The Stem Cell State

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Abstract

This volume describes the latest findings on transcriptional and translational regulation of stem cells. Both transcriptional activators and repressors have been shown to be crucial for the maintenance of the stem cell state. A key element of stem cell maintenance is repression of differentiation factors or developmental genes – achieved transcriptionally, epigenetically by the Polycomb complex, and post-transcriptionally by RNA-binding proteins and microRNAs. This volume takes two approaches to this topic – (1) illustrating the general principles outlined above through a series of different stem cell examples – embryonic, iPS and adult stem cells, and (2) describing several molecular families that have been shown to have roles in regulation of multiple stem cell populations.

Keywords

Clonogenicity • History • Niche • Pluripotency • Repression

1.1 The History of Stem Cells

The term "stem cell" has had a variety of meanings over the past decades and its history is intertwined with the concept of cell potency. These ideas can be traced back to the work of Hans Driesch in the early 1890s who used vigorous shaking to isolate blastomeres from two-cell sea urchin embryos and was then able to demonstrate that these single blastomeres were totipotent and could develop into complete larvae [1]. The pluripotent nature of cells in the vertebrate blastula was elucidated by Robert Briggs and Thomas King in 1952 by transfer of Xenopus blastula cells into enucleated oocytes [2]. This work was extended by John Gurdon in the late 1950s-early 1960s in a now famous series of experiments that resulted in the cloning of Xenopus by nuclear transfer [3, 4]. The pluripotency of mammalian embryonic cells was initially demonstrated by

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G. Hime and H. Abud (eds.), *Transcriptional and Translational Regulation of Stem Cells*, Advances in Experimental Medicine and Biology 786, DOI 10.1007/978-94-007-6621-1_1,

transplantation of teratocarcinoma cells into blastocysts by Beatrice Mintz and Karl Illmensee in 1975 [5].

The pluripotential capacity of differentiated adult mammalian cells became clear with the generation of the sheep named "Dolly" by the group of Ian Wilmut in 1996 using somatic cell nuclear transfer [6]. The foundations for the generation of Dolly go back to the 1928 studies of Hans Spemann (published in his 1938 book "Embryonic development and induction") who was the first to transfer a nucleus from one cell to another in a salamander embryo [7]. Direct re-programming of differentiated adult mouse cells was achieved by Shinya Yamanaka and colleagues in 2006 to produce induced pluripotent stem cells (iPS cells) followed by similar studies from human cells in 2007 [8, 9].

The first description of a cell as a stem cell (or Stammzelle) was by Alexander Maximow in his 1909 reference to the lymphocyte as a common element to all blood cell types [10]. Experimental evidence for the existence of stem cells *in vivo* was not obtained until 1963 when work from the laboratory of Ernest McCulloch and James Till showed that cells isolated from bone marrow when transplanted into irradiated mice formed nodules in the spleen in proportion to the number of cells first injected [11]. The term "embryonic stem cell" is credited to Gail Martin. In 1981 both Martin and the team of Martin Evans and Matthew Kaufman independently derived methods of extracting embryonic stem cells from the inner cell mass of mouse blastocysts [12, 13]. In 1998 James Thomson established the first human embryonic stem cell lines [14]. Stem cells therefore have a long experimental history but the criteria used to define them have varied over this period.

1.2 What Is a Stem Cell?

Stem cells have traditionally been defined by a variety of functional assays leading to some differences in whether specific cells are considered as stem cells. Clonogenicity has long been considered a "gold standard" for identifying if stem cells are present in a population of cells and as a surrogate method of determining the number of stem cells in the population [11, 15]. These experiments conducted with haematopoietic tissue demonstrated the self-renewing capacity of stem cells (required for generation of a transplant colony, either *in vitro* or *in vivo*) and the ability of multiple lineages to be derived from the stem cell founders. Multipotency should not be regarded as a condition of all stem cells as transplantable spermatogenic stem cells are present in the testis [16] that only produce sperm precursors under normal conditions. The nature of the assay used to define or culture stem cells is of critical importance when defining the characteristics of the stem cell population as even unipotent spermatogenic stem cells can be induced to differentiate into cells with characteristics of all germ layers when cultured under specific conditions not normally found in the seminiferous tubules of the testis environment [17].

