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On Stem Cells, Organoids and Human Disease

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Stem cells build our bodies during development, starting from a fertilized oocyte. Subsequently, they maintain and repair our organs for a lifetime. Thus, stem cells hold the promise of eternal life. While this may still be in the distant future, stem cells are rapidly changing the face of biomedical science and are finding applications in the clinic. In this article, I discuss the principles of stem cell biology and the mini-organs that can be grown from stem cells in a petri dish.

Stem cells first came to prominence in the Second World War. After two nuclear bombs were dropped on Hiroshima and Nagasaki, radiation sickness was first described by the Red Cross Hospital Surgeon, Terufumi Sasaki. As a major symptom, he noticed the loss of white blood cells from the blood of patients within weeks after the bombings. Subsequent experiments in the 1950s established that intravenous injection of bone marrow cells reversed the loss of blood cells. It was thus found that a population of radio-protective cells exists within the bone marrow, capable of generating all types of circulating blood cells as well as of self-renewing this population. Thomas and colleagues (1957) were prompted by these observations to develop therapeutic bone marrow transplantation. For this, they turned to identical twins, the recipient of the bone marrow stem cells being a leukaemia patient.

The transplantation technology that was developed from this allowed scientists to replace a qualitative phenomenon, the rescue of blood cell production by donor-derived bone marrow, with the properties of a physical entity, i.e. the rare, almost magical, multipotent hematopoietic stem cell (HSC). Till and McCulloch (1961) in Canada then developed a surrogate, quantitative assay for HSCs in vivo, the spleen focus-forming assay. Various in vitro assays for clonogenic HSCs and lineage-restricted progenitors followed, and the HSC field was born.

Fifty years after the initial findings, stem cell transplantation is now one of the pillars of the treatment of leukaemia. The scientific field has achieved broad consensus on the defining characteristics of HSCs and on the design of the cellular

differentiation hierarchy fuelled by these stem cells. A stem cell has the unique, defining capacity to self-renew, and its lineage is therefore long-lived. Individual HSCs can generate all blood lineages – in other words, they are multipotent. While daughter cells continue to divide, they migrate down the hierarchy and become progressively lineage restricted. This occurs through a well-orchestrated series of discrete steps and, eventually, yields mature blood cells of the various types.

Much more recently, it has become feasible to identify specialized stem cells in other tissues and organs. From a stem cell perspective, solid tissues come in two 'flavours'. Some (such as liver, pancreas or lung) display very little cell proliferation in their steady state but undergo bursts of proliferation upon damage. Others (such as the epidermis, testis or the epithelial lining along the length of the intestinal tract) are constantly self-renewing with tissue replacement rates of the order of days, weeks or months. The latter tissues resemble the bone marrow in their continuous generation of daughter cells.

Our work on the inner lining of the small intestine (the epithelium) has revealed many unexpected aspects of the behaviour and use of adult tissue stem cells. The epithelium of the small intestine is organized into large numbers of self-renewing crypt-villus units. Villi are finger-like protrusions of the gut wall that project into the gut lumen to maximize the available absorptive surface area. A villus is covered by a simple non-dividing layer of cells, underneath which capillaries and lymph vessels mediate transport of absorbed nutrients into the body. The base of each villus is surrounded by multiple epithelial invaginations, termed crypts of Lieberkühn, after their discoverer Jonathan Nathanael Lieberkühn (1711–1756), who used wax injections to reveal anatomical structures. It has long been known that crypts are home to a population of vigorously proliferating epithelial cells, which fuel the active self-renewal of the epithelium. The most populous cell on the villus is the absorptive enterocyte, a highly polarized columnar cell, characterized by an elaborate luminal brush border. Goblet cells and enteroendocrine cells secrete mucus and a variety of hormones, respectively, and are located both on villi and in crypts. Paneth cells occupy the bottom positions in the crypt and have long been known to secrete bactericidal products such as lysozyme and defensins. And finally, microfold (M) cells reside in the specialized epithelium that overlies Peyer's patches, lymphoid accumulations that play a key role in mucosal immunity. M cells are believed to serve as portals for luminal antigens.

In 1947, Stevens and Leblond (1947) published a landmark study on the rate and mechanism of self-renewal of the epithelium. They concluded that full-grown rats continue to produce large numbers of cells in crypts throughout life and that the life cycle of an individual cell is of the order of days, a notion that was met with disbelief at the time. Also, they realized that this high birth rate of crypt cells should be balanced by a graveyard located elsewhere. They concluded, '... the cells formed in the crypts of Lieberkühn move upward along the side of the villi to be ejected when they reach the villi tips.' From these observations, it followed logically that stem cells fuelling this rapid self-renewal process should reside somewhere near the crypt bottoms. Such stem cells should display two basic

characteristics: self-renewal and multipotency. In other words, they should persist for the lifetime of the mouse while producing all other cell types of the epithelium. It was again the Leblond lab that was the first to investigate the identity of the crypt stem cell. Cheng and Leblond (1974) noted that the crypt base is not exclusively populated by Paneth cells. Wedged between these prominent postmitotic cells, electron microscopy revealed the presence of diminutive cells that are continuously cycling, the so-called crypt base columnar (CBC) cells.

