 (Studer). A similar approach is being developed for treating long-term cognitive and motor deficits resulting from brain damage caused by radiation during cancer treatment (Viviane Tabar, Memorial Sloan Kettering Cancer Centre, New York, USA). Animal data indicate that both neural stem cells and the progenitors for
oligodendrocytes, the cells responsible for myelination in the brain, are affected by radiation. Oligodendrocyte progenitors derived from hESC have been successfully grafted into rat cerebral cortex after irradiation: nerve fibres are remyelinated and performance on behavioural tests recovers. Pituitary cells are also being derived from hESC to develop a treatment for reduced pituitary function resulting from radiation damage.

An important consideration for using stem cells clinically is that they have the potential to form tumours (Tabansky; Rizzoti). Benign tumours are relatively common in the pituitary, causing either increased or reduced hormonal output, with metabolic and cognitive problems. These tumours contain stem cells characterised by factors that drive tumour growth and expansion, which need to be eradicated to stop the tumour developing (Vankelecom). One class of these tumours have cells with a mutation in the β-catenin gene and pituitary stem cells containing this mutation can induce tumour formation in vivo (Andoniadou). However, the mutation is not in the bulk of tumour cells but specifically in Sox2+ pituitary stem cells. These stem cells carrying the mutation do not directly give rise to the tumour mass – rather, they secrete factors that stimulate surrounding cells to divide and form the tumour (Andoniadou). Further investigations will determine which type of cell originally responds to factors secreted by the mutated stem cells.

**Disease in a dish**

As well as their potential therapeutic applications, stem cells are valuable tools for modelling disease mechanisms, beautifully illustrated here in a study of depression (Patricia Zunszain, Institute of Psychiatry, London, UK). Stem cells in the adult hippocampus are known to provide a source of new neurons involved in memory processing. Now it seems that this neurogenesis is reduced by circulating glucocorticoid stress hormones, inflammation and, to some extent, by diet – all factors that may contribute to depression. Conversely, antidepressants, exercise, environmental stimulation and some aspects of diet increase neurogenesis. These effects are being more closely defined using human hippocampal stem cells in vitro: antidepressants increase and glucocorticoids decrease the proliferation of progenitor cells, whereas differentiation into neurons and their subsequent maturation is reduced by the inflammatory cytokine interleukin 1β, which stimulates a neurotoxic pathway. Similar experiments with depressive and anti-depressive stimuli are being carried out with iPSCs from healthy controls. The hope is that having established such parameters, iPSCs from patients could one day be used to assess how an individual will respond to drug treatments, which varies considerably between patients.

**Stem cells in the clinic**

In some fields, stem-cell therapy is relatively advanced. Bone-marrow transplants, essentially stem-cell grafts, are a well-established treatment for certain blood cancers and a stem-cell treatment for the retinal disease macular degeneration is in clinical trials (Rizzoti). Much of the effort in pituitary stem-cell research is directed towards treatment for under-active pituitary, or hypopituitarism, a serious neuro-endocrine disturbance that affects quality of life and causes both morbidity and mortality. It is a
moderately frequent congenital disorder but can also be acquired as a result of pituitary tumours, brain damage and radiotherapy. Current treatment employs hormone substitution, which does not mimic normal patterns of secretion, is costly and has side effects. Grafts that integrate functionally into the host tissue, of either progenitors or hormone-secreting cells produced in vitro, offers a much more satisfactory alternative (Rizzoti).

Although cells can be grafted ectopically, animal experiments show that control of hormone secretion is best when the graft is close to the hypothalamus (Rizzoti). As pituitary surgery is routinely performed through the transnasal route using endoscopy to remove tumours, once cells are available, grafting into the gland should pose little problem (Tabar). Similar opportunities exist for developing treatments for hypothalamic deficiencies and could in future be employed for conditions including obesity, infertility, sleep disorders and age-related conditions. Here the evidence indicates that grafts sited in the brain’s third ventricle are the most effective (Rizzoti).

Two obstacles to grafting are immune rejection and the danger of tumour formation (Tabansky). Whereas cells derived from hESCs carry the risk of triggering immune responses, the recent development of iPSCs, which can be derived from a patient’s own cells, circumvents the problem for treating accidental damage. Two difficulties remain: first, using stem-cell derived cells for treating autoimmune diseases, where the patients’ immune systems destroy their own cells; here, techniques being developed for modifying the immune system may be the answer (Tabansky). Second, congenital malfunction that results from a deficit of a specific hormone-producing cell type cannot be repaired with iPSCs from the patient, which would only replicate the deficit. The recently introduced technique of gene editing could be used to repair the deficiency in iPSCs in vitro before cells are differentiated for grafting. Gene editing may similarly be employed to reduce the risk of tumour formation (Rizzoti).

A developing field

Given the short time since the discovery of neuroendocrine stem cells, substantial progress has been made. Even so, much of the talk at the meeting was about potential. Because the neuroendocrine system regulates such a range of body activities, the prospect of precise interventions, not only to repair damage but also to improve a less-than-adequate function, is powerful (Pfaff). These aims are being facilitated by new tools for manipulating cells: gene editing in vitro is one example; another is the use of viral constructs to deliver growth factors to stem cells in vivo, either to stimulate differentiation or to alter their developmental fates (Tabansky). Techniques like opto-genetic manipulation of neurons are being used to test the function of tissue derived from grafted cells in animal models (Studer), while micro-fluidics, nanoparticles and designer receptors activated only by matching designer drugs (DREADDS) are enabling the precise targeting of chemicals to selected cells in vivo to alter their function (Pfaff). These developments come with ethical and safety challenges but the enthusiasm evident at the meeting will no doubt rapidly drive the field forward in the next few years.

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