Another characteristic associated with stem cells is that they are long lived and in many organs are essentially immortal, persisting for the lifetime of the host organism. Perhaps it is more appropriate to consider the lifespan of the stem cell pool in an organ as recent lineage tracing studies in the mouse intestinal epithelium have demonstrated turnover of individual stem cell clones in intestinal crypts while maintaining a steady state stem cell pool [18]. Some cell types capable of self-renewal and production of differentiated daughters only exist for a limited number of cell divisions during developmental processes, for example embryonic neuroblasts of *Drosophila melanogaster*, and have been referred to as progenitor cells rather than stem cells [19].

A decrease in stem cell activity or loss of stem cell pools has been thought to be associated with tissue aging. An experimental demonstration of the principle can be observed by transplantation of purified spermatogonia from differently aged mice into young recipient testes and counting numbers of subsequent graft colonies. Spermatogonia from aged mice produce far less grafts and hence can be considered to contain fewer spermatogonial stem cells. This correlates with decreasing fertility

observed in aged animals [20]. However, if purified spermatogonia from young males are serially transplanted into young testes they can produce engraftment rates similar to those from young animals even when the serial grafts pass 3 years of age. This has been interpreted as evidence that the stem cells are not aging but the somatic support cells lose the capacity to maintain the stem cell population as tissues age [20].

Stem cells do not appear to be associated with any specific mode of cell division. Germline stem cells in *Drosophila* and *C. elegans* cycle continuously [21] while many vertebrate stem cell populations are regarded as quiescent and capable of being marked by long term retention of radiolabelled nucleotide analogues [22, 23]. These species-specific distinctions are now less clear as rapidly cycling stem cell populations have been isolated from vertebrate organs [24] and there are indications that different stem cell types may play separate roles, or cycle differently during homeostasis and tissue repair [25].

Stem cells by definition must be undifferentiated cells as their main role is to provide a pool of cells that can regenerate components of a tissue via a series of steps that involve tightly regulated division and differentiation. The cellular environment, or stem cell niche, regulates stem cell behaviour by providing appropriate signals that influence maintenance, proliferation and differentiation. This hypothesis was first proposed by Schofield [26] and experimental evidence for the existence of the niche came from genetic studies in the *Drosophila* female germline [27]. Even this simple functional relationship between stem cell and niche has now become confused as evidence has been obtained that stem cell progeny can contribute to the niche (reviewed in [28]) and it is now known that stem cells of one lineage can act as niche cells for stem cells of another lineage. For example, populations of germline and somatic stem cells co-exist in the apical tip of the adult *Drosophila* testis. The somatic stem cells secrete a BMP-family signal that is critical for maintenance of the germline stem cell population in addition to acting as a precursor to differentiated somatic cyst cells [29, 30].

1.3 Stem Cell Maintenance Involves Repression of Differentiation

As is described in the chapters of this volume, stem cells are found in tissues derived from all germ layers, either quiescent or cycling, and associated with varied niches. It follows suit that different stem cells pools are regulated by different molecular mechanisms and few generalities can be drawn regarding this regulation. There does not appear to be a general factor that promotes "stemness" in a population of cells. What can be said in a general fashion is that stem cells must remain undifferentiated if the pool is to be maintained and molecular mechanisms that promote stem cell maintenance must repress differentiation. The first studies to show global repression of developmental genes (i.e. those that promote differentiation of various tissues and organs) in stem cells were conducted in mouse and human embryonic stem cells [31, 32]. These studies demonstrated that the Polycomb group proteins act as repressor complexes to suppress transcription of mainly developmental genes in ES cells without affecting genes required for nucleic acid metabolism, cell cycle and protein synthesis. The following chapters will show that we now know much more about the transcriptional circuitry that regulates stem cell behaviour but that this is only one layer of regulation imposed upon stem cells. microRNAs and translational activators/repressors also play key roles in promoting stem cell maintenance and controlling differentiation. The Polycomb proteins recruit factors that modulate histone methylation and hence play an epigenetic role in maintaining patterns of gene expression. This mechanism appears not to be restricted to embryonic stem cells but epigenetic regulation of stem cell maintenance is a more general phenomenon [33].

This volume describes different stem cell populations and the varied molecular genetic mechanisms that have been associated with their regulation.