Much later, in 2007, we discovered Lgr5 as an exquisite marker for the CBC cell. Using a series of mouse models, we could prove that the CBC cell, marked by the molecular marker Lgr5, represents the stem cell of the intestinal crypt.

It is generally assumed that adult tissue cells cannot be cultured for prolonged periods of time. Indeed, after six decades of bone marrow transplantation, it has remained impossible to significantly expand hematopoietic stem cells in culture. Because Lgr5 stem cells divide every day, they complete around 1000 cell divisions in the lifetime of a laboratory mouse. Based on the growth factor requirements observed in vivo, we have established a Matrigel-based culture system that allows the formation of ever-expanding 'organoids' (or 'mini-guts') in vitro from a single Lgr5 stem. An essential component of these cultures is the Wnt agonist Rspondin1, the ligand of Lgr5. The other constituents are EGF and the BMP inhibitor Noggin. The mini-guts faithfully recapitulate the central features of normal gut epithelium. They consist of crypts (with resident Lgr5 cells, Paneth cells, and TA cells) that feed into a central lumen lined by mature epithelial cells of all villus lineages. Self-renewal kinetics resemble the in vivo situation: cells are born in the crypts, proliferate, differentiate, and are shed into the central lumen about 5 days later. Clonal organoids expanded from a single adult colonic $Lgr5^{+}$ cell have been transplanted into multiple recipient mice in which epithelial damage had been induced by chemical treatment. The grafted organoids remained healthy and functional for at least 6 months after transplantation. Since then, similar cultures conditions have been developed for a variety of other tissue stem cells, invariably leading to the growth or organoids recapitulating central features of the tissue from which the stem cells were originally derived. The past 9–10 years have witnessed an explosion of technologies and discoveries around this concept. An organoid is now defined as a 3D structure grown from stem cells and consisting of organ-specific cell types that self-organizes through cell sorting and spatially restricted lineage commitment. Iconic examples of organoids derived from pluripotent stem cells are the so called 'mini-brains' of Lancaster *et al.* (2013), and the mini-kidneys of Takasato *et al.* (2016) from Melbourne.

Human organoids have rapidly gained ground in basic biomedical research, as alternatives to animal experimentation and to the classical cancer cell line models. They are also increasingly used to model a broad range of human diseases: In infectiology, organoids allow the study of a variety of pathogens, be it bacteria such as the stomach-ulcer causing Helicobacter, or Noro- and influenza viruses. In human genetics, they can model hereditary diseases such as Cystic fibrosis. In oncology, organoids can be directly grown from tumours and they are now believed to faithfully recapitulate tumour cells of individual patients.

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Organoids have turned out to be very useful for personalized medicine approaches, i.e. to tailor the prescription of drugs to each individual patient. Organoid technology allows rapid ex vivo testing of drug responses on the affected tissue of individual patients. As a first example, a colon organoid-based Cystic Fibrosis (CF) test can be read out in weeks after obtaining a tiny amount of living colon tissue from individual CF patients. The test allows an almost black-and-white readout to determine if a given patient will or will not respond to a given drug. The approach has already been applied for identification and successful treatment of patients with very rare versions of Cystic Fibrosis, who otherwise have no access to the recently introduced, very expensive CF drugs. Indeed, organoid testing is now part of the clinical routine: when CF patients with a rare version of the disease yield a positive organoid test, they will be given the expensive CF drug and the insurance will refund the cost.

The feasibility of culturing various solid tumours directly from the patient in the form of tumour organoids (see above) holds a similar promise. Initial studies are already indicating that tumour-derived organoids can predict with high precision whether a patient will or will not respond to a given drug or drug combination. This would be clinically extremely important given (1) the general disease severity of cancer; (2) the extensive side effects of chemotherapy; and (3) the cost of cancer drugs. Currently, cancer patients are subjected to specific therapeutic regimens based on statistical considerations only: an individual cancer patient is carefully classified by pathology (and sometimes by DNA analysis). It is then known what statistically represents the most promising treatment for patients in a particular class, yet is unknown which individual from that class will and which individual will not respond. Tumour organoid technology promises that multiple treatments can be tested in the lab on the tumour cells of a given patient and that the treatment that is proven to eradicate the tumour cells in the lab will subsequently be given to the pertinent patient. It is anticipated that it will take several years before this approach will be validated and can become clinical practice.

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About the Author

Hans Clevers obtained his MD and PhD degrees from Utrecht University (the Netherlands). After a postdoc at Harvard University, he returned to Utrecht where he has held several positions since. He studies cancer and stem cells. He is a member of several international academies, served as the president of the Royal Netherlands Academy of Arts and Science (2012–2015) and has won numerous awards for his work on stem cells, cancer and organoids. He is Chevalier de la Légion d'Honneur and Knight in the Order of the Netherlands Lion.