References

1. Driesch H (1892) The potency of the first two cleavage cells in echinoderm development. Experimental production of partial and double formation (reprinted translation). In: Oppenheimer JM (ed) Foundations of experimental embryology, part 2. Hafner, New York, pp 39–50
2. Briggs R, King TJ (1952) Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. Proc Natl Acad Sci USA 38(5):455–463
3. Gurdon JB (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J Embryol Exp Morphol 10:622–640
4. Gurdon JB, Elsdale TR, Fischberg M (1958) Sexually mature individuals of Xenopus laevis from the transplantation of single somatic nuclei. Nature 182(4627):64–65
5. Mintz B, Illmensee K (1975) Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proc Natl Acad Sci USA 72(9):3585–3589
6. Campbell KH, McWhir J, Ritchie WA, Wilmut I (1996) Sheep cloned by nuclear transfer from a cultured cell line. Nature 380(6569):64–66
7. Spemann H (1938) Embryonic development and induction. Yale University Press, New Haven
8. Takahashi K, Tanabe K, Ohnuki M, Narita M et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872
9. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
10. Maximow A (1909) The lymphocyte as a stem cell common to different blood elements in embryonic development and during the post-fetal life of mammals. Originally in German. Folia Haematol 8:125–134 [English translation (2009) Cell Ther Transplant 1(3):14–18]
11. Becker AJ, McCulloch EA, Till JE (1963) Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197:452–454
12. Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292(5819):154–156
13. Martin GR (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci USA 78(12):7634–7638
14. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA et al (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147
15. Moore MA, Metcalf D (1970) Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. Br J Haematol 18(3):279–296
16. Brinster RL, Zimmermann JW (1994) Spermatogenesis following male germ-cell transplantation. Proc Natl Acad Sci USA 91(24):11298–11302
17. Simon L, Ekman GC, Kostereva N, Zhang Z et al (2009) Direct transdifferentiation of stem/progenitor spermatogonia into reproductive and nonreproductive tissues of all germ layers. Stem Cells 27(7):1666–1675
18. Snippert HJ, van der Flier LG, Sato T, van Es JH et al (2010) Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell 143(1):134–144
19. Chia W, Somers WG, Wang H (2008) Drosophila neuroblast asymmetric divisions: cell cycle regulators, asymmetric protein localization, and tumorigenesis. J Cell Biol 180(2):267–272
20. Ryu BY, Orwig KE, Oatley JM, Avarbock MR et al (2006) Effects of aging and niche microenvironment on spermatogonial stem cell self-renewal. Stem Cells 24(6):1505–1511
21. Spradling A, Fuller MT, Braun RE, Yoshida S (2011) Germline stem cells. Cold Spring Harb Perspect Biol 3(11):a002642
22. Cotsarelis G, Sun TT, Lavker RM (1990) Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell 61(7):1329–1337
23. Potten CS, Booth C, Pritchard DM (1997) The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol 78(4):219–243
24. Barker N, van Es JH, Kuipers J, Kujala P et al (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449(7165):1003–1007
25. Li L, Clevers H (2010) Coexistence of quiescent and active adult stem cells in mammals. Science 327(5965):542–545
26. Schofield R (1978) The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells 4(1–2):7–25
27. Xie T, Spradling AC (2000) A niche maintaining germ line stem cells in the drosophila ovary. Science 290(5490):328–330
28. Hsu YC, Fuchs E (2012) A family business: stem cell progeny join the niche to regulate homeostasis. Nat Rev Mol Cell Biol 13(2):103–114
29. Leatherman JL, Dinardo S (2008) Zfh-1 controls somatic stem cell self-renewal in the drosophila testis and nonautonomously influences germline stem cell self-renewal. Cell Stem Cell 3(1):44–54
30. Leatherman JL, Dinardo S (2010) Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in drosophila testes. Nat Cell Biol 12(8):806–811
31. Boyer LA, Plath K, Zeitlinger J, Brambrink T et al (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441(7091):349–353
32. Lee TI, Jenner RG, Boyer LA, Guenther MG et al (2006) Control of developmental regulators by polycomb in human embryonic stem cells. Cell 125(2):301–313
33. Jepsen K, Solum D, Zhou T, McEvilly RJ et al (2007) SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature 450(7168):415